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Original Article

A *TaqI* polymorphism of vitamin D receptor is associated with Alzheimer's disease in Korean population: a case-control study

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Abstract: Alzheimer's disease (AD) is the most common type of dementia, although its symptoms, causes, and risk factors were discovered only in the last 30 years. Several studies have investigated the associations between vitamin D receptor (*VDR*) polymorphisms and the risk of AD. In addition, a recent meta-analysis suggested that *Apa*I and *Taq*I polymorphisms are associated with the risk of AD. In this study, the associations between four *VDR* polymorphisms and the risk of AD in a Korean population were investigated. Also, the association between *VDR* polymorphisms and the risk of AD was subjected to meta-analysis. The study enrolled 144 AD patients and 335 healthy controls without dementia. The four *VDR* gene polymorphisms were genotyped by PCR sequencing. The meta-analysis was performed using the PubMed, Science Direct, Scopus, and Google Scholar databases up to December 2015 using the search terms "vitamin D receptor or VDR" and "variant or polymorphism or SNP" in combination with "Alzheimer's disease". The results showed that only the *Taq*I polymorphism was significantly associated with an increased risk of AD in Koreans. Furthermore, the meta-analysis indicated that the *Taq*I polymorphism was significantly associated with an increased risk of AD in the overall population. Therefore, the *Taq*I polymorphism of VDR may be closely associated with the risk of AD, and so could facilitate monitoring of AD. Large-scale studies are necessary to evaluate further the association between VDR gene polymorphisms and the risk of AD. Furthermore, various environmental risk factors for AD should be examined.

Keywords: Alzheimer's disease, vitamin D receptor, case-control study, meta-analysis, Korean population

Introduction

Worldwide, 35.6 million people were estimated to have dementia in 2010, and that number is projected to increase to 65.7 million by 2030 and to 115.4 million by 2050. Approximately 7.7 million new cases of dementia are diagnosed annually worldwide [1]. Alzheimer's disease (AD) is the most common type of dementia, although its symptoms, causes, and risk factors were discovered only in the last 30 years [2]. Several genetic risk factors for AD have been identified. Polymorphisms in amyloid precursor protein (APP), presenilin-1 (PSEN1), and presenilin-2 (PSEN2) are associated with susceptibility to early onset familial AD (EOFAD) [3]. In addition, the ε4 variant of apolipoprotein E (ApoE) is closely associated with an increased risk of late-onset Alzheimer's disease (LOAD) [4]. Many candidate genes have also been identified in genome-wide association studies [5-7].

Vitamin D regulates the transcription of target genes by binding to the vitamin D receptor (VDR), which is involved in the endocrine system, insulin-like growth factor signaling, and estrogen-related pathways [8]. Moreover, vitamin D monitors calcium levels by regulating intracellular calcium buffering systems as well as the expression of L-type voltage-sensitive calcium channels (L-VSCCs) [9]. Calcium signaling is important in neurons for a variety of functions, including synaptic plasticity, neurotransmitter release, and neuronal excitability [10]. In addition, vitamin D is associated with produc-

