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Vitamin D Receptor Gene Haplotype Is Associated with Late-Onset Alzheimer's Disease

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Vitamin D_3 is a neurosteroid that mediates its effects via the vitamin D receptor (VDR). The VDR gene is located on chromosome 12q13 and consists of 9 exons. VDR contains the DNA-binding site encoded by exons 2 and 3 and the ligand-binding site encoded by exons 4 - 9. Our earlier study showed that the ApaI polymorphic site of the VDR gene is associated with late-onset Alzheimer's disease (AD). Here, we investigated the association between additional polymorphisms of the VDR gene and AD using the same samples. Two single nucleotide polymorphisms (SNPs) in intron 8 (BsmI and Tru9I polymorphisms) and one in exon 2 (FokI polymorphism) of the VDR gene were examined in up to 108 AD patients and 115 age-matched controls. Genotypes were determined with polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. Haplotype analysis also included the previously studied polymorphic sites that were recognized by TaqI (in exon 9) and ApaI (in intron 8) restriction enzymes. There was no significant difference between AD patients and controls when their genotypes for BsmI, Tru9I and FokI polymorphic sites were compared. However, the frequency of "TaubF" haplotype (alleles of TaqI, ApaI, Tru9I, BsmI and FokI, respectively), which was determined by analyzing 5 polymorphisms together, was significantly higher in the AD patient group, suggesting that this haplotype is a risk factor in AD. Our results point out a possible link between AD and certain VDR polymorphisms and indicate that individuals with these polymorphisms might be vulnerable to AD.

Keywords: Alzheimer's disease; haplotype; neurodegeneration; vitamin D; vitamin D receptor
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Alzheimer's disease (AD) is the most common cause of cognitive decline in elderly individuals, constituting approximately 60-70% of dementia cases (Emilien et al. 2004). It is a chronic, degenerative illness with a typically insidious onset (Rocca et al. 1991). Extracellular amyloid plaques and intracellular neurofibrillary tangles are two major pathological hallmarks of AD (Hardy 1997). Extracellular and intracellular fibrillar aggregations cause disruption of axonal transport, interneuronal signal transduction and neurotrophic factor synthesis, alteration of neuronal calcium homeostasis, and induction of oxidative stress, each of which may eventually cause neurons to die (Hardy 1997). Thus, the key aim in therapeutic strategies of AD is to decrease neuronal loss (Tuszynski et al. 2002). We hypothesized that a candidate molecule capable of treating AD should have control over the mechanisms of survival and detoxification mechanisms of neurons and therefore we focused on vitamin D, a neurosteroid hormone and its intracellular pathway (Dursun et al. 2011, Gezen-Ak et al. 2011).

An active form of vitamin D (1,25-dihydroxyvitamin D_3) consists of a broken cholesterol backbone and has steroid-like effects, such as regulating the expression of over 1000 genes. Vitamin D exerts its effects via its nuclear hormone receptor, the vitamin D receptor (VDR). Vitamin D receptor binds to DNA as VDR/VDR homodimers (Freedman et al. 1994) or VDR/RXR (retinoid × receptor) heterodimers in order to regulate gene expression (Issa et al. 1997). Recent insights suggest less known roles for the active form of vitamin D, which include modulation of the immune system, the renin-angiotensin system and cell cycle control, protection against cardiovascular disease and can-

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cer, and regulation of neuromuscular function and cognitive functions (Holick 1995; Garcion et al. 2002; Cekic et al. 2009).

Poduslo et al. (2001) suggested a susceptibility locus for developing late-onset AD on chromosome 12q where vitamin D receptor gene was also located. Our earlier study has shown that a polymorphism of the VDR gene might increase the risk of AD 2.3 times (Gezen-Ak et al. 2007). Beecham et al. (2009) showed that in addition to Apolipoprotein E (APOE), there is a risk locus on 12q13 and that among the number of nearby candidate genes in this region, VDR is the most probable risk gene for developing AD according to their genome wide association (GWA) study that was conducted on 518 late-onset AD cases analyzing 555.000 SNPs. Furthermore, recent studies indicated an association between VDR polymorphisms and cognitive decline (Kuningas et al. 2009; Beydoun et al. 2012), AD (Lehmann et al. 2011), and Parkinson's disease (PD) (Butler et al. 2011).

