

# Vitamin D deficiency might pose a greater risk for ApoE $\epsilon$ 4 non-carrier Alzheimer's disease patients

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**Abstract** Vitamin D is a secosteroid hormone that shares a synthetic pathway with cholesterol. ApoE, which is involved in the transport of cholesterol, is the most significant genetic risk factor for sporadic Alzheimer's disease (AD). Surprisingly, recent studies have indicated the presence of an evolutionary juncture between these two molecules. To demonstrate this possible relationship, we investigated serum levels of 25-hydroxyvitamin-D<sub>3</sub> (25OHD) in patients with early onset-AD (EOAD; *n*:22), late onset-AD (LOAD; *n*:72), mild cognitive impairment (MCI; *n*:32) and in healthy subjects (*n*:70). We then analyzed the correlation between 25OHD and cytokines, BDNF and Hsp90 with respect to ApoE alleles, as these molecules were investigated in our previous studies. The LOAD patients had low levels of 25OHD, but these low levels originated only from ApoE $\epsilon$ 4 non-carrier patients. Negative correlations were observed between serum 25OHD and TNF $\alpha$ , IL-1 $\beta$  or IL-6 levels in healthy subjects or MCI patients, but these same correlations were positive in LOAD patients. ApoE alleles indicated that these positive correlations exist only in  $\epsilon$ 4 carrier LOAD patients. Consequently, our results indicate that vitamin D deficiency presents a greater risk for ApoE $\epsilon$ 4 non-carrier AD patients than for  $\epsilon$ 4 carriers. Therefore, it might be

beneficial to monitor the vitamin D status of ApoE $\epsilon$ 4 allele non-carrier AD patients.

**Keywords** Alzheimer's disease · 25OHD · Vitamin D · ApoE · Cytokine · Biomarker

## Introduction

ApoE functions as a component of plasma lipoproteins in the transport of lipids (e.g., cholesterol) [1, 2]. Recent studies suggested a relationship between ApoE and vitamin D by indirect synthesis or reabsorption mechanisms. These studies indicated a protective effect of ApoE $\epsilon$ 4 against vitamin D deficiency [3], higher levels of vitamin D in ApoE $\epsilon$ 4 carriers [4], and an association of vitamin D with chylomicrons or lipoproteins [5]. In addition, vitamin D is a secosteroid hormone that consists of a broken cholesterol backbone. The synthetic pathway of the precursor of vitamin D is highly conserved and is the same as that of cholesterol [6]. These data might indicate the transport of vitamin D via ApoE chylomicrons or lipoprotein particles in brain or circulating blood. If such suggestions are supported, we should be able to discern a relationship between ApoE alleles and vitamin D levels in AD just as we observe such a relationship between ApoE and cholesterol in cardiovascular disease. Thus, our aim in this study was (1) to investigate the serum levels of 25OHD in patients with AD, MCI, and in age-matched healthy controls; (2) to compare the 25OHD levels in these patients according to ApoE $\epsilon$ 4 allele status to investigate any correlation of ApoE-dependent genetic vulnerability to AD and circulating vitamin D levels; (3) to investigate the correlation of serum cytokine and serum 25OHD levels in regard to the ApoE $\epsilon$ 4

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allele status to determine any potential consequences of a vitamin D-ApoE relationship on immune response.

## Methods

### The patients and control groups

The patients, including AD or MCI, recruited to study at the Behavioral and Movement Disorders Unit of the Istanbul Faculty of Medicine at Istanbul University. AD patients were clinically diagnosed according to DSM-IV criteria. No patient with advanced disease stage was included. Clinical Dementia Ratings (CDR) Scale scores of AD patients were 1 and 2. Thus, none of the patients were bedridden or had any significant disability. All MCI patients had mild cognitive symptoms and had a global score of 0.5 in the CDR scale [7]. Three patient groups were established according to the age and disease status after the exclusion of patients with inflammatory diseases, autoimmune disease, infectious or psychiatric diseases, non-Alzheimer's dementia (other than MCI), chronic heart disease, patients taking antibiotics or non-steroidal anti-inflammatory drugs, and those with significant laboratory abnormalities. The patients included in the study had erythrocyte sedimentation rates within the reference values. The healthy controls were free from any neurodegenerative disorders. A neuropsychological assessment using the mini mental status examination (MMSE) was performed for the patients with AD and MCI and for all controls. The demographics of each group are given in Table 1.

### DNA isolation and ApoE genotyping

DNA was extracted from peripheral blood samples using a QIAamp DNA Mini Kit (Cat.No.51304, QIAGEN, Germany). Genotypes of *ApoE* were determined by real-time polymerase chain reaction (RT-PCR) using a LightMix<sup>®</sup> Kit *ApoE-C112R-R158C* (Cat.No.40-0445-16, TibMolBiol, Germany) in a LightCycler<sup>®</sup> 480 InstrumentII (Roche Diagnostics, Germany) as previously described [8]. Melting curve analyses were performed for genotyping.

### CLIA and ELISA assays

The quantitative determination of 25OHD in serum samples was performed by the chemiluminescent immunoassay (CLIA) technology using the DiaSorin Liaison 25(OH)D TOTAL assay (DiaSorin, USA) in a Liaison analyzer. ELISA was used to measure the levels of BDNF, Hsp90, CFH, TNF $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, and  $\alpha_2$ M in the serum samples, as described in our previous studies [9, 10].

### Statistical analysis

The distribution of *ApoE* alleles in regard to the presence of the  $\epsilon 4$  allele in the patient groups was analyzed by Chi-square ( $\chi^2$ ) test using SPSS 21.0 software. All data are expressed as the mean  $\pm$  the standard deviation (SD). *p* values less than 0.05 were considered statistically significant.