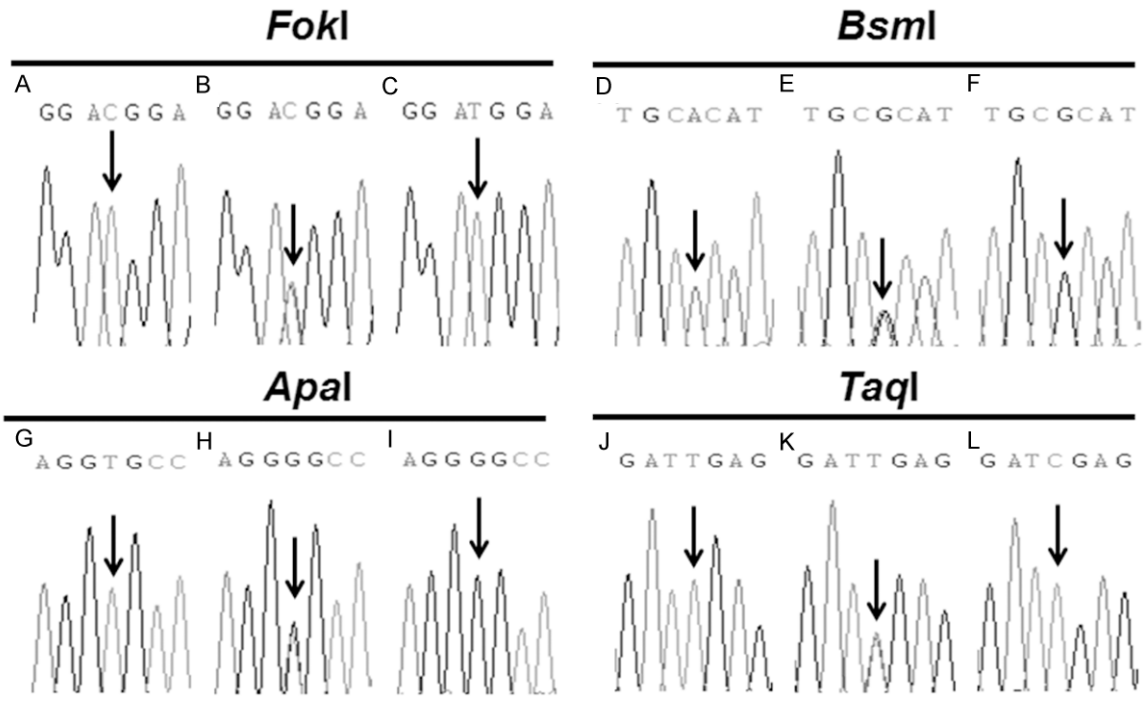

Figure 1. Sequencing results of the four VDR gene polymorphisms. A-C. Are FF, Ff and ff of FokI. D-F. Are BB, Bb and bb of BsmI. G-I. Are AA, Aa and aa of ApaI. J-L. Are TT, Tt and tt of TaqI. Black arrows indicate polymorphisms.

tion of neurotrophins and neurotrophic factors, including neurotrophin 3 (NT3), neurotrophin 4 (NT4), and glial cell line-derived neurotrophic factor (GDNF) [11]. Moreover, regulation of neurotrophin production by vitamin D exerts a neuroprotective effect in some cases [12]. A functional study reported that VDR overexpression or vitamin D treatment is significantly associated with decreased expression of APP in neuroblastoma cells [13]. In addition, several genetic studies have examined the association between VDR polymorphisms and the risk of AD [14-20]. Two studies reported that *Apa*I and *Taq*I polymorphisms of the VDR gene were significantly associated with AD risk [14, 15].

Therefore, we hypothesized that vitamin D/VDR binding is a potential risk factor for AD. This study focused on four genetic polymorphisms of the VDR-*Fok*I, *Bsm*I, *Apa*I, and *Taq*I. The associations between the four polymorphisms of VDR and the risk of AD in a Korean population were evaluated. In addition, the associations between the four polymorphisms of the VDR gene and the risk of AD in seven published case-control studies and this work were subjected to meta-analysis.

Materials and methods

Subjects

The study enrolled 144 AD patients and 335 healthy controls without dementia. The mean age of the AD group (119 females and 25 males) was 79.82 ± 7.02 years and that of the controls (166 females and 169 males) was 68.94 ± 6.10 years. The average education level of the AD group (2.92 ± 3.59 years) was lower than that of the control group (8.29 ± 5.26 years). The clinical dementia rating (CDR) of AD patients was as follows: 58 patients had a score of 0.5, 51 patients a score of 1, 28 patients a score of 2, and 7 patients had a score of 3. Among the healthy controls, 321 had a score of 0 and 14 a score of 0.5. AD patients were recruited from a dementia clinic at Dankook University Hospital, South Korea. The cognitively normal control participants were community-dwelling elderly who had participated in the Korean Longitudinal Study on Cognitive Aging and Dementia (KLOSCAD) and the Nationwide Dementia Screening and Registration Program (NDSRP) [21]. All subjects underwent standardized clinical interviews and neurological and physical examinations, administered by geriat-