Our recent in vitro experiments also provided some explanation for the biological function of the vitamin D-VDR pathway in AD and neurodegeneration (Dursun et al. 2011; Gezen-Ak et al. 2011). We showed that amyloid beta suppressed the expression of VDR in cortical neurons and suggested that vitamin D supplementation may prevent amyloid induced cytotoxicity (Dursun et al. 2011). On the other hand, we also found that disruption of the vitamin D-VDR pathway and beta amyloid induced toxicity have very similar effects in cortical neurons regarding L-type voltage sensitive calcium channels and nerve growth factor (NGF) synthesis (Gezen-Ak et al. 2011). Thus any alteration in the vitamin D-VDR pathway (for example: decreases in vitamin D levels, decreases in VDR expression, affinity changes of VDR to vitamin D, to DNA or to other closely related molecules like RXR), might result in "inefficient utilization of vitamin D" and may influence the vulnerability of neurons to aging and neurodegeneration.

Single nucleotide polymorphisms (SNPs) in the VDR gene might also be a cause for some of the alterations in the vitamin D-VDR pathway. FokI, ApaI, TaqI, Tru9I and BsmI are restriction enzymes that have been used to determine the most common polymorphisms in the VDR gene and these polymorphisms have been studied for a long time in a vast variety of diseases (Uitterlinden et al. 2004). The only polymorphism that changes the protein structure of VDR is known to produce an elongated form of VDR (by three additional amino acids) and is determined by the FokI restriction enzyme in exon 2 of the VDR gene (Uitterlinden et al. 2004). There are three polymorphisms in intron 8 recognized by BsmI, Tru9I, and ApaI restriction enzymes and one polymorphism in exon 9 recognized by TaqI. The intronic polymorphisms have strong linkage disequilibrium with the polymorphisms in the 3'-untranslated region that is known to be involved in the regulation of expression of the VDR gene (Uitterlinden et al. 2004).

Based on these biological and genetic backgrounds, in this study we have focused on the other polymorphisms which we have not previously studied (Gezen-Ak et al. 2007) in the ligand and DNA binding sites of the VDR gene. We aimed to expand the VDR polymorphisms data in AD and to determine whether there is an association between the VDR gene BsmI, Tru9I and FokI SNPs and late onset AD in the slightly increased samples which were used for the ApaI and TaqI SNPs in our earlier study (Gezen-Ak et al. 2007).

Materials and Methods

One hundred and eight late-onset AD patients and 115 age-matched controls free from any neurodegenerative disorders (mean ages 74 ± 4.2, with age ranging from 65 to 94 and 75.2 ± 6.8 years, with age ranging from 65 to 90, respectively) were included in this study. Depending on the amount of DNA sample, each polymorphism analysis had a different sample size. Thus, the BsmI SNP was analyzed in 107 patients and 114 controls; the FokI SNP, 108 patients and 112 controls; and the Tru9I SNP, 106 patients and 104 controls. As a result, our earlier study (Gezen-Ak et al. 2007) has been expanded with 3 other polymorphic sites. Also the size of ApaI and TaqI samples were slightly increased (ApaI included 108 patients and 115 controls; TaqI included 108 patients and 115 controls). Patients were diagnosed at Istanbul University, Cerrahpasa Faculty of Medicine, Department of Geropsychiatry and Istanbul Faculty of Medicine, Department of Neurology, Behavioral and Movement Disorders Unit according to DSM-IV criteria.

The subjects of this study were treated according to the World Medical Association Declaration of Helsinki ethical principles for medical research involving human subjects and the Ethics Committee of Istanbul University approved the study, thus appropriate approval and procedures were used concerning human subjects. Written informed consent was obtained from all human subjects included in this study.