Each 25OHD test run included case-control sets, two blinded quality control (QC) specimens from our study as

**Table 1** The demographics of the patient and control groups

Groups	<i>n</i> (number of individuals); Apo $\epsilon 4$ + ( <i>n</i> of Apo $\epsilon 4$ carriers)	Age (mean $\pm$ SD)	Age onset	MMSE score (mean $\pm$ SD)
EOAD	<i>n</i> = 22 Apo $\epsilon 4$ + = 14	61 $\pm$ 4 (aged between 52 and 70)	<65 (age at AD onset 50–63)	17 $\pm$ 5
LOAD	<i>n</i> = 72 Apo $\epsilon 4$ + = 24	76 $\pm$ 7 (aged between 67 and 83)	>65 (age at AD onset 65–82)	17 $\pm$ 6
MCI	<i>n</i> = 32 Apo $\epsilon 4$ + = 10	74 $\pm$ 8 (aged between 66 and 80)	age at MCI onset 60–78	28 $\pm$ 2
Control for EOAD	<i>n</i> = 40 Apo $\epsilon 4$ + = 5	57 $\pm$ 9 (aged between 50 and 73)	–	29 $\pm$ 2
Control for LOAD and MCI	<i>n</i> = 30 Apo $\epsilon 4$ + = 7	73 $\pm$ 3 (aged between 68 and 78)	–	29 $\pm$ 2

The groups were as follows: early onset Alzheimer's disease (EOAD), late onset Alzheimer's disease (LOAD), mild cognitive impairment (MCI), healthy control individuals who were age-matched to the patients in the EOAD group, and healthy control individuals who were age-matched to patients in the LOAD and MCI groups. No significant differences were noted among the ages of LOAD and MCI patients and age-matched controls ( $p > 0.05$ ) or between the ages of EOAD patients and age-matched controls ( $p > 0.05$ ). The MMSE scores of the EOAD and LOAD groups were significantly lower than those of the MCI patients and those of the corresponding age-matched controls ( $p < 0.001$  Dunn's multiple comparisons test)

well as two inter assay controls and six calibrators. The test detection range was 4–150 ng/ml. The intra-assay coefficient variation was 8.51 % for low controls and 9.93 % for high controls. Both the low and high controls were in the range of the calibrators in each run. 25OHD values were season-adjusted according to the cosinor model of Sachs et al. [11]. No significant difference was observed between the adjusted and unadjusted 25OHD values; thus, all comparisons were made with the adjusted values.

The raw data for each group were analyzed using GraphPad InStat DTCC 3.06. Data are presented as the mean  $\pm$  SD in the text and figure legends. Group comparisons of patients with LOAD and MCI with the age-matched controls were performed by one-way ANOVA (more than 2 groups) when appropriate and by the Kruskal–Wallis test (non-parametric ANOVA) when the data were not normally distributed. Group comparisons between patients with EOAD and age-matched controls were generated by unpaired *t* test (with two groups), with a Welch correction (when required) or by Mann–Whitney *U* test when the data were not normally distributed. The comparisons were also made with respect to the presence of the ApoE $\epsilon$ 4 allele. Any significant result for multiple parameters within group comparisons was analyzed again with Dunn's multiple comparisons test or with the Tukey–Kramer multiple comparisons test to avoid type I errors; in addition, *p* values are given under the graphs. Multiple regression analysis was used to compare the significant contribution of the serum 25OHD levels to the constant parameters (serum BDNF, Hsp90, CFH, TNF $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, and  $\alpha_2$ M levels [9, 10]). If any positive data were obtained from the multiple regression analysis, the analysis was repeated with Pearson's correlation coefficient to keep most of the data available, given that multiple regression analysis excludes any patients with missing data. The correlations of 25OHD with serum BDNF, Hsp90, CFH, TNF $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, and  $\alpha_2$ M levels were also assessed with respect to the presence of the ApoE $\epsilon$ 4 allele. The Bonferroni correction, which lowers the significance level ( $\alpha_c$  value) to avoid a type I error, was performed for the *p* values of Table 3; the data were considered significant at  $p < \alpha_c$ . Retrospective power analysis was also performed.

## Results

### ApoE allele frequencies

The frequency of the ApoE $\epsilon$ 4 allele in groups was as follows: 89 % for  $\epsilon$ 4 non-carriers, 11 % for  $\epsilon$ 4 carriers out of all of the healthy controls; 79 % for  $\epsilon$ 4 non-carriers, 21 % for  $\epsilon$ 4 carriers in all patients with AD. The frequency of

ApoE $\epsilon$ 4 allele carriers out of all AD patients was significantly higher than that of all healthy controls ( $p = 0.01$ ; 95 % CI: 1.2–3.7; OR 2.1). The numbers of ApoE  $\epsilon$ 2 allele carriers were not sufficient for statistical comparison, given that these individuals were evaluated in the  $\epsilon$ 4 non-carrier group.

### Serum 25OHD levels

No significant difference was observed between the unadjusted and the season-adjusted 25OHD levels in each group ( $p > 0.05$ ). Given that all of the comparisons were generated with season-adjusted 25OHD levels, henceforth, all '25OHD levels' reflect the adjusted values.

Comparison of the unadjusted and season-adjusted levels of serum 25OHD between the age-matched control groups and the disease groups was given in Table 2. Multiple comparisons of the season-adjusted levels of serum 25OHD between groups and the same analysis depending on the ApoE $\epsilon$ 4 allele presence were given in Fig. 1a, b. Retrospective power analysis of adjusted 25OHD levels of AllAD vs all control indicated the power of the study to be 83.1 %.

### Correlation analysis of 25OHD with age, age of onset and MMSE with respect to ApoE $\epsilon$ 4 allele status

No correlation was found between the serum 25OHD levels and age or age of onset in any of the healthy controls or in patients with MCI or LOAD ( $p > 0.05$ ). On the contrary, positive correlations were found between the serum 25OHD levels and age ( $r = 0.48$ , 95 % CI 0.06–0.75,  $p = 0.03$ ) as well as age of onset ( $r = 0.67$ , 95 % CI 0.33–0.85,  $p = 0.0009$ ) in the EOAD group. No significant difference was observed between the serum 25OHD levels and MMSE scores in LOAD patients or in healthy subjects, whereas positive correlations were observed between the serum 25OHD levels and MMSE scores in the EOAD ( $r = 0.51$ , 95 % CI 0.1–0.77,  $p = 0.01$ ) and MCI groups ( $r = 0.38$ , 95 % CI 0.01–0.66,  $p = 0.04$ ).