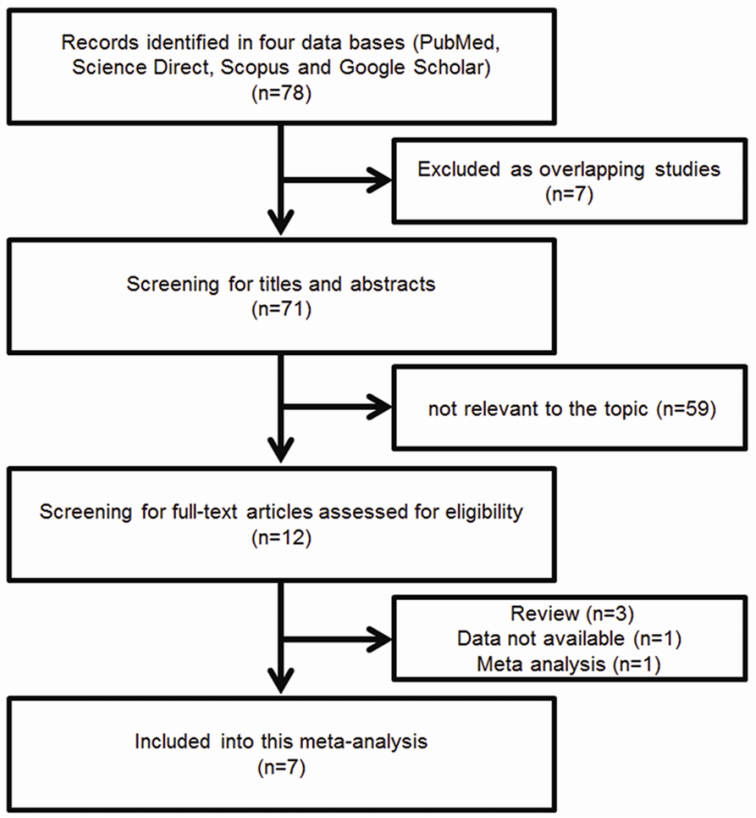

Figure 2. Flow chart of the selection of studies for inclusion in our meta-analysis.

ric psychiatrists with advanced training in neuropsychiatry and dementia research. Dementia was diagnosed using the Korean version of the protocol of the clinical assessment battery established by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD-K) [22]. Dementia was diagnosed using the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), and probable or possible AD was diagnosed using the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [23]. All participants were fully informed of the aims of the study, and each participant or his/her legal guardian provided written informed consent.

Assessment of neuropsychological functions

Global cognitive function was evaluated via the mini-mental status examination (MMSE) [22, 24]. To assess memory, the word-list memory test (WLMT), the word-list recall test (WLRT), and the word-list recognition test (WLRcT) of the Korean version of the CERAD Neuropsychological Assessment Battery (CERAD-K-N) were administered. Language function was evaluated using the verbal fluency test (VFT). The 15-item modified Boston Naming Test (mBNT) from the CERAD-K-N was also em ployed [22, 24]. Visuospatial function was evaluated using the constructional praxis test (CPT) of the CERAD-K-N. Frontal function was evaluated using trail-making tests A and B of the CERAD-K-N [22, 24].

Genotyping

DNA was extracted from nucleated peripheral blood cells using the salting-out method [25]. The four polymorphisms of the VDR gene were genotyped by PCR sequencing. The primers used for genotyping were designed manually, and their sequences were as follows: *Fok*I forward, 5'-GGTGGGTGGCACCAAGGATG-3' and reverse, 5'-GTGAAAGCCAGTGGCTCGGTC-3'; *Bsm*I forward, 5'-CTGCCCTTAGCTCTGCCTTG-3' and reverse, 5'-CATCACCGACATCATGTCCCC-3'; *Apa*I forward, 5'-GTGTTGCCAGGAATGGCCTT-3' and reverse, 5'-CACAAGGGGCGTTAGCTTCATG-3'; and *Taq*I forward, 5'-GTGTTGCCAGGAATGGCCTT-3' and reverse, 5'-CACAAGGGGCGTTAGCTTCATG-3'. All PCRs were performed on a C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA) in a total volume of 25 μL containing 2.5 mM $MgCl_2$, 2.5 mM each dNTP, 100 μM of each primer, 50 ng template DNA, and 1 U *Taq* DNA polymerase. The PCR protocol was as follows: initial denaturation at 94°C for 5 min; followed by 33 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 45 s; with a final extension at 72°C for 3 min. PCR products were directly sequenced using a BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI 3730XL DNA sequencer (Applied Biosystems, Foster City, CA, USA). Uppercase (F or B or A or T) and lowercase (f or b or a or t) letters indicate the absence and presence of the restriction enzyme recognition site, respectively.

Table 1. The association between four polymorphisms of VDR gene and risk of AD in Korean population

