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Association between the Val66Met polymorphism (rs6265/G196A) of the BDNF gene and cognitive performance with SSRI use in Arab Alzheimer's disease patients
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ABSTRACT

Brain derived neutrophic factor (BDNF) is a protein and a member of the neurotrophin family of growth factors, supports the survival of existing neurons and encourages the growth and differentiation of new neurons and synapses. The BDNF gene Val66Met polymorphism (rs6265/G196A) is responsible for BDNF synthesis that impact BDNF function that includes memory and cognition.

This study investigated whether the BDNF gene Val66Met polymorphism (rs6265/G196A) is associated with cognitive function changes in both Alzheimer disease (AD) patients and elderly participants. In addition the impact of SSRI use on cognition improvement will be assessed. Healthy young, middle ages (25-59 years old) and elderly (more than 60 years old) participants (140) as well as 40 AD patients of whom are both of Saudi Arabian origin were recruited. The genotyping for the association study was performed by real-time PCR using Taqman chemistry in the ABI Prism 7900HT Sequence Detection System. Both Mini-Mental Status Examination (MMSE) and Clinical Dementia Rating (CDR) were used to assess cognitive function of healthy and AD participants, respectively.

The findings showed that the BDNF Val66Met genotype distributions and allele frequencies have significant association with cognitive performance in both elderly control group and AD patients. The main findings showed that carriers of GG homozygotes (Val/Val) have superior cognitive performance among AD patients and elderly control subjects. In addition the use of SSRIs in 13 AD patients and 17 elderly participants positively improved cognitive function in elderly (p>0.001) but not in AD patients (p=0.1).

INTRODUCTION

The past three decades of genetic research in Alzheimer's disease (AD) have transformed the understanding of its causes and enhanced the innovation and development of novel therapeutics aimed at the treatment and prevention of this disease. The BDNF gene represents an interesting, potential genetic mechanism for the risk of late-onset AD (Matrisciano *et al.*, 2009).

The most common BDNF polymorphism is Val66Met (rs6265, G196A). The A allele of rs6265 has been correlated with worse episodic memory, abnormal hippocampal activation and reduced hippocampal n-acetyl aspartate (NAA) in human subjects (Egan *et al.*, 2003). There have been studies of many polymorphisms of the BDNF gene, such as rs11030104, rs16917204, rs7103411, rs6265 and rs2030324. A meta-analysis conducted in 2014, including 21 articles on rs6265 and 22 articles on rs2030324, found that there was no evidence for an association between rs2030324 and AD (Lin *et al.*, 2014). However, rs6265 conveys an increased risk for AD in Caucasian females but not in Asians. Moreover, the A allele of rs6265 significantly contributes to the increased risk of AD in female late onset AD patients (Li *et al.*, 2014).

Genotype and allele frequencies for the BDNF Val66Met polymorphism in a sample of 102 Colombian AD patients and 168 healthy volunteer were examined. A significant association between A carrier genotypes and familial AD was observed (Forero et al., 2006). However, in Turkey, Solmaz et al. found that there was no association between AD and the BDNF gene Val66Met polymorphism (Solmaz et al., 2017).

Use of antidepressants, especially selective serotonin reuptake inhibitors (SSRIs), is frequent for treating depression in AD patients. A meta-analysis conducted by Yan et al., to evaluate the efficacy of antidepressants with respect to the BDNF Val66Met polymorphism in Asian populations found that Met carriers exhibited a better response rate than Val/Val carriers. In Asians, Met carriers were positively associated with the response rate in the SSRI group and with treatments ≥6 weeks. Ethnic origin of patients may be a factor that influence the effect of antidepressants since BDNF had a low

influence on the response in mixed-race studies. Moreover, the duration of treatment plays an important role in the response and remission rate of antidepressant efficacy (Yan et al., 2014).

In addition, the BDNF Val66Met polymorphism influences antidepressant response and remission in Caucasian patients. Val/Val patients have a higher probability of a three-month response to SSRIs compared to serotonin and noradrenalin reuptake inhibitor/tricyclic antidepressants (SNRI/TCA). Carriers of the Met allele have a higher probability of a six-month remission rate with SNRI/TCA compared to SSRIs. This effect is unrelated to antidepressant side effects. Thus, the results of the study suggest that SSRIs could be recommended for Val/Val patients and that, conversely, SNRI/TCA could be beneficial in Met patients (Colle et al., 2015).