No correlation was found between the serum 25OHD levels and age, age of onset or MMSE scores of the  $\epsilon$ 4 non-carriers, the  $\epsilon$ 4 carrier healthy subjects, the MCI patients or the LOAD patients ( $p > 0.05$ ). No correlation was observed between the serum 25OHD levels and age or MMSE scores of the  $\epsilon$ 4 non-carriers or the  $\epsilon$ 4 carrier EOAD patients ( $p > 0.05$ ), but positive correlations were observed between the serum 25OHD levels and age of onset in both the  $\epsilon$ 4 non-carriers and the  $\epsilon$ 4 carrier EOAD patients ( $r = 0.57$ , 95 % CI 0.07–0.85,  $p = 0.03$ ;  $r = 0.83$ , 95 % CI 0.20–0.97,  $p = 0.02$ , respectively).

**Table 2** Comparison of the unadjusted and season-adjusted levels of serum 25OHD between the age-matched control groups and the disease groups

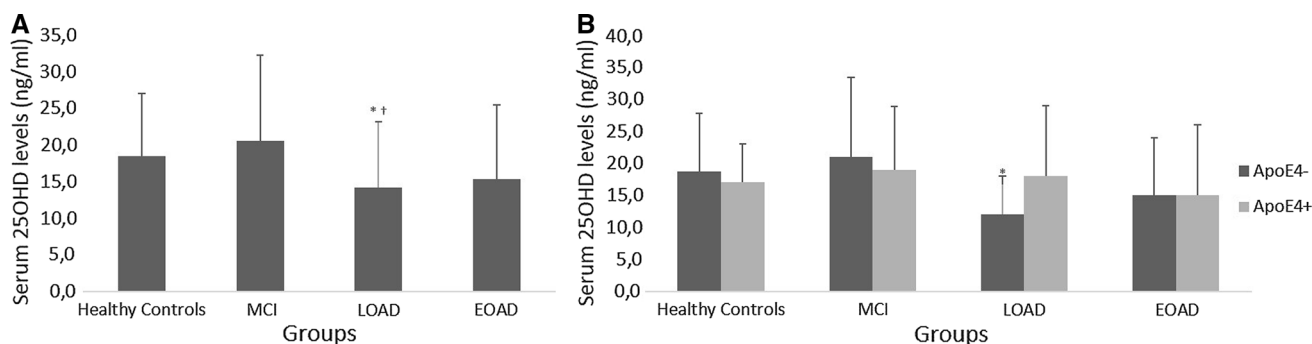
Groups	n	Unadjusted 25OHD levels			Season-adjusted 25OHD levels		
		Mean ± SD	95 % CI	p	Mean ± SD	95 % CI	p
All AD patients	94	13.8 ± 9.5	11.9–15.8	<0.0001**	14.4 ± 9.3	12.5–16.3	0.0001**
All controls	70	19.9 ± 8.2	17.9–21.8		18.5 ± 8.6	16.5–20.6	
EOAD patients	22	15.3 ± 9.9	10.9–19.8	0.01*	15.1 ± 10.2	10.6–19.6	0.05*
Control for EOAD	40	19.5 ± 8.5	16.8–22.2		18.3 ± 8.9	15.5–21.2	
LOAD patients	72	13.4 ± 9.4	11.2–15.6	<0.0001**	14.2 ± 9.1	12.0–16.3	0.001**
Control for LOAD or MCI	30	20.3 ± 8.1	17.3–23.3		18.8 ± 8.4	15.6–21.9	
MCI patients	32	20.9 ± 12.0	16.6–25.2	>0.05	20.5 ± 11.7	16.3–24.8	>0.05
Control for LOAD or MCI	30	20.3 ± 8.1	17.3–23.3		18.8 ± 8.4	15.6–21.9	

The Mann–Whitney test was used for paired comparisons of age-matched groups

Multiple comparison results for season-adjusted 25OHD levels are given in Fig. 1a

\* Significant difference

\*\* Extremely significant difference



**Fig. 1 a** Multiple comparisons of the season-adjusted levels of serum 25OHD between groups. Serum 25OHD levels of LOAD patients were significantly lower than that of all healthy controls (\* $p < 0.01$ ) and MCI patients ( $\dagger p < 0.05$ ). No difference was observed between EOAD patients and other groups in terms of 25OHD levels ( $p > 0.05$ ) (Kruskal–Wallis with Dunn’s multiple comparisons). If these groups were compared again with the appropriate age-matched controls, the serum 25OHD levels were nearly significantly decreased in the EOAD group compared with the age-matched controls ( $p = 0.05$ ). The serum 25OHD levels of the LOAD group were significantly lower than the levels in the corresponding age-matched controls ( $p < 0.001$ ). No significant difference was observed in the 25OHD levels between patients with MCI and the age-matched control group ( $p > 0.05$ ). The serum 25OHD levels were significantly decreased in all AD patients (LOAD and EOAD) compared with all healthy

subjects ( $p < 0.0001$ ) (Table 2). All unadjusted and season-adjusted serum 25OHD comparisons are given in greater detail in Table 2. **b** Multiple comparisons of the season-adjusted levels of serum 25OHD between groups depending on ApoE4 allele status. The serum 25OHD levels of  $\epsilon 4$  non-carrier LOAD patients were significantly lower than those of  $\epsilon 4$  non-carrier healthy subjects and MCI patients (\* $p < 0.001$ ,  $p < 0.01$ , respectively, Kruskal–Wallis with Dunn’s multiple comparisons). No significant difference was observed in the serum 25OHD levels between  $\epsilon 4$  carrier healthy subjects and any of the patient groups ( $p = 0.68$ ). The serum 25OHD levels of  $\epsilon 4$  non-carrier LOAD patients were significantly lower than those of  $\epsilon 4$  carrier LOAD patients (\* $p = 0.03$ ). A similar difference was not observed within any other group. The numbers of ApoE4 carriers were given in Table 1

### Correlation analysis of 25OHD with certain serum parameters with respect to ApoE4 allele status

We investigated the correlation of serum 25OHD levels and certain serum parameters (BDNF, HSP90, CFH, TNF $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-10,  $\alpha_2$ M), which were investigated in our previous studies [9, 10], with respect to

the presence of the ApoE4 allele. The correlation analysis of these parameters was performed only in individuals who were included in our previous studies. The numbers of individuals who were included in the correlation analysis were as follows: all healthy subjects, nmax:47; MCI patients, nmax:29; LOAD patients, nmax:52; EOAD patients, nmax:21.