	Locus	Genotype	Case (n=144)	Control (n=329)	Crude OR (95% CI)	*p*-value	HWE in control (p)
Univariate analysis	*Fok*I	FF	43	129	1		
		Ff	77	148	1.561 (1.004-2.427)	0.048	
		ff	24	52	1.385 (0.764-2.508)	0.283	0.382
	*Bsm*I	BB	0	1			
		Bb	19	34	NA		
		bb	125	294			0.987
	*Apa*I	AA	3	12	1		
		Aa	62	129	1.908 (0.519-7.006)	0.330	
		aa	79	188	1.690 (0.464-6.152)	0.426	0.074
	*Taq*I	TT	125	296	1		
		Tt	19	32	1.359 (0.744-2.481)	1.000	
		tt	0	1			0.891
	Locus	Genotype	Case (n=144)	Control (n=329)	Adjusted OR (95% CI)[a]	*p*-value	
Multivariate analysis	*Fok*I	FF	43	129	1		
		Ff	77	148	1.794 (0.967-3.328)	0.064	
		ff	24	52	1.621 (0.721-3.644)	0.242	
	*Bsm*I	BB	0	1			
		Bb	19	34	NA		
		bb	125	294			
	*Apa*I	AA	3	12	1		
		Aa	62	129	1.847 (0.343-9.941)	0.475	
		aa	79	188	1.217 (0.229-6.477)	0.818	
	*Taq*I	TT	125	296	1		
		Tt	19	32	2.830 (1.184-6.766)*	0.019	
		tt	0	1	0	0.000	

[a]Logistic regression adjusted for age, gender and levels of education. *Statistically significant ($P < 0.05$).

Identification and eligibility of relevant studies

Two clinical researchers independently searched and reviewed the literature. A meta-analysis of the published literature was conducted to analyze the associations between VDR gene polymorphisms and the risk of AD. Search sources included the PubMed, Science Direct, Scopus, and Google Scholar databases. The search was conducted up to December 2015, and the following search terms were used: "vitamin D receptor or VDR" and "variant or polymorphism or SNP" in combination with "Alzheimer's disease". The reference lists in the published articles were also reviewed to identify any studies missed by the database search. The workflow of the literature search is shown in **Figure 2**.

Inclusion criteria

All articles reporting the genotype frequencies of the following VDR single nucleotide polymorphisms (SNPs) were included: *Fok*I (rs2228570), *Bsm*I (rs1544410), *Apa*I (rs7975232), and *Taq*I (rs731236). As the studies were heterogeneous in terms of the number of cases and controls, racial composition, and the polymorphisms analyzed, the following inclusion criteria were applied: hospital-based or population-based case-control studies of the associations of VDR gene polymorphisms with AD, genotype frequencies of each polymorphism for cases and controls, genotype distribution in the control group confirmed by Hardy-Weinberg equilibrium (HWE), and English-language articles only. If overlapping cases and controls between studies were

Table 2. Previous studies of the association between VDR gene polymorphisms and risk of AD

Locus	First author	Study region	Ethnicity	No. of case/control	Diagnostic criteria	Genoytping methods	HWE in control (p)
*Fok*I	Gezen-Ak (2012)	Turkey	Caucasian	108/112	DSM-IV	PCR-RFLP	0.585
	Luedecking-Zimmer (2003)	US	Caucasian	536/492	NINCDS-ADRDA	PCR-RFLP	0.924
	Łaczmański (2015)	Poland	Caucasian	108/77	/	PCR-sequencing	0.742
	Our study (2016)	Korea	Asian	144/329	NINCDS-ADRDA/CERAD/ DSM-IV	PCR-sequencing	0.382
*Bsm*I	Gezen-Ak (2012)	Turkey	Caucasian	107/114	DSM-IV	PCR-RFLP	< 0.001*
	Łaczmański (2015)	Poland	Caucasian	108/77	/	PCR-sequencing	0.122
	Our study (2016)	Korea	Asian	144/329	NINCDS-ADRDA/DSM-IV	PCR-sequencing	0.987
*Apa*I	Gezen-Ak (2007)	Turkey	Caucasian	104/109	DSM-IV	PCR-RFLP	0.549
	Khorshid (2013)	Iran	Caucasian	145/162	DSM-IV	PCR-RFLP	0.925
	Lehmann (2011)	UK	Caucasian	255/260	NINCDS-ADRDA	Amplifluor SNP Genotyping System	0.209
	Łaczmański (2015)	Poland	Caucasian	108/70	/	PCR-sequencing	0.138
	Esfehani (2011)	Iran	Caucasian	101/109	DSM-IV	PCR-RFLP	0.707
	Our study (2016)	Korea	Asian	144/329	NINCDS-ADRDA/CERAD/DSM-IV	PCR-sequencing	0.074
*Taq*I	Gezen-Ak (2007)	Turkey	Caucasian	104/109	DSM-IV	PCR-RFLP	0.040*
	Khorshid (2013)	Iran	Caucasian	145/162	DSM-IV	PCR-RFLP	0.237
	Lehmann (2011)	UK	Caucasian	255/260	NINCDS-ADRDA	Amplifluor SNP Genotyping System	0.410
	Łaczmański (2015)	Poland	Caucasian	108/77	/	PCR-sequencing	0.463
	Esfehani (2011)	Iran	Caucasian	101/109	DSM-IV	PCR-RFLP	0.630
	Our study (2016)	Korea	Asian	144/329	NINCDS-ADRDA/CERAD/DSM-IV	PCR-sequencing	0.891

DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; NINCDS-ADRDA: National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism; NA: not applicable. *Deviated from HWE.