DNA was extracted from peripheral blood samples by the salting-out method. Two SNPs in intron 8 (BsmI and Tru9I) between the exons of the ligand binding site and one SNP in exon 2 (FokI) in the DNA binding site of the VDR gene were examined. Polymerase chain reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) was performed in order to determine the presence of BsmI (Fermentas, ER0961), FokI (Fermentas, FD2144) and Tru9I (Roche, 11464 817 001) restriction sites (Table 1). PCR products are digested into pieces by restriction enzymes and they are separated according to their lengths by gel electrophoresis in RFLP method. The method basically helps to reveal the allelic discrimination of the individuals for each SNP that is studied. The recognition site of each enzymes and the corresponding nucleotide substitution for each polymorphisms were as follows: FokI restriction enzyme recognizes *5′.. GGATG(N)9..3′* sequence and cut if T nucleotide is present, it reveals C/T substitution in exon 2 of the VDR gene. BsmI restriction enzyme recognizes *5′...GAATGCN...3′* sequence and cut it if G nucleotide is present and reveals the A/G substitution in intron 8. Tru9I restriction enzyme recognizes *5′...TTAA...3′* sequence and cut it if A nucleotide is present and reveals the G/A substitution in intron 8. ApaI restriction enzyme recognizes *5′...GGGCCC ...3′* sequence and cut it if C nucleotide is present and reveals the C/A substitution in intron 8. TaqI restriction enzyme recognizes *5′...TCGA ...3′* sequence and cut it if C nucleotide is present and reveals the T/C substitution in exon 9.

Table1. The PCR primers and RFLP conditions of BsmI, FokI and Tru9I polymorphisms.

Polymorphic site	Primers for PCR and product size	Alleles according to the restriction sites		RFLP conditions
BsmI SNP ID number: rs1544410 Substitution: A/G Recogtion site: 5′…GAATGCN…3′	F (5′- CCAAGACTACAAGTACC GCGTCAGTGA -3′) R (5′- AACCAGCGGAAGAGG TCAAGGG -3′) PCR product: 825 bp	650 and 175 bp fragments ***b***	825 bp fragment ***B***	37°C and overnight
FokI SNP ID number: rs2228570 Substitution: C/T Recogtion site: 5′…GGATG(N)$_9$…3′	F (5′- AGCTGGCCCTGGCACTG ACTCTGCTCT -3′) R (5′- ATGGAAACACCTTGCTT CTTCTCCCTC-3′) PCR product: 265 bp	196 and 69 bp fragments ***f***	265 bp fragment ***F***	55°C and overnight
Tru9I SNP ID number: rs757343 Substitution: G/A Recogtion site: 5′…TTAA…3′	F(5′- GCAGGGTACAAAACT TTGGAG -3′) R(5′-CCTCATCACCGACAT CATGTC -3′) PCR product: 177 bp	91 and 86 bp fragments ***u***	177 bp fragment ***U***	65°C and 90 minutes

The substituted nucleotide was underlined in recognition site of restriction enzyme.

Alleles of the each polymorphic site named as follows: for TaqI restriction site "t" represents the presence of C nucleotide, "T" the presence of T nucleotide; for ApaI restriction site "a" represents presence of C nucleotide, "A" the presence of A nucleotide, for Tru9I restriction site "u" represents the presence of A nucleotide, "U" the presence of G nucleotide, for BsmI restriction site "b" represents the presence of G nucleotide, "B" the presence of A nucleotide, and for FokI restriction site "f" represents the presence of T nucleotide, "F" the presence of C nucleotide.

All samples were tested twice by different researchers. DNA fragments were visualized by ultraviolet illumination and fragment size was estimated by comparing with a ladder of 50 bp.