The potential association between single nucleotide polymorphisms (SNPs) of the BDNF gene (G11757 C, C270T, G196A, G-712A) and Alzheimer's disease-related depression (AD-D) has been investigated (Zhang et al., 2011). Participants included 336 patients with AD, 128 of whom had AD-D. Response to an eight-week paroxetine (SSRI) treatment was assessed. The frequency of the 11757 C allele was significantly higher in AD-D patients than in AD patients without depression. Furthermore, the 196A allele occurred with significantly higher frequency in AD-D patients than in AD non-depressed patients, and carriers of the A allele of G196A responded better to paroxetine treatment. These findings support an important role for BDNF polymorphisms in AD-D (Zhang et al., 2011). Moreover, a recent study identified a relationship between gender differences and the risk for AD in correlation with neurotrophin gene polymorphisms, which recommended examining the role of potential modifying factors, such as diet, exercise, antidepressant medications and hormone-replacement use, in addition to gene–gene interactions, to clarify the role of neurotrophins and risk for AD (Matyi *et al.*, 2017)

According to the literature, the association between the BDNF gene Val66Met polymorphism and AD is inconsistent. This work examined the following:

1. Is there an association between the BDNF gene Val66Met polymorphism and AD in Saudi patients?

2. Is SSRI use improve cognition performance in AD patients?

3. Is this improvement in cognition related to certain type of BDNF gene polymorphism?

METHODOLOGY

Participants

This was an observational cross-sectional study where all participants provided DNA samples. Both AD patients (40) and healthy control groups (140) were Saudi recruited from King Fahad Medical City (KFMC) and King Salman Social Center (KSSC), respectively. DNA extraction was performed at King Faisal Specialist Hospital & Research Center (KFSH&RC), Riyadh, Saudi Arabia. Institutional review board approval was obtained for this study from KFMC, KSSC and KFSH&RC. This study was performed in accordance with the regulations of the Institutional Research Advisory Council in compliance with the Helsinki Declaration principles (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects). Mean age at onset of AD patients was 78.2±5.2 years, while healthy control mean age was 74.4 ± 6.9 years.

AD patients were diagnosed and met the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorder Association (NINCDS/ADRDA) for possible AD. The age at onset of obvious cognitive dysfunction, including memory problems, was obtained from spouses or other relatives. Patients with evidence of vascular and "mixed" dementia were excluded.

Exclusion criteria for both groups included recent (within 6 months) alcohol or drug abuse as defined by DSM-IV criteria, concurrent psychotherapeutic interventions, poor medical health or abnormal clinical laboratory results, active suicidality, and the use of medications that could interfere with the study, such as anxiolytics, antiepileptics, and cholinesterase inhibitors. All subjects were screened for medical illness by completing a medical review sheet, involving cardiopulmonary and physical examinations. All participants signed an informed consent, and a brief explanation of the study was delivered to each of them. Participant demographics and clinical data were obtained retrospectively through medical record review or through an interview during blood sample collection.

Cognition tests

1. Mini-Mental Status Examination (MMSE)

The MMSE tool was used to evaluate patients' cognitive function (Folstein et al., 1975). The MMSE is a short, widely used cognitive screening test that evaluates general cognitive function, primarily those that predominate in the left cerebral hemisphere classified within a range of 0 to 30 points. The MMSE cutoff point was 24. It is composed of questions on orientation for time and space, memory, attention/calculation, call-up, language, and praxes. It is available in Arabic (El-Hayeck et al., 2019, Al-Rajeh et al., 1999).

2. Clinical Dementia Rating (CDR)

The tool used for the assessment of the patients' cognitive performance was the Arabic version of the Clinical Dementia Rating (CDR) scale for AD (Morris J., 1993, Karam et al., 2018). This instrument was developed by the Memory and Aging Project at the Washington University School of Medicine in 1979 for staging the severity of dementia. The CDR is a 5-point scale used to characterize six domains of cognitive and functional performance applicable to AD and related dementias: memory, orientation, judgment and problem solving, community affairs, home and hobbies and personal care. The necessary information for each rating is obtained through a semi-structured interview of the patients and care providers. Each domain is rated on a 5-point scale of function as follows: 0, no impairment; 0.5, questionable impairment; 1, mild impairment; 2, moderate impairment; and 3, severe impairment (personal care is scored on a 4-point scale without a 0.5 rating available). The global CDR score was computed via an algorithm, and higher scores indicate more severe dementia. Herein, each global score was calculated using the Washington University online algorithm

(http://www.biostat.wustl.edu/~adrc/cdrpgm/index.html).