Table 3. The associations between VDR gene polymorphisms and the AD risk by meta-analysis

SNP	Genetic models	Pooled OR (95% CI)		Heterogeneity		Publication bias	Deviated from HWE
		Fixed effect model	Random effect model	I^2 value	*P*-value	*P*-value	
FokI	Homozygote model (ff vs. FF)	1.09 (0.82-1.45)	1.09 (0.82-1.45)	0%	0.850	0.899	
	Heterozygote model (Ff vs. FF)	0.98 (0.81-1.20)	1.04 (0.76-1.43)	49%	0.110	0.502	
	Dominant model (ff/Ff vs. FF)	0.91 (0.75-1.10)	0.91 (0.75-1.10)	0%	0.940	0.198	
	Recessive model (ff vs. Ff/FF)	1.08 (0.83-1.40)	1.08 (0.83-1.40)	0%	0.990	0.149	
ApaI	Homozygote model (aa vs. AA)	0.81 (0.60-1.10)	0.85 (0.58-1.23)	24%	0.250	0.122	
	Heterozygote model (Aa vs. AA)	0.99 (0.79-1.25)	1.06 (0.71-1.58)	60%	0.030	0.336	
	Dominant model (aa/Aa vs. AA)	0.95 (0.76-1.19)	1.02 (0.71-1.49)	59%	0.030	0.268	
	Recessive model (aa vs. Aa/AA)	0.86 (0.69-1.08)	0.86 (0.68-1.10)	4%	0.390	0.881	
TaqI	Homozygote model (tt vs. TT)	1.37 (0.96-1.95)	1.37 (0.96-1.95)	0%	0.690	0.225	Gezen-AK 2007
	Heterozygote model (Tt vs. TT)	1.43 (1.10-1.85)*	1.43 (1.10-1.85)	0%	0.740	0.138	
	Dominant model (tt/Tt vs. TT)	1.41 (1.10-1.80)*	1.41 (1.10-1.81)	0%	0.610	0.089	
	Recessive model (tt vs. Tt/TT)	1.04 (0.80-1.35)	1.04 (0.80-1.35)	0%	0.820	0.709	

*Statistically significant ($p < 0.05$).

identified, only the most complete study was included in the meta-analysis.

Data extraction

Data were extracted by two reviewers. The following data were extracted from each study: last name of the first author, publication year, study region, participants' ethnicity, sample size, genotype distribution of the four polymorphisms of the VDR gene in cases and controls, genotyping methods, and *p*-values for the HWE of genotype distribution of controls (*p*-value < 0.05 for HWE was considered to indicate significance).

Statistical analysis

Case-control study: All statistical analyses were performed using the SPSS version 23.0 software (SPSS Inc., Chicago, IL, USA). The chi-squared test was used to determine whether genotype distributions in the controls were in Hardy-Weinberg Equilibrium (HWE). Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to estimate the associations between different genotypes in AD patients and healthy controls. Univariate analyses were conducted to investigate the effect of each polymorphism separately. Multivariate analyses were conducted using genetic polymorphisms as independent variables and all the significant variables including age, gender and education levels. A *p*-value < 0.05 was considered to indicate statistical significance. A *p*-value < 0.05 was considered to indicate statistical significance.

Meta-analysis: The chi-squared test was used to determine whether the distribution of genotypes in the control group was in HWE. Pooled ORs and 95% CIs were calculated to assess the associations between the four VDR gene polymorphisms and the risk of AD under heterozygous, homozygous, dominant, and recessive models using fixed-effects (Mantel-Haenszel method) and random-effects (Mantel-Haenszel method) models. Statistical heterogeneity between studies was evaluated using the I^2 statistic. A random-effects model was used to calculate the pooled ORs and 95% CIs. I^2 values of > 50% were considered indicative of significant heterogeneity among studies, and I^2 values of < 50% low heterogeneity among studies. The risk of bias due to a small sample size, such as that of publication bias, was assessed using funnel plots and further evaluated by Egger's linear regression test. It was assumed that large-sample studies would plot close to the mean in the absence of publication bias, whereas small-sample studies would be spread evenly on both sides of the mean. All meta-statistical analyses were performed using the RevMan ver. 5.1 software (Cochrane Collaboration, Copenhagen, Denmark) and confirmed using the trial version of the Comprehensive Meta-Analysis software. A *p*-values < 0.05 were considered to indicate statistical significance.