Statistical analyses were performed by UNISTAT 6.0® and SPSS 15® software. The distributions of alleles, genotypes and combined genotypes were statistically analyzed with Chi square test. All data were given as mean ± standard error (S.E.). The data were considered significant at $\alpha = 0.05$. Bonferroni correction, which lowers the significance level (α_c value) in order to avoid a type I error, was performed for the p values of combined genotype assignments and the data were considered significant at $p < \alpha_c$ value. While determining risk (odds ratio estimates) of alleles and genotypes as independent variables and patients/controls as dependent variables, logistic regression was performed as well as Wald's confidence intervals (CI). An exact test for Hardy-Weinberg Equilibrium (HWE) was also performed. Haplotype analysis was performed by "Haploview 4.2" software with chromosome positions of each SNP taken as reference. Chromosome position of VDR TaqI site is 46525024, ApaI polymorphic site is 46525104, Tru9I site is 46525942, BsmI site is 46526102 and FokI site is 46559162. SNP Identification numbers (rs) of VDR TaqI site is rs731236, ApaI polymorphic site is rs7975232, Tru9I site is rs757343, BsmI site is rs1544410, and FokI site is rs2228570.

Results

Genotype data

The alleles of the controls for polymorphisms were in Hardy Weinberg Equilibrium. There was no significant difference in the frequency of the BsmI genotypes, FokI genotypes in patients vs. controls ($p = 0.48$, $\chi^2 = 1.48$ and $p = 0.91$, $\chi^2 = 0.195$, respectively). There was no significant difference in the frequency of the Tru9I genotypes in patients vs. controls ($p = 0.13$, $\chi^2 = 4.101$) (Table 2).

No significant difference was found for the distribution of BsmI (B,b), FokI (F, f), and Tru9I (U,u) alleles ($p = 0.683$, $p = 0.796$, and $p = 0.837$ respectively).

The combined genotypes for three SNPs (BsmI, FokI and Tru9I) did not significantly differ. As ApaI SNP is the only significantly different genotype in our samples published in our previous study (Gezen-Ak et al. 2007), we also compared that data with BsmI, FokI and Tru9I combined genotypes. The AAbb and AAFF combined genotypes were observed with the highest frequency in healthy controls ($p = 0.0048$ and $p = 0.028$, respectively) (Tables 3, 4), in which the AaUu combined genotype was observed with the highest frequency in patients ($p = 0.015$) (Table 5).

The logistic regression of genotypes was not given as no significant difference was observed.

Haplotype data

When patient and control groups were compared for the frequency of VDR gene haplotypes, "**TaubF**" haplotype was found to be significantly higher in the patients ($p = 0.0258$, $\chi^2 = 4.967$) (Table 6) and the LD between the five SNPs was shown in Fig. 1. Removing the furthest located SNP (FokI, rs2228570) from the haplotype analysis revised the "Taub" haplotype data with the $p = 0.0263$, $\chi^2 = 4.938$ in patients and a nearly significant difference for the "TAUB" haplotype was seen in the healthy controls ($p = 0.0753$, $\chi^2 = 3.163$).

Updating the data for the ApaI and TaqI genotypes

The distribution of the ApaI and TaqI genotypes

Table 2. The distribution of VDR gene BsmI, FokI and Tru9I genotypes.

VDR polymorphic sites	Genotypes	Patients	Controls	Significance
BsmI n (%)	BB (AA)*	30 (28.0)	34 (29.8)	$p = 0.48$, $\chi^2 = 1.48$
	Bb (AG)	38 (35.5)	32 (28.1)	
	bb (GG)	39 (36.4)	48 (42.1)	
FokI n (%)	FF (CC)	52 (48.1)	51 (45.5)	$p = 0.91$, $\chi^2 = 0.195$
	Ff (CT)	46 (42.6)	51 (45.5)	
	ff (TT)	10 (9.3)	10 (8.9)	
Tru9I n (%)	UU (GG)	50 (47.2)	56 (53.8)	$p = 0.13$, $\chi^2 = 4.101$
	Uu (GA)	52 (49.1)	39 (37.5)	
	uu (AA)	4 (3.8)	9 (8.7)	

*The corresponding nucleotides of each genotype were given in paranthesis.