AD patients and elderly control subjects underwent CDR and MMSE cognitive assessment tests, respectively. Controls were given clinical, mental and neurological examinations to rule out AD, healthy control had Mini-Mental Status Examination >27. The Arabic MMSE & CDR versions are available upon request from the corresponding author.

Sample collection, DNA extraction and genotyping:
A five ml sample of peripheral blood was obtained by venipuncture from AD patients and healthy control into purple top vacutainer tubes (Beckton Dickenson, Franklin Lakes, NJ USA) containing K2EDTA to a final concentration of 1.5- 2.0 mg/ml blood.

Samples were processed immediately or stored at 4°C for subsequent DNA extraction (maximum 72 hours).

DNA was extracted using the Gentra Puregene DNA Purification Blood Core Kit C (Qiagen Sciences, Germantown, Maryland, USA).

The BDNF Val66Met polymorphism (rs6265) was genotyped using TaqMan allelic discrimination with the ABI Prism®7900HT Sequence Detection System (Life Technologies).

Association studies

Genotyping for the association study was performed by real-time PCR using Taqman chemistry on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems Inc. CA, USA). Primers and TaqMan probes were designed using the Primer Express software V2.0 (Applied Biosystems, Foster City, CA, USA). Real-time PCR assay and probe melting point hybridization analyses were used to detect allelic changes.

Statistical Analysis

 1. Power analysis and sample size calculation

Sample size was calculated based on a power of 80% using the assumption that SNPs are informative when they are present in 10% of the sample against a one-sided alternative with the size of the critical region = 5%.

2. Association statistics

The frequencies of BDNF alleles and genotypes were estimated by gene counting. Comparisons of genotype and allele frequencies were made using analysis of variance (ANOVA) or Student's t-test where appropriate. Chi-square test or Fisher's exact test was used for categorical variables. Interactions between cognitive stage, age, gender and use of SSRIs with both genotype frequencies and allele distributions were analyzed by the General Linear Model. Statistical significance was set at $p < 0.05$. All statistical analyses were performed with Minitab 18 software for Windows.

RESULTS

The total participants were 180, 40 of them were AD patients and 140 were controls. The controls were healthy young and middle age (75) and elderly (65). The elderly control were healthy (48) whom are not using SSRI and 17 were elderly control using SSRI. The demographic and clinical characteristics of the AD patients and the healthy elderly controls represented in Table 1 showed that there were no significant differences between the two groups.

1. BDNF Val66Met (rs6265/G196A) genotype frequency and allele distributions among all participants

The frequency of the BDNF Val66Met genotype among all participants (n=180) was divided into 72 (40%) participants who were AA homozygotes (Met/Met), 60 (33.4%) participants who were AG heterozygotes (Met/Val) and 48 (26.6%) who were GG homozygotes (Val/Val). The distribution of BDNF Val66Met (rs6265/G196A) alleles among all participants (360 alleles in total) were 205 (56.94%) A allele (Met) and 155 (43.06 %) G allele (Val).
Results revealed that age did not significantly affect genotype frequency. However, when considering allele distributions (N=360), there was a significant difference between the different age groups (p=0.048). The frequency of the G allele was lower in the older age group.

Neither gender nor the use of SSRIs showed significant difference in genotype frequency. There was a significant difference in genotype and allele frequency between the different cognition groups (p= 0.004). Because of these findings, it was appropriate to explore the effects of age, gender and SSRI use with respect to genotype and cognition status (Table 2).

2. BDNF Val66Met (rs6265/G196A) genotype frequency and allele distributions among AD patients and control elderly participants

To further investigate differences in genotypes between AD patients and elderly participants, the data were divided into two groups: AD patients (N=40) and elderly control age matched participants above 60 years old (N=65). Interestingly, the findings revealed that there was no significant difference between BDNF Val66Met genotype frequency and allele distributions between the two groups (Table 3).

4. Association of Gender & cognition performance and BDNF Val66Met (rs6265/G196A) genotype distributions and allele frequencies in AD patients and the age matched control group.

Gender was not significantly associated with BDNF Val66Met genotype frequency or allele distributions between AD and elderly groups.

The genotype frequency for the age matched control group (n=65) and MMSE scores revealed a significant association (F=4.38, P=0.017). The elderly control group showed that GG carriers have higher MMSE scores both than AA carriers and AG carriers (Figure 1).