Results

Association between VDR polymorphisms and the risk of AD in a Korean population

Sequencing results were showed in **Figure 1**. The genotype distributions in AD patients and controls did not deviate from HWE ($P > 0.05$). The genotyping failure rate was 1.25%. The results of univariate analysis showed four poly-

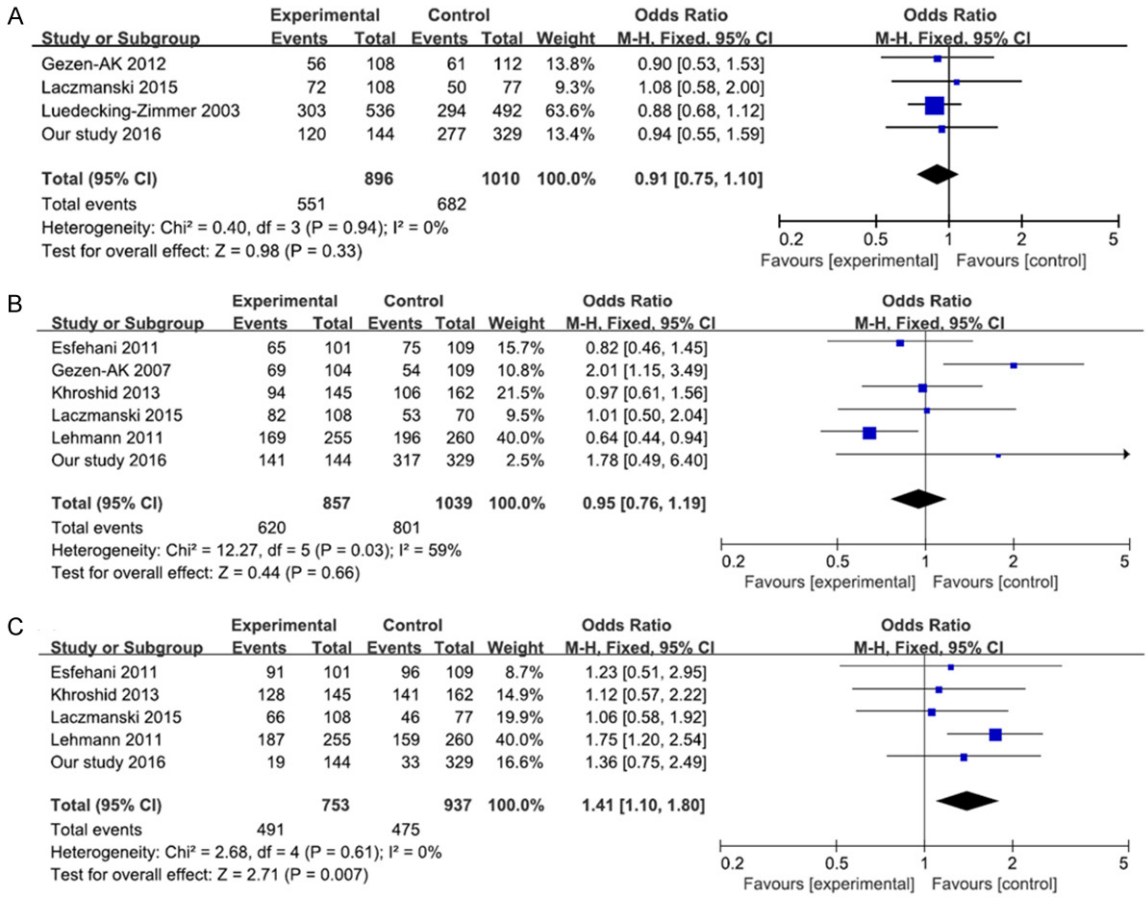

Figure 3. Forest plot for the association between the dominant models of *VDR* gene polymorphisms and risk of AD (A) *Fok*I, (B) *Apa*I and (C) *Taq*I.

morphisms of VDR gene were not related with risk of AD. The multivariate analysis indicate that the *Fok*I polymorphism is not associated with an increased risk of AD (Ff allele: adjusted OR=1.794, 95% CI=0.967-3.328, *p*-value=0.064; and ff allele: adjusted OR=1.621, 95% CI=0.721-3.644, *p*-value=0.242). Similarly, the *Apa*I polymorphism was not associated with an increased risk of AD (Aa allele: adjusted OR=1.863, 95% CI=0.346-10.030, *p*-value=0.064; and aa allele: adjusted OR=1.212, 95% CI=0.228-6.448, *p*-value=0.821). In addition, no BB alleles of the *Bsm*I polymorphism were detected in the AD patients. However, the *Taq*I polymorphism was significantly associated with an increased risk of AD (Tt allele: adjusted OR=2.839, 95% CI=1.187-6.791, *p*-value=0.019) (**Table 1**).