Table 3. The distribution of ApaI-Bsm1 combined genotypes.

	Genotype					
Group	AABB (AAAA)** *n* (%)	AABb (AAAG) *n* (%)	AAbb (AAGG) *n* (%)	Aabb (ACGG) *n* (%)	AaBb (ACAG) *n* (%)	aabb (CCGG) *n* (%)
Patients	28 (26.7)	5 (4.8)	2 (1.9)	31 (29.5)	33 (31.4)	6 (5.7)
Controls	34 (30.8)	11 (9.7)	*14 (12.3)	23 (20.2)	21 (18.4)	11 (9.7)

*$p = 0.0048$, $\chi^2 = 16.81$, $\alpha_c = 0.0083$ (Bonferroni adjustment), $p < \alpha_c$ AaBB, aaBB, aaBb combined genotypes were excluded in the table as none observed in the subjects or the size was not enough for statistical comparisions. The difference resulting from AAbb was determined by proceeding Chi-square test. Reanalyzing the data after excluding AAbb genotype values became $p = 0.087$, $\chi^2 = 8.1$. **The corresponding nucleotides of each combined genotype were given in paranthesis.

Table 4. The distribution of ApaI-Fok1 combined genotypes.

	Genotype					
Group	AAFF (AACC)** *n* (%)	AAFf (AACT) *n* (%)	AAff (AATT) *n* (%)	AaFF (ACCC) *n* (%)	AaFf (ACCT) *n* (%)	Aaff (ACTT) *n* (%)
Patients	14 (13.7)	16 (15.8)	5 (5.0)	35 (34.7)	26 (25.8)	5 (5.0)
Controls	*28 (30.8)	23 (9.7)	8 (12.3)	21 (20.2)	19 (18.4)	2 (9.7)

*$p = 0.028$, $\chi^2 = 12.49$, $\alpha_c = 0.0083$ (Bonferroni adjustment), $p > \alpha_c$

aaFF, aaFf, aaff combined genotypes were excluded in the table as none observed in the subjects or the size was not enough for statistical comparisons. The difference resulting from AAFF was determined by proceeding Chi-square test. Reanalyzing the data after excluding AAFF genotype values became $p = 0.015$, $\chi^2 = 6.65$. **The corresponding nucleotides of each combined genotype were given in paranthesis.

slightly differed from our previous study (Gezen-Ak et al. 2007) with the data of additional patient and control samples. The distribution of the ApaI genotypes was 32.4% for AA, 61.1% for Aa, 6.5% for aa in the 108 patients and 52.2% for AA, 38.3% for Aa, 9.6% for aa in the 115 healthy controls. The highly significant difference in the frequency of the Aa genotype in patients vs. controls remains in the revised data set ($p = 0.003$, $\chi^2 = 11.66$, OR = 2.57). The distribution of the Taq I genotypes was 38.0% for TT, 47.2% for Tt, 14.8% for tt in the 108 patients and 47.0% for TT, 36.5% for Tt, 16.5% for tt in the 115 healthy controls. There was no significant difference in the frequency of the TaqI genotypes ($p = 0.26$, $\chi^2 = 2.69$). Allele distribution of ApaI (A, a) and TaqI (T,t) (the nucleotide substitution for ApaI SNP is C/A and for TaqI SNP is T/C) was also revised as ($p = 0.061$, $p = 0.425$, respectively), indicating no significant difference.

Discussion

Wider biological roles for vitamin D secosteroid hor-

Table 5. The distribution of ApaI-Tru91 combined genotypes.