The genotype distributions for the AD group (n=40) and CDR scores demonstrated significant association (F=6.80, p<.001). CDR score means for AD patients with no SSRIs were higher in both AA carriers (12.24± 4.13 SD) and AG carriers (10.57± 6.47 SD) compared to AD patients with SSRIs. However, GG carriers exhibited lower scores among AD patients compared to AD patients with no SSRIs (3.5± 0.54 SD).

5. Association of BDNF Val66Met polymorphism (rs6265/G196A) with SSRI use

Next, the association between the use of SSRIs and BDNF Val66Met genotype distributions and allele frequencies in the elderly group and AD patients was examined. First, the association between the use of SSRIs and genotype distribution within the elderly group was performed by dividing the elderly group into two groups. The first group was the control (n=48), and the second group (n=17) was those who were using SSRIs. Chi-square test revealed significant differences between the two groups regarding both genotype distributions and allele frequencies (p=0.031 and p=0.017, respectively) (Table 4). However, the association between the use of SSRIs and genotype distribution within AD patients was not significant. AD patients with SSRIs had a higher BDNF GG homozygote distribution than elderly controls using SSRIs, which may indicate that AD patients with BDNF GG homozygote distribution are more prone to depression (p-value=0.001) (Table 5).

6. The effects of SSRI use and genotype on CDR scores

Next, the effects of SSRI use and genotype distributions on CDR scores (after conversion of the MMSE scores into CDR scores) were explored.
Notably, for elderly and AD patient groups, results revealed that CDR scores were influenced by the use of SSRIs (p<0.001), regardless of the genotype distributions (p-value=0.301). There was an interaction between the effects of SSRI use and genotype (Table 6).

DISCUSSION

The results of this study demonstrate significant differences in BDNF Val66Met genotype and allele distributions among cognitive impairment groups. This work proved that BDNF Val66Met genotype distribution is a significant contributing factor to the development of AD stages. BDNF Val66Met genotype distributions and allele frequencies were significantly associated with cognitive performance in both the elderly control group and AD patients using the Clinical Rating Scales and MMSE tests. The primary findings showed that GG homozygotes (Val/Val) have superior cognitive performance among AD patients and the elderly control group, which was previously documented in healthy young

adults and psychiatric patients (lamb.et al., 2015, Kambeitz *et al.*, 2012). However, in depressed geriatric patients, Benjamin et al. revealed that there was no association between cognitive performance and BDNF genotypes (Benjamin *et al.*, 2010).

A number of studies suggest that the Val66Met BDNF SNP is linked to brain morphology (Tanzi, 2012). Evidence from neuroimaging studies demonstrate that BDNF A allele (Met) carriers have significantly greater reductions in the dorsolateral prefrontal cortex, caudate nucleus, and frontal grey matter volume, as well as smaller hippocampus volumes, compared to BDNF G allele (Val) carriers (Sleegers & Van Duijin 2001; Tanzi, 2012; Lee *et al.*, 2005). It has been suggested that the BDNF Val66Met genotype is an important factor in AD pathology and that cognitive decline is explained by higher brain BDNF expression (Buchman *et al.*, 2016). However, Lim et al. suggested that the BDNF Val66Met gene polymorphism could be a factor in moderating cognitive performance through Amyloid-B in preclinical AD. Therefore, individuals with the A allele (Met) can expect to show clinically significant memory impairment within 3 years, whereas BDNF G allele (Val) individuals did not show a similar degree of impairment until 10 years later (Lim et al., 2015). Additionally, research findings broadly support the work of other studies in this area linking the BDNF Val66Met polymorphism and cognitive performance in antipsychotic-naïve patients with schizophrenia (Bian et al., 2005).

Regarding the association of age and allele distribution, there were no significant associations with aging changes. These findings are supported by a recent study that found no association between BDNF polymorphism and age in AD patients (Solmaz *et al.*, 2017). However, another study found a significant interaction between age and baseline whole brain volume and the BDNF rs6265 SNP, showing that GG homozygote (Val/Val) individuals have reduced whole brain volumes with increasing age compared to AA homozygote (Met/Met) and A allele (Met) carriers (Honea *et al.*, 2013).