### Correlation analysis of 25OHD with BDNF or Hsp90 or immune response components (CFH, TNF $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, and $\alpha_2$ M) with respect to *ApoE $\epsilon$ 4* allele status

Correlation analysis of 25OHD with specified parameters with respect to *ApoE $\epsilon$ 4* allele status was given in Table 3. The levels of serum parameters with respect to the presence of the *ApoE $\epsilon$ 4* allele were also assessed for each group (Fig. 2).

### Discussion

The mechanisms of ApoE in AD are not fully understood. Likewise, whether a deficiency in vitamin D is a casual factor or is the result of the disease is a matter of debate. Surprisingly, recent studies have indicated the presence of an evolutionary juncture of these two factors, which encompasses latitude-UVB radiation, the immune system, and dietary habits [3, 4].

Data from our cohort of Turkish EOAD and LOAD patients supported the suggestion that 25OHD deficiency is frequent among patients with cognitive disorders [12–15]. In regard to the healthy subjects, we should note that several Turkish studies presented mean 25OHD values [16–18] that were similar to those in our current study, but the 25OHD levels in the Turkish population seem to be lower than the mean levels in other populations [13, 19]. Our study indicated that although EOAD and LOAD patients had lower 25OHD levels than the corresponding age-matched healthy controls, the MCI patients had similar values of circulating 25OHD as the healthy subjects and both have significantly higher MMSE scores than EOAD or LOAD patients. Furthermore, increased serum 25OHD has the potential to positively influence MMSE scores given that they are correlated in MCI and EOAD patients, but not in LOAD patients.

It has been hypothesized that the *ApoE $\epsilon$ 4* allele protects against a deficiency in vitamin D in circumstances of high geographical latitude, dark skin or insufficient dietary supplementation where vitamin D synthesis is compromised [3, 4]. Higher levels of vitamin D were indicated in *ApoE $\epsilon$ 4* carrier healthy subjects compared with non-carrier ones [4], and the higher 25OHD level in  $\epsilon$ 4 allele carriers was associated with higher memory functions [20]. This may be interpreted as the capability of these individuals to better endure low UV concentrations, which may be an advantage of the ancestral *ApoE $\epsilon$ 4* allele compared with the evolutionally young  $\epsilon$ 3 allele [21]. This concept is known as the UVB radiation hypothesis of ApoE [3, 21].

The vitamin D status was also assessed in our AD patients with respect to the presence of the *ApoE $\epsilon$ 4* allele.

First, if the patient groups were compared with all healthy subjects independent of age discrimination, only LOAD patients had significantly low levels of 25OHD. No significant difference was observed between the serum 25OHD levels in any of the patient groups and healthy subjects who carried at least one *ApoE $\epsilon$ 4* allele. Surprisingly, the 25OHD status of LOAD patients indicated that the significantly low levels seen in LOAD originated from  $\epsilon$ 4 non-carrier patients and not from  $\epsilon$ 4 carriers. At first glance, our study supports the suggestions by Huebbe [4] and Maddock [20] studies, which showed high levels of 25OHD in  $\epsilon$ 4 allele carriers. However, they demonstrated this alteration in healthy subjects, whereas we demonstrated it in LOAD patients. The differences between the 25OHD levels in the *ApoE $\epsilon$ 4* allele carriers and non-carrier healthy subjects of the Maddock and Huebbe studies and those in our study might be explained by the differences in sample number or the geographical locations of the populations.

From a different point of view, our findings might also be interpreted as low levels of 25OHD in *ApoE $\epsilon$ 4* allele non-carriers. This finding presents an opportunity to claim vitamin D deficiency to be a casual factor for individuals with the sporadic AD phenotype who have no  $\epsilon$ 4 allele. The biological relevance of this relationship is unclear. Yet, there are a limited number of studies indicating a possible mechanistic link between vitamin D and ApoE: ApoE-deficient mice had lower 1,25(OH) $_2$ D $_3$  levels and VDR silencing in endothelial cells resulted in decreased ApoE expression [22]. ApoE suggested to be an upstream regulator of vitamin D metabolism via Lrp2 (megalin) which is a known vitamin D transporter and a receptor for ApoE-lipoproteins [23], or via VDBP [4]. ApoE functions in the transport of cholesterol [1, 2]. Basically, vitamin D and cholesterol originate from the same molecule (7-dehydrocholesterol), and they differ from each other only by the presence of two hydrogens [24, 25]. In addition, serum 25OHD levels are positively correlated both with ApoA-I and LpA-I [26] and the total cholesterol level after UVB radiation [25] and negatively correlated with the ratio of LDL to HDL [26]. In addition to this possible relation, one can speculate that the levels of a certain molecule may also depend on transport mechanisms: a system may exist for the simultaneous transport of vitamin D and cholesterol via ApoE-bearing lipoproteins in the CNS or plasma. This suggestion is relevant considering the limited (5 % or less) ability of 25OHD [27, 28] and cholesterol [29] to cross the blood brain barrier. If we assume that vitamin D and ApoE coexist in the same cellular mechanisms or pathways, then we have to consider that either the effect of vitamin D or the effect of ApoE, for example, on the immune response might require each other. *ApoE $\epsilon$ 4* has been reported to be associated with an inflammatory response [30], and vitamin