	Genotype						
Group	AAUU (AAGG)** *n* (%)	AAUu (AAGA) *n* (%)	AAuu (AAAA) *n* (%)	AaUU (ACGG) *n* (%)	AaUu (ACGA) *n* (%)	aaUU (CCGG) *n* (%)	aaUu (CCGA) *n* (%)
Patients	16 (15.7)	17 (16.7)	1 (0.9)	31 (30.4)	*30 (29.4)	3 (3.0)	4 (3.9)
Controls	23 (22.3)	23 (22.3)	8 (7.8)	27 (26.2)	13 (12.6)	6 (5.8)	3 (2.9)

* $p = 0.015$, $\chi^2 = 15.736$, $\alpha_c = 0.0071$ (Bonferroni adjustment), $p > \alpha_c$

Aauu, aauu combined genotypes were excluded in the table as none observed in the subjects or the size was not enough for statistical comparisons. The difference resulting from AaUu was determined by proceeding Chi-square test. Reanalyzing the data after excluding AaUu genotype values became $p = 0.213$, $\chi^2 = 7.107$. ** The corresponding nucleotides of each combined genotype were given in paranthesis.

Table 6. The distribution of VDR haplotypes.

VDR haplotypes	Patients Frequency	Controls Frequency	Total Frequency	χ^2	*p* value
tAUBF (CAGAC)	0.191	0.174	0.182	0.202	0.6533
TaUbF (TCGGC)	0.173	0.164	0.168	0.061	0.8048
tAUBf (CAGAT)	0.126	0.100	0.113	0.699	0.4031
TaUbf (TCGGT)	0.103	0.093	0.098	0.108	0.7421
TAUbF (TAGGC)	0.078	0.112	0.095	1.385	0.2393
TAubF (TAAGC)	0.075	0.109	0.092	1.412	0.2348
TaubF (TCAGC)	0.067	0.022	0.045	4.967	***0.0258**
tAuBF (CAAAC)	0.038	0.034	0.036	0.054	0.8162
TAuBF (TAAAC)	0.037	0.030	0.034	0.19	0.663
TAuBf (TAAAT)	0.019	0.033	0.026	0.733	0.3918
TAUBF (TAGAC)	0.014	0.036	0.025	2.063	0.1509
TAubf (TAAGT)	0.019	0.027	0.023	0.288	0.5915
TAUbf (TAGGT)	0.009	0.022	0.015	1.0	0.3173
tAuBf (CAAAT)	0.011	0.011	0.011	0.0010	0.9754

The corresponding nucleotides of VDR haplotypes were given in paranthesis.

mone in tissues, which are not primarily related to mineral metabolism, have been shown within last 30 years. Vitamin D is suggested to act as a neuroactive steroid due to the local bioactivation of the pre-vitamin D hormone in the central nervous system (CNS) and the widely distributed localization of nuclear receptor throughout the brain (Luine et al. 1987; Musiol et al. 1992; Elaroussi et al. 1994; Johnson et al. 1996; Veenstra et al. 1998; Prufer et al. 1999; Miller and Portale 2000; Langub et al. 2001; Garcion et al. 2002; Burkert et al. 2003; Eyles et al. 2005; McCann and Ames. 2008; Cekic et al. 2009; Dickens et al. 2011).

Keisala et al. (2009) suggested that VDR genetic ablation promotes premature aging in mice, and vitamin D homeostasis regulates physiological aging. Moreover, Féron et al (2005) showed animals that were exposed to transient early vitamin D deficiency had larger lateral ventricles, reduced NGF protein content, and reduced expression of a number of genes involved in neuronal structure. They conclude that transient early life hypovitaminosis D_3 not only disrupts brain development but leads to persistent changes in the adult brain. Vitamin D deficiency has also been suggested to elevate brain damage and attenuate the affects of other treatments in traumatic brain injury cases (Cekic et al. 2009). One study showed that vitamin D potentiates axon regeneration by significantly increasing axogenesis and axon diameter in a rat model of peripheral nerve injury (Chabas et al. 2008). Thus vitamin D may have a potential role in the treatment of CNS injuries (Kiraly et al. 2006; Cekic et al. 2009).