Other than early death, another possible explanation of the lack of GG individuals amongst the elderly participants is population changes in Saudi Arabia. In Saudi Arabia, immigration could be a factor explaining the lower prevalence of GG homozygotes (Val/Val) in elderly participants. Most Saudis are ethnically Arabs, the majority of whom are tribal Bedouins. According to a random survey, most would-be Saudis come from

the Indian subcontinent and Arab countries. Many Arabs from nearby countries, particularly Egypt, are employed in the kingdom; the Egyptian community has developed gradually from the 1950s onwards. In the 1970s and 1980s, there was also a significant community of South Korean migrant laborers, numbering in the hundreds of thousands, but most have since returned home. The South Korean government's statistics showed only 1,200 of their nationals living in the kingdom as of 2005. There are also significant numbers of Asian expatriates from India, Pakistan, Bangladesh, Indonesia, Philippines, and recently refugees from Syria and Yemen. Petryshan et al. reported that the G allele is more prevalent in the Asian than in the African population (Petryshen *et al.*, 2010). Immigration of Asians into Saudi Arabia could account for an increase in the prevalence of the G allele, as evidenced by higher frequencies in younger populations.

According to Kambeitz et al., variations in BDNF Val66Met (rs6265/G196A) allele and genotype frequencies may mediate critical neurocognitive impairments observed in various neuropsychiatric conditions (Kambeitz *et al.*, 2012).

Furthermore, our results found that GG homozygote (Val/Val) frequency was higher in female geriatric controls compared to female AD cases, which seems to suggest a protective effect of the BDNF GG genotype from AD in females. This could be explained by the work of Egan et al. who found that the BDNF A allele (Met) was associated with poor episodic memory, abnormal hippocampal activation and reduced hippocampal n-acetyl aspartame in human subjects (Egan *et al.*, 2003). These results revealed that in males, the GG genotype frequency was lower than in females in both AD patients and the geriatric group, indicating that in males, neither BDNF A allele (Met) carriers nor BDNF G allele (Val) carriers experienced any protective effects against AD. This finding contrasts with the hypothesis that the BDNF Val66Met polymorphism may affect susceptibility to regional white matter hyperintensity (WMH) volume and that such a genotype-by-WMH interaction is correlated with cognitive decline in non-demented elderly males, in which the Met allele plays a protective role (Huang *et al.*, 2014).

In BDNF GG homozygote (Val/Val) carriers, this study found that the most important clinically relevant finding was that delayed memory index scores were significantly lower in BDNF GG homozygote (Val/Val) carriers than in BDNF AA homozygotes (Met/Met) in

AD patients on SSRIs. BDNF GG homozygotes (Val/Val) performed better in the aged control group with respect to cognition, while in AD patients, BDNF GG homozygotes (Val/Val) were lacking, suggesting possible early death or depression. In short; these studies indicate that the BDNF Met-66 variant may influence memory in humans with or without AD.

According to these data, we can infer that BDNF GG homozygote (Val/Val) genotypes lose their effects with neurodegenerative diseases, i.e. GG is protective in elderly patients but not in AD patients. Together, these results provide an important insight into BDNF Val66Met genotype distributions and allele frequencies in elderly participants and AD patients, as well as their association with SSRI use. AD patient carriers of BDNF GG homozygote (Val/Val) exhibited more depressive symptoms than the elderly control group. In contrast, findings from a meta-analysis of geriatric depression, with AD patients excluded, found an association between geriatric depression and the BDNF Val66Met polymorphism. From five case control studies, it was established that BDNF AA homozygotes (Met/Met) exhibited increased risk for geriatric depression compared to GG. Furthermore, the Met allele was associated with decreased activity of the BDNF system (Pei *et al.*, 2012).

Although much remains to be determined regarding the risks of AD and how cognitive function can be improved in AD patients, this study has produced important results on the effects of SSRIs in AD patients. However, several concerns about limitations must be noted, primarily patient sample size and the possibility of selection bias due to non-random selection of patients and control. The duration period of this study as well as the geographical location of the AD patients.

Conclusions

This work obtained the following results:

- The GG genotype may lead to enhanced cognition among elderly control and AD patients;
- The GG genotype may result in early death among AD patients;
- SSRIs have an effect on cognitive performance in elderly controls only.