**Table 3** Correlation analysis of 25OHD with serum parameters with respect to ApoEε4 allele status

	25OHD/BDNF	25OHD/Hsp90	25OHD/CFH	25OHD/TNFα	25OHD/IL-1α	25OHD/IL-1β	25OHD/IL-6	25OHD/IL-10	25OHD/α <sub>2</sub> M
<b>All healthy subjects</b>									
ε4 non-carrier	$r = -0.29$	$r = -0.04$	$r = -0.17$	$r = -0.02$	$r = -0.22$	$r = -0.41$	$r = -0.14$	$r = -0.04$	$r = -0.13$
	95 % CI = -0.57 to 0.04 $p = 0.08$ ( $n = 35$ )	95 % CI = -0.36 to 0.29 $p = 0.84$ ( $n = 36$ )	95 % CI = -0.48 to 0.17 $p = 0.32$ ( $n = 34$ )	95 % CI = -0.35 to 0.32 $p = 0.92$ ( $n = 34$ )	95 % CI = -0.51 to 0.11 $p = 0.19$ ( $n = 36$ )	95 % CI = -0.66 to -0.06 $*p = 0.02$ ( $n = 33$ )	95 % CI = -0.45 to 0.21 $p = 0.44$ ( $n = 35$ )	95 % CI = -0.38 to 0.29 $p = 0.79$ ( $n = 34$ )	95 % CI = -0.44 to 0.20 $p = 0.43$ ( $n = 36$ )
ε4 carrier	$r = -0.32$	$r = 0.37$	$r = -0.03$	$r = 0.15$	$r = -0.34$	$r = -0.23$	$r = -0.59$	$r = 0.06$	$r = 0.14$
	95 % CI = -0.77 to 0.34 $p = 0.33$ ( $n = 11$ )	95 % CI = -0.29 to 0.79 $p = 0.25$ ( $n = 11$ )	95 % CI = -0.65 to 0.61 $p = 0.93$ ( $n = 10$ )	95 % CI = -0.49 to 0.68 $p = 0.67$ ( $n = 11$ )	95 % CI = -0.78 to 0.32 $p = 0.30$ ( $n = 11$ )	95 % CI = -0.75 to 0.47 $p = 0.52$ ( $n = 11$ )	95 % CI = -0.89 to 0.05 $p = 0.06$ ( $n = 11$ )	95 % CI = -0.55 to 0.64 $p = 0.84$ ( $n = 11$ )	95 % CI = -0.50 to 0.68 $p = 0.68$ ( $n = 11$ )
Total sample	$r = -0.31$	$r = 0.04$	$r = -0.17$	$r = -0.18$	$r = -0.02$	$r = -0.34$	$r = -0.30$	$r = -0.02$	$r = -0.15$
	95 % CI = -0.55 to -0.01 $*p = 0.04$ ( $n = 46$ )	95 % CI = -0.25 to 0.32 $p = 0.80$ ( $n = 47$ )	95 % CI = -0.45 to 0.14 $p = 0.27$ ( $n = 44$ )	95 % CI = -0.46 to 0.12 $p = 0.23$ ( $n = 44$ )	95 % CI = -0.46 to 0.09 $p = 0.18$ ( $n = 47$ )	95 % CI = -0.58 to -0.43 $*p = 0.03$ ( $n = 44$ )	95 % CI = -0.55 to -0.002 $*p = 0.04$ ( $n = 46$ )	95 % CI = -0.31 to 0.28 $p = 0.90$ ( $n = 45$ )	95 % CI = -0.42 to 0.14 $p = 0.32$ ( $n = 47$ )
<b>MCI patients</b>									
ε4 non-carrier	$r = -0.30$	$r = 0.29$	$r = 0.23$	$r = -0.44$	$r = 0.42$	$r = -0.44$	$r = -0.02$	$r = 0.11$	$r = 0.09$
	95 % CI = -0.66 to 0.16 $p = 0.19$ ( $n = 20$ )	95 % CI = -0.16 to 0.65 $p = 0.20$ ( $n = 20$ )	95 % CI = -0.25 to 0.62 $p = 0.34$ ( $n = 19$ )	95 % CI = -0.73 to 0.003 $p = 0.05$ ( $n = 20$ )	95 % CI = -0.04 to 0.73 $p = 0.07$ ( $n = 20$ )	95 % CI = -0.76 to 0.05 $p = 0.06$ ( $n = 18$ )	95 % CI = -0.43 to 0.43 $p = 0.91$ ( $n = 19$ )	95 % CI = -0.34 to 0.53 $p = 0.63$ ( $n = 20$ )	95 % CI = -0.36 to 0.52 $p = 0.68$ ( $n = 20$ )
ε4 carrier	$r = -0.11$	$r = 0.12$	$r = 0.16$	$r = -0.63$	$r = 0.27$	$r = -0.41$	$r = -0.54$	$r = -0.52$	$r = 0.27$
	95 % CI = -0.72 to 0.59 $p = 0.76$ ( $n = 9$ )	95 % CI = -0.59 to 0.72 $p = 0.76$ ( $n = 9$ )	95 % CI = -0.56 to 0.75 $p = 0.67$ ( $n = 9$ )	95 % CI = -0.91 to 0.06 $p = 0.07$ ( $n = 9$ )	95 % CI = -0.54 to 0.82 $p = 0.52$ ( $n = 9$ )	95 % CI = -0.86 to 0.42 $p = 0.32$ ( $n = 8$ )	95 % CI = -0.19 to 0.13 $p = 0.13$ ( $n = 9$ )	95 % CI = -0.89 to 0.28 $p = 0.17$ ( $n = 8$ )	95 % CI = -0.48 to 0.79 $p = 0.47$ ( $n = 9$ )
Total sample	$r = -0.27$	$r = 0.21$	$r = 0.23$	$r = -0.46$	$r = -0.29$	$r = -0.39$	$r = -0.21$	$r = -0.09$	$r = 0.17$
	95 % CI = -0.58 to 0.11 $p = 0.15$ ( $n = 29$ )	95 % CI = -0.17 to 0.54 $p = 0.26$ ( $n = 29$ )	95 % CI = -0.16 to 0.56 $p = 0.24$ ( $n = 28$ )	95 % CI = -0.71 to -0.11 $*p = 0.01$ ( $n = 29$ )	95 % CI = -0.08 to 0.59 $p = 0.12$ ( $n = 29$ )	95 % CI = -0.67 to 0.0003 $*p = 0.05$ ( $n = 26$ )	95 % CI = -0.54 to 0.18 $p = 0.29$ ( $n = 28$ )	95 % CI = -0.45 to 0.28 $p = 0.63$ ( $n = 28$ )	95 % CI = -0.21 to 0.50 $p = 0.39$ ( $n = 29$ )