Characteristics of the included studies

A total of 78 papers published before December 2015 were identified in the four databases. After reviewing the full texts, seven publications were found to satisfy the inclusion criteria. A total of 1,385 cases and 1,355 controls from seven studies that reported associations, together with the subjects of the present study (144 cases and 335 controls), were included in the meta-analysis. The majority of the studies involved Caucasian populations (Turkey, Iran, UK, US, and Poland). Only this study was performed in an Asian population (**Table 2**).

Meta-analysis of the association between VDR polymorphisms and risk of AD

Three individual studies of the *Fok*I polymorphism (788 cases and 933 controls), two individual studies on the *Bsm*I polymorphism (251 cases and 443 controls), four individual studies on the *Apa*I polymorphism (749 cases and 969 controls), and four individual studies on the *Taq*I polymorphism (754 cases and 964 controls) were included in the meta-analysis. There were insufficient data for meta-analysis of the

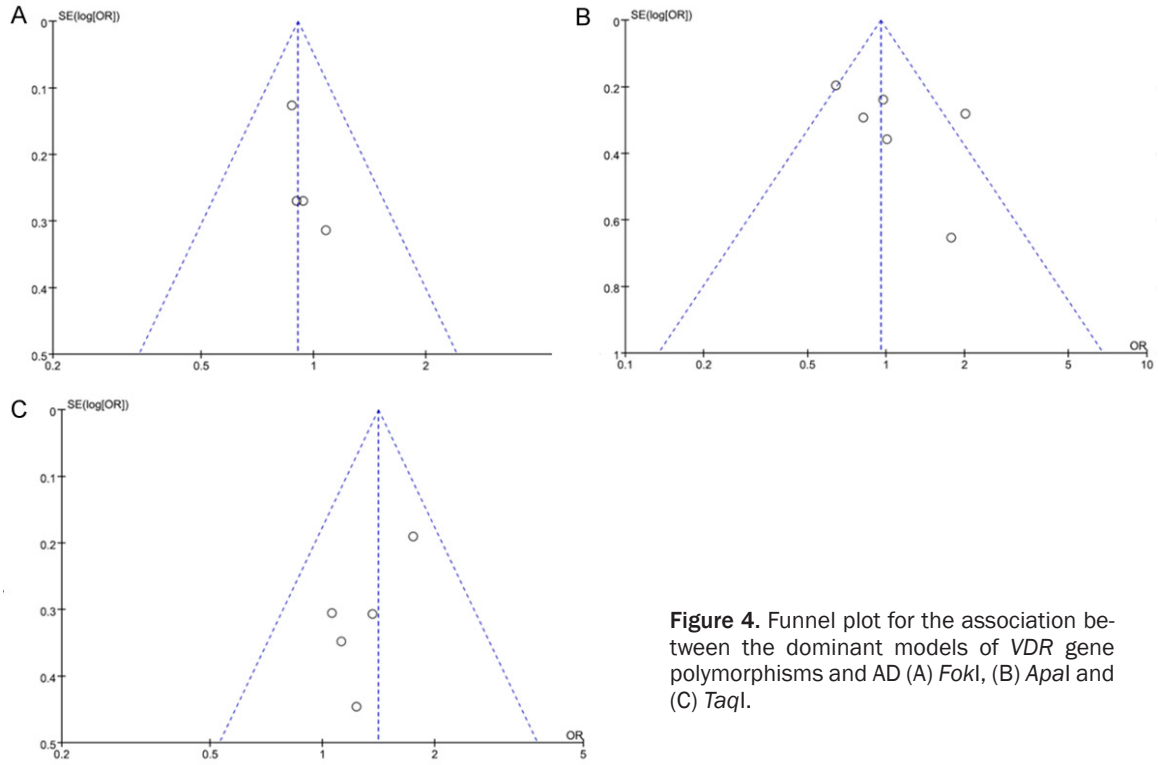

Figure 4. Funnel plot for the association between the dominant models of *VDR* gene polymorphisms and AD (A) *Fok*I, (B) *Apa*I and (C) *Taq*I.

association between the *Bsm*I polymorphism and risk of AD (one study deviated from HWE). One study on the *Taq*I polymorphism also deviated from HWE. The meta-analysis indicated that all genetic models of *Fok*I and *Apa*I were not associated with the risk of AD. In contrast, the heterozygote and dominant models were significantly associated with an increased risk of AD (Tt vs. TT: OR=1.43, 95% CI=1.10-1.85; and tt/Tt vs. TT: OR=1.41, 95% CI=1.10-1.80) (**Table 3**) (**Figure 3**).