We showed that amyloid beta suppresess the expression of VDR in cortical neurons and amyloid induced cytotoxicity can be prevented by vitamin D treatment (Dursun et al. 2011). Furthermore our results also indicated beta amyloid induced alterations and VDR silencing have very similar effects in cortical neurons regarding L type voltage sensitive calcium channels and NGF synthesis (Gezen-Ak et al. 2011). A recent study supporting our results suggested that a vitamin D_3-enriched diet correlates with a decrease in the number of amyloid plaques, a decrease in beta amyloid peptides, a decrease in inflammation, and an

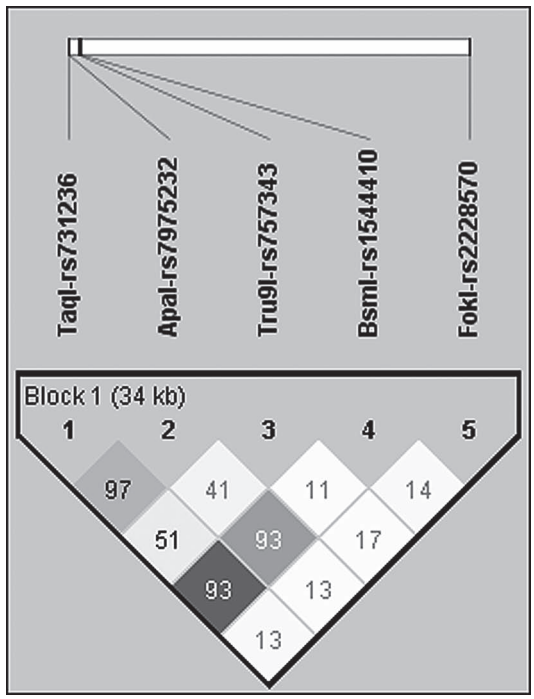

Fig. 1. Pairwise LD plot for examined *VDR* SNPs. The darker color is representing higher *r*2 value. The chromosome positions of each SNP taken as reference. Chromosome position of VDR TaqI site is 46525024, ApaI polymorphic site is 46525104, Tru9I site is 46525942, BsmI site is 46526102 and FokI site is 46559162. SNP Identification numbers (rs) of VDR TaqI site is rs731236, ApaI polymorphic site is rs7975232, Tru9I site is rs757343, BsmI site is rs1544410 and FokI site is rs2228570. The figure was created by Haploview 4.2 software.

increase in NGF in the brains of amyloid precursor protein (APP) transgenic mice (Yu et al. 2011).

In addition to animal findings, recent human studies reported low levels of the plasma 25-dihydroxyvitamin D_3 (25OHD) for individuals suffering mood disorders, PD, AD or cognitive decline (Wilkins et al. 2006; Cherniack et al. 2008; Evatt et al. 2008; McCann and Ames 2008; Llewellyn et al. 2009; Annweiler et al. 2010; Llewellyn et al. 2010). Another study showed significantly higher Mini-Mental State Examination (MMSE) scores for vitamin-D-sufficient patients compared to vitamin-D insufficient ones (Oudshoorn et al. 2008). Valuable, though limited in number, longitudinal studies indicated low levels of vitamin D were associated with substantial cognitive decline in the elderly population (Llewellyn et al. 2010) and baseline vitamin D deficiency predicted the onset of non Alzheimer dementias (Annweiler et al. 2012b). Importantly, Annweiler et al. (2012a) showed the association of elevated serum 25OHD concentration with a lower risk of mild cognitive impairment (MCI) and the association of low 25OHD concentrations with MCI status in older non-demented people with subjective memory complaint, suggesting that hypovitaminosis D may participate in the dementia process from prodromal stages. Recent remarkable studies showed that vitamin D strongly stimulated phagocytosis and clearance of beta amyloid while protecting against apoptosis in AD patients' macrophages (Masoumi et al. 2009; Mizwicki et al. 2012). Fiala and Mizwicki (2011) suggested that increased consumption of vitamin D and fish oil could prevent neurodegeneration in some subjects by maintaining adequate endocrine, paracrine, and/or autocrine production of vitamin D and docosahexaenoic acid (DHA)-derived lipidic modulators. Finally, Annweiler et al (2011) published their novel AD treatment protocol (AD-IDEA) by combining vitamin D and memantine (a commonly used AD drug) assay.