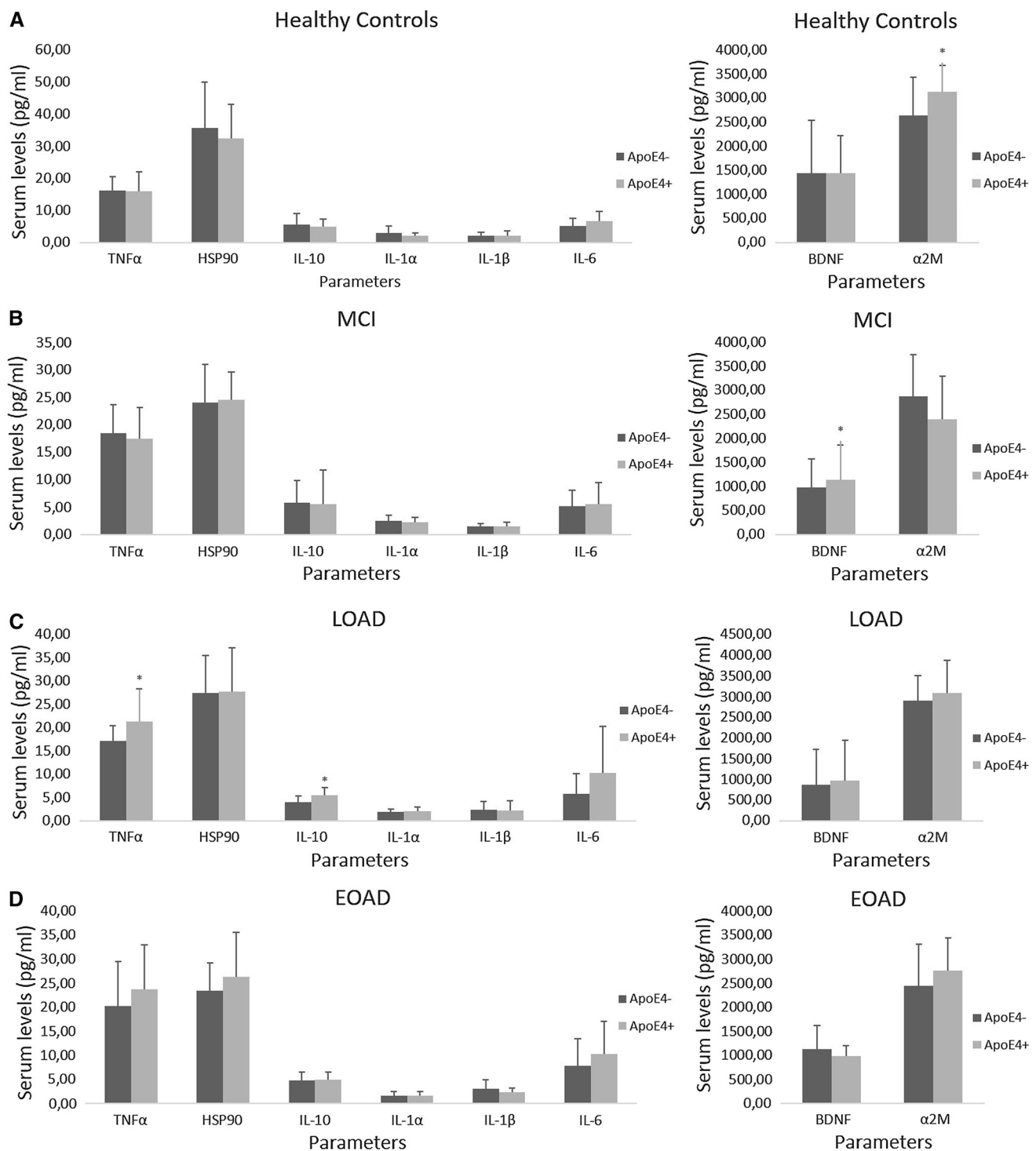
**Table 3** continued

	25OHD/BDNF	25OHD/Hsp90	25OHD/CFH	25OHD/TNF $\alpha$	25OHD/IL-1 $\alpha$	25OHD/IL-1 $\beta$	25OHD/IL-6	25OHD/IL-10	25OHD/ $\alpha_2$ M
<b>LOAD patients</b>									
$\epsilon$ 4 non-carrier <i>n</i> = 35	<i>r</i> = -0.03	<i>r</i> = 0.11	<i>r</i> = -0.19	<i>r</i> = 0.02	<i>r</i> = 0.04	<i>r</i> = 0.09	<i>r</i> = 0.09	<i>r</i> = 0.03	<i>r</i> = -0.02
	95 % CI = -0.37 to 0.31 <i>p</i> = 0.85 ( <i>n</i> = 33)	95 % CI = -0.24 to 0.43 <i>p</i> = 0.54 ( <i>n</i> = 34)	95 % CI = -0.49 to 0.16 <i>p</i> = 0.28 ( <i>n</i> = 35)	95 % CI = -0.32 to 0.34 <i>p</i> = 0.93 ( <i>n</i> = 35)	95 % CI = -0.34 to 0.41 <i>p</i> = 0.83 ( <i>n</i> = 27)	95 % CI = -0.24 to 0.42 <i>p</i> = 0.57 ( <i>n</i> = 35)	95 % CI = -0.24 to 0.41 <i>p</i> = 0.58 ( <i>n</i> = 35)	95 % CI = -0.24 to 0.36 <i>p</i> = 0.86 ( <i>n</i> = 35)	95 % CI = -0.31 to 0.31 <i>p</i> = 0.91 ( <i>n</i> = 35)
$\epsilon$ 4 carrier <i>n</i> = 17	<i>r</i> = 0.15	<i>r</i> = -0.01	<i>r</i> = 0.001	<i>r</i> = 0.55	<i>r</i> = -0.05	<b><i>r</i> = 0.64</b>	<i>r</i> = 0.63	<i>r</i> = 0.51	<i>r</i> = -0.25
	95 % CI = -0.36 to 0.59 <i>p</i> = 0.57 ( <i>n</i> = 17)	95 % CI = -0.50 to 0.49 <i>p</i> = 0.96 ( <i>n</i> = 16)	95 % CI = -0.48 to 0.48 <i>p</i> = 0.99 ( <i>n</i> = 17)	95 % CI = 0.06 to 0.82 <i>p</i> = 0.02 ( <i>n</i> = 17)	95 % CI = -0.52 to 0.44 <i>p</i> = 0.83 ( <i>n</i> = 17)	<b>95 % CI = 0.22 to 0.86</b> <b>**<i>p</i> = 0.006</b> ( <i>n</i> = 16)	95 % CI = 0.19 to 0.85 <i>p</i> = 0.008 ( <i>n</i> = 17)	95 % CI = 0.04 to 0.79 <i>p</i> = 0.03 ( <i>n</i> = 17)	95 % CI = -0.66 to 0.26 <i>p</i> = 0.32 ( <i>n</i> = 17)
Total sample	<i>r</i> = 0.17	<i>r</i> = 0.05	<i>r</i> = -0.11	<b><i>r</i> = 0.39</b>	<i>r</i> = 0.33	<i>r</i> = 0.33	<b><i>r</i> = 0.52</b>	<i>r</i> = 0.28	<i>r</i> = -0.08
	95 % CI = -0.11 to 0.43 <i>p</i> = 0.24 ( <i>n</i> = 50)	95 % CI = -0.23 to 0.32 <i>p</i> = 0.73 ( <i>n</i> = 50)	95 % CI = -0.37 to 0.17 <i>p</i> = 0.45 ( <i>n</i> = 52)	95 % CI = 0.13 to 0.60 <b>**<i>p</i> = 0.004</b> ( <i>n</i> = 52)	95 % CI = -0.26 to 0.33 <i>p</i> = 0.79 ( <i>n</i> = 46)	95 % CI = 0.05 to 0.55 <i>p</i> = 0.02 ( <i>n</i> = 51)	<b>95 % CI = 0.29 to 0.69</b> <b>**<i>p</i> &lt; 0.0001</b> ( <i>n</i> = 52)	95 % CI = 0.02 to 0.52 <i>p</i> = 0.04 ( <i>n</i> = 52)	95 % CI = -0.34 to 0.19 <i>p</i> = 0.57 ( <i>n</i> = 52)