Publication bias, heterogeneity and sensitivity

Publication bias is shown graphically as a funnel plot (**Figure 4**). Publication bias was confirmed using Egger's linear regression test, as the funnel plot shapes did not exhibit a distinct symmetry in all genetic models. No evidence of publication bias was found in the majority of the genetic models. Significant heterogeneity was found in the heterozygote and dominant models for the *Apa*I polymorphism. Therefore, fixed-effect and random-effect models were applied in the meta-analysis (**Table 3**). Moreover, a sensitivity test was performed to assess the stability and reliability of the results by sequentially deleting each subgroup study from the meta-analysis. None of the subgroup studies influenced the statistical significance.

Discussion

Genetic variants are common throughout the genome and often indicate connections between certain genes and disease. Of the several types of genetic variants, polymorphisms are apparent in at least 1% of the population. Polymorphisms in the regulatory regions of genes may affect the level of gene expression and protein function [26, 27]. The vitamin D receptor (VDR) gene is located on chromosome 12 (12q14), and contains two promoter regions, six untranslated exons (exon 1a-1f), and eight coding exons (exons 2-9) [28, 29]. Molecular-based epidemiological studies have identified several VDR gene polymorphisms, including Cdx-2 between exon 1f and 1e, *Fok*I in exon 2, Tru9I, *Bsm*I and *Apa*I in intron 8, and *Taq*I in exon 9 [30].

Numerous epidemiological studies have reported associations between VDR gene polymorphisms and an increased risk of several diseases, including cancer, osteoarthritis, diabetes

mellitus type 2, and multiple sclerosis [31-36]. Furthermore, several studies have reported associations between VDR gene polymorphisms and an increased risk of neurodegenerative diseases (AD, amyotrophic lateral sclerosis, and Parkinson's disease) [14-20, 37-46]. Of them, seven studies examined the relationship between VDR polymorphisms and the risk of AD. The first study of the association between VDR polymorphisms and AD risk showed that the *Fok*I polymorphism was not associated with the AD risk in a US population [17]. Similarly, studies in Turkey and Poland reported that *Fok*I polymorphisms were not associated with AD risk [19, 20]. In addition, two studies of *Bsm*I polymorphisms reported no association with the risk of AD [16, 19]. However, a Turkish study deviated from HWE (p-value < 0.001). Based on these results, *Fok*I and *Bsm*I polymorphisms are not related with AD risk. In contrast, the Turkish study suggested that the *Apa*I polymorphism was significantly associated with the risk of AD. However, the frequency of the *Taq*I genotype deviated from HWE (p-value 0.040) [14]. In addition, a UK study reported that *Apa*I and *Taq*I polymorphisms were associated with a highly increased risk of AD [15]. However, studies of *Apa*I and *Taq*I polymorphisms performed in Iran and Poland indicated that *Apa*I polymorphisms were not related to AD risk [16, 18, 20]. Thus, the association between *Apa*I and *Taq*I polymorphisms and the risk of AD is controversial. Therefore, in this work the association between VDR polymorphisms and AD risk by means of a case-control study and meta-analysis was investigated. The results showed that the *Taq*I polymorphism was associated with an increased risk of AD, but the other three polymorphisms were not. In addition, the meta-analysis suggested that the dominant model of *Taq*I polymorphism was significantly associated with the risk of AD.

This case-control and meta-analysis study had several limitations. First, the majority of the studies included had small sample sizes; this led to a low statistical power. Second, AD is a multifactorial disease; gene-gene or gene-environmental interactions such as smoking, alcohol status, and disease progression were not considered. Third, the studies included in the meta-analysis were limited to published reports. Unpublished reports or those published in non-international journals were not included. This limitation may have affected the stability of the meta-analysis data. Nevertheless, this case-control study and meta-analysis improves our understanding of the associations between the four VDR gene polymorphisms and the risk of AD.

In summary, our results suggest that the four polymorphisms were not associated with the risk of AD in Caucasians. However, the *Taq*I polymorphism of the VDR may be closely associated with the risk of AD, and so may facilitate monitoring of AD in the Korean population. Large-scale studies are needed to confirm the associations between VDR gene polymorphisms and AD, and further investigations should consider other factors that contribute to the disease.

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Disclosure of conflict of interest

None.

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