Strong evidence indicated that additional risk genes exist on chromosome 12 for AD (Blacker et al. 1998; Hollenbach et al. 1998; Poduslo and Yin 2001; Luedecking-Zimmer et al. 2003; Beecham et al. 2009). The VDR gene is very close to this region and our earlier study provided the first evidence for a possible genetic association between AD and VDR (Gezen-Ak et al. 2007). Beecham et al. (2009) reported that VDR is the most appealing of the candidate genes for the newly identified risk locus on chromosome 12q13 for AD regarding their genome wide association study results and our previous results. Butler et al. (2011) found the most significant SNP (rs7968585) in their Parkinson's patients data set was in high linkage disequilibrium (LD) with the ApaI polymorphism.

This present study updates the data of our earlier study in 2007 (Gezen-Ak et al. 2007) with the results of three other polymorphisms. The p values and the OR for ApaI genotypes were also updated with the slightly increased number of patients but no association between BsmI, FokI and Tru9I polymorphisms and AD was observed. FokI, which is also known as a start codon polymorphism, is the only known protein polymorphism in VDR where the presence of FokI restriction site results in a 3-amino-acid longer form of the VDR protein. ApaI, TaqI, Tru9I and BsmI have no known functional consequences. However they are located near the 3′-end of the VDR gene and have strong LD with the polymorphisms in the 3′-untranslated region which is known to be involved in the regulation of the expression of the VDR gene (Uitterlinden et al. 2004).

Our results suggest that "A" is a protective allele, as the AAbb and AAFF combined genotypes were observed with the highest frequencies in healthy controls. On the other hand, the "a" allele may be the risk allele because of the AaUu combined genotype, which was seen with the highest frequency in patients. These findings were also supported by the haplotype results. "TaubF" haplotype is the most common haplotype for the patients. Removing the furthest located SNP (FokI) from the haplotype analysis made almost no effect on the p value and "Taub" haplotype remained significantly higher in patients. Additionally a nearly significant difference for the "TAUB" haplotype was seen in the healthy controls. These findings correlate with our previous study (Gezen-Ak et al. 2007). Another conc-

lusion from the results of this study is that TaqI alleles may have no effect in our sample. Thus, we suggest that the polymorphism in the ligand-binding site of the VDR gene increases the risk for AD development. Our findings are in correlation with Beydoun's study, which has the largest sample size, as it observed greater cognitive decline with "Tab" haplotype (Beydoun et al. 2012). On the contrary, Lehmann et al. (2011) suggest VDR SNPs are also associated with AD but this association is due to "tA" haplotype, and Kuningas et al. (2009) suggest "tAB" haplotype of VDR is associated with worse cognitive performance. Although limited numbers of studies have focused on VDR SNPs and AD as well as cognitive performance and PD, with albeit controversial results, these studies are highly remarkable in detecting associations between VDR SNPs and neurodegenerative disorders.

Our previous findings and recent studies suggest that any alteration in the vitamin D-VDR pathway such as vitamin D levels, VDR expression or affinity changes of VDR to vitamin D, DNA or another molecule like RXR may result in inefficient utilization of vitamin D and may make neurons vulnerable to aging and neurodegeneration. While molecular studies are ongoing to explain the implications of vitamin D to AD pathogenesis, additional population studies should be carried out to determine the genetic associations between VDR and AD and other neurodegenerative diseases.

Determining the roles of vitamin D on signaling pathways, calcium metabolism, neurotrophic factor production, synapthogenesis, and inflammation in brain may help to elucidate the neuropathological consequences of aberrant vitamin D regulation or vitamin D deficiency.

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Conflict of Interest

All authors declare no conflict of interest.

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