<b>EOAD patients</b>									
$\epsilon$ 4 non-carrier	<i>r</i> = -0.16	<i>r</i> = 0.53	<i>r</i> = 0.27	<i>r</i> = -0.15	<i>r</i> = 0.18	<i>r</i> = 0.19	<i>r</i> = 0.33	<i>r</i> = -0.05	<i>r</i> = 0.56
	95 % CI = -0.65 to 0.42 <i>p</i> = 0.59 ( <i>n</i> = 13)	95 % CI = -0.03 to 0.83 <i>p</i> = 0.06 ( <i>n</i> = 13)	95 % CI = -0.30 to 0.70 <i>p</i> = 0.35 ( <i>n</i> = 14)	95 % CI = -0.64 to 0.44 <i>p</i> = 0.62 ( <i>n</i> = 13)	95 % CI = -0.38 to 0.65 <i>p</i> = 0.52 ( <i>n</i> = 14)	95 % CI = -0.43 to 0.68 <i>p</i> = 0.55 ( <i>n</i> = 12)	95 % CI = -0.30 to 0.76 <i>p</i> = 0.29 ( <i>n</i> = 12)	95 % CI = -0.57 to 0.48 <i>p</i> = 0.83 ( <i>n</i> = 14)	95 % CI = 0.85 to 0.85 <i>p</i> = 0.04 ( <i>n</i> = 14)
$\epsilon$ 4 carrier	<i>r</i> = -0.34	<i>r</i> = 0.04	<i>r</i> = -0.22	<i>r</i> = -0.07	<i>r</i> = -0.14	<i>r</i> = -0.20	<i>r</i> = 0.54	<i>r</i> = -0.33	<i>r</i> = -0.19
	95 % CI = -0.90 to 0.65 <i>p</i> = 0.51 ( <i>n</i> = 6)	95 % CI = -0.79 to 0.82 <i>p</i> = 0.95 ( <i>n</i> = 6)	95 % CI = -0.83 to 0.64 <i>p</i> = 0.64 ( <i>n</i> = 7)	95 % CI = -0.84 to 0.78 <i>p</i> = 0.88 ( <i>n</i> = 6)	95 % CI = -0.97 to 0.95 <i>p</i> = 0.85 ( <i>n</i> = 7)	95 % CI = -0.87 to 0.73 <i>p</i> = 0.70 ( <i>n</i> = 6)	95 % CI = -0.48 to 0.94 <i>p</i> = 0.27 ( <i>n</i> = 6)	95 % CI = -0.65 to 0.90 <i>p</i> = 0.52 ( <i>n</i> = 6)	95 % CI = -0.87 to 0.73 <i>p</i> = 0.70 ( <i>n</i> = 7)
Total sample	<i>r</i> = -0.20	<i>r</i> = 0.25	<i>r</i> = 0.12	<i>r</i> = -0.09	<i>r</i> = 0.33	<i>r</i> = 0.07	<i>r</i> = -0.39	<i>r</i> = 0.09	<i>r</i> = -0.27
	95 % CI = -0.60 to 0.27 <i>p</i> = 0.40 ( <i>n</i> = 19)	95 % CI = -0.23 to 0.63 <i>p</i> = 0.31 ( <i>n</i> = 19)	95 % CI = -0.33 to 0.53 <i>p</i> = 0.59 ( <i>n</i> = 21)	95 % CI = -0.53 to 0.38 <i>p</i> = 0.70 ( <i>n</i> = 19)	95 % CI = -0.11 to 0.67 <i>p</i> = 0.14 ( <i>n</i> = 21)	95 % CI = -0.41 to 0.52 <i>p</i> = 0.77 ( <i>n</i> = 18)	95 % CI = -0.09 to 0.72 <i>p</i> = 0.11 ( <i>n</i> = 18)	95 % CI = -0.37 to 0.51 <i>p</i> = 0.71 ( <i>n</i> = 20)	95 % CI = -0.21 to 0.64 <i>p</i> = 0.27 ( <i>n</i> = 21)

$\alpha_c$  = 0.006 (Bonferroni correction)

\* Nearly significant 0.05 > *p* >  $\alpha_c$  (italics)

\*\* Significant *p* <  $\alpha_c$  (dark gray)



**Fig. 2** Serum levels of immune response components, BDNF and HSP90 with respect to ApoE $\epsilon$ 4 allele status. **a** All healthy controls: serum  $\alpha$ 2M levels of ApoE $\epsilon$ 4 carrier healthy controls ( $n$ :11, 95 % CI 2804.3–3458.3) were significantly higher than those of ApoE $\epsilon$ 4 non-carrier healthy controls ( $n$ :36, 95 % CI 2804.3–3458.3) ( $*p = 0.04$ , Mann–Whitney  $U$ ). **b** MCI patients: serum BDNF levels of ApoE $\epsilon$ 4 carrier MCI patients ( $n$ :9, 95 % CI 978.9–2082.2) were significantly higher than those of ApoE $\epsilon$ 4 non-carrier MCI patients ( $n$ :20, 95 % CI 701.8–1249.1) ( $*p = 0.019$ , Mann–Whitney  $U$ ). **c** LOAD patients:

serum TNF $\alpha$  and IL-10 levels of ApoE $\epsilon$ 4 carrier LOAD patients ( $n$ :17, 95 % CI 17.6–25.1;  $n$ :17, 95 % CI 4.6–6.3, respectively) were significantly higher than those of ApoE $\epsilon$ 4 non-carrier LOAD patients ( $n$ :35, 95 % CI 16.1–18.2;  $n$ :35, 95 % CI 3.6–4.5, respectively) ( $*p = 0.03$  Unpaired  $T$  test with Welch,  $p = 0.004$ , Mann–Whitney  $U$ , respectively). **d** EOAD patients: no significant difference was seen between the ApoE $\epsilon$ 4 carrier EOAD patients and the non-carrier patients in terms of any of the above-mentioned parameters



D has been consistently known to display anti-inflammatory effects [31]. In PBMCs, ApoE secretion is restricted to CD14<sup>+</sup> monocytes [32] and CD14 was suggested as the most suitable marker for the description of the vitamin D status of blood samples [33]. ApoE promotes the conversion of macrophages from the proinflammatory M1 to the anti-inflammatory M2 phenotype [34]. Vitamin D can stimulate the macrophages in AD patients, so that they function in amyloid beta clearance, and as a result of this response, vitamin D stimulates cytokines, which contrasts with its usual anti-inflammatory role [35]. ApoE affects the levels of cytokines, but TNF- $\alpha$  and IL-1 $\beta$  can suppress ApoE production [36]. Due to these overlapping effects of vitamin D and ApoE on the immune system, we also investigated the correlation of serum 25OHD levels and several serum parameters (BDNF, HSP90, CFH, TNF $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-10,  $\alpha_2$ M), which were investigated in our previous studies [9, 10], with respect to the presence of the *ApoE $\epsilon$ 4* allele. We have not observed any study that has focused on 25OHD and cytokines in AD patients.

In this study, our results revealed a common pattern in groups with relatively higher 25OHD levels (healthy subjects or MCI patients) and supported the negative effect of vitamin D on the expression of TNF $\alpha$ , IL-1 $\beta$  and IL-6; however, the effects were no longer significant after Bonferroni adjustment. The negative correlation of serum 25OHD levels and IL-1 $\beta$  in healthy subjects originated from the *ApoE $\epsilon$ 4* non-carrier subjects. The correlations of serum 25OHD and either TNF $\alpha$  or IL-6 were independent from the *ApoE* allele status in healthy subjects and MCI patients. These negative correlation patterns between the serum levels of 25OHD and the serum levels of TNF $\alpha$ , IL-1 $\beta$  or IL-6 were altered in groups with low 25OHD levels (EOAD and LOAD). Intriguingly, the negative correlations became positive between 25OHD, and all three parameters are in  *$\epsilon$ 4* carrier LOAD patients. Thus, even though this is speculative, higher vitamin D levels in *ApoE $\epsilon$ 4* carriers might be one way to compensate for the lack of an anti-inflammatory response. In addition, vitamin D might induce cytokines to suppress the production of ApoE only in *ApoE $\epsilon$ 4* carriers to prevent the elevation of amyloid beta accumulation in the brain or other parts of the body [37] given the higher affinity of ApoE $\epsilon$ 4 for amyloid plaques. This suggestion may explain why we see a positive correlation of serum 25OHD levels and serum TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-10 levels only in *ApoE $\epsilon$ 4* carrier LOAD patients but not in healthy controls or MCI patients.

The reason that the EOAD group did not present such correlations or any difference between 25OHD levels with respect to the presence of the  *$\epsilon$ 4* allele might be due to the effect of age on the immune system. This may also be explained by different genetic backgrounds, the different roles of vitamin D over different periods of time throughout life

[15] or the low number of EOAD samples. Our results also indicate that the correlations between 25OHD levels and age, age of onset or MMSE scores in EOAD patients, and the correlations between 25OHD levels and MMSE scores in MCI patients were independent of the *ApoE* allele status.

The fundamental finding of this study was that LOAD patients had very low levels of vitamin D, but that these low levels originated from only the *ApoE $\epsilon$ 4* non-carrier patients. This finding was the first observation for ApoE and vitamin D relation in AD. Thus, the vitamin D status should be monitored in AD patients who are non-carriers of the *ApoE $\epsilon$ 4* allele to confirm our data.

Vitamin D has existed on earth for more than 750 billion years and affects many cellular mechanisms [38–40]. Moreover, given its shared synthesis pathway with cholesterol, it would be more logical to reconsider and combine the ApoE perspective with that of vitamin D deficiency in AD.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. In addition, the study was approved by the Ethics Committee of Istanbul University. Signed informed consent was obtained from all study participants.

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