CONFLICTS

KEY WORDS

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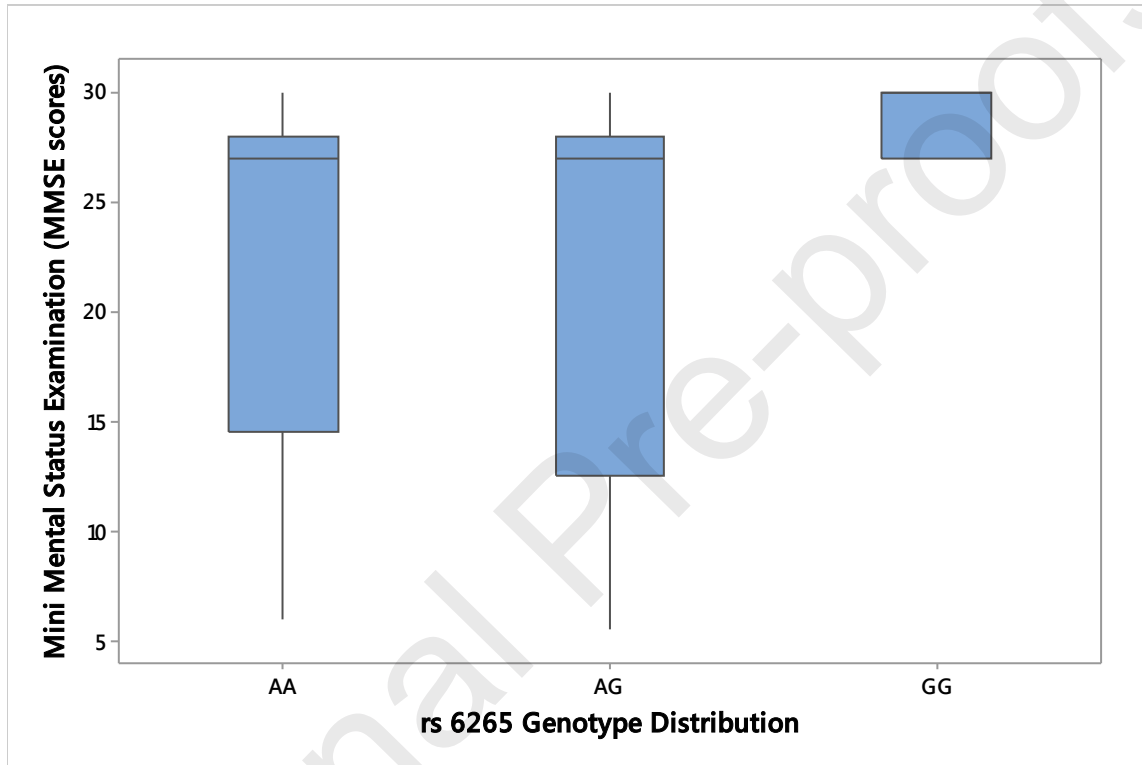
Figure 1: Elderly control group genotype distribution and cognition performance (N=65, 48 of them were healthy)

	Alzheimer's patients (N=27)	Elderly healthy control (N=48)	P-Value
Age (years)	78.9±5.28	69.8±6.45	0.45
Gender	M=14(51.8)	M=12 (25)	0.96

M, F (%)	F=13(48.2)	F=36(75)	0.001
BMI (kg/m^2)	25.1±3.472	29.7±5.30	0.53
HbAlc (%)	6.7±1.177	7.9±10.09	0.75
White Blood Cell Count (x 10^9 per liter L)	6.4± 2.03	8.0 ±2.45	0.67
Red Blood Cell Count (10^6/µL)	4.6± 0.09	4.3±0.46	0.92
Hemoglobin level (gm/dL)	14.0±0.27	13.1±1.40	0.86
Platelet count (10^9/L)	274.0± 10.3	270.8±61.51	0.89
Use of medication (%)	55.6%	50%	0.62
Cognition test	CDR Score 9.3±4.36	MMSE Score 28±1.25	-

Table 1: Demographic and clinical characteristics of AD patients and elderly healthy controls (both not using SSRI)

Variables	Genotype			p-value	Allele		p-value
	AA (%)	AG (%)	GG (%)		A (%)	G (%)	
Age	N=180			0.231	N=360		0.048
Young (25-35 y.o)	10(29)	13(38)	11(32)		34(50)	34(50)	
Middle (36-59 y.o)	14(34)	12(29)	15(37)		40(49)	42(51)	
Elderly (60 y.o and above)	48(46)	35(33)	22(21)		131(62)	79(38)	
Gender	N=180			0.227	N=360		0.369
Male	37(40)	35(38)	20(22)		109(59)	75(41)	
Female	35(40)	25(28)	28(32)		96(55)	80(45)	
Use of SSRIs	N=180			0.445	N=360		0.167
No	57(38)	51(34)	42(28)		166(77)	134(23)	
Yes	15(50)	9(30)	6(20)		39(65)	21(35)	

Cognition stages	N=180			0.004	N=360		<0.001
No cognition changes	42(34)	41(33)	40(33)		125(51)	121(49)	
Very Mild	6(38)	3(19)	7(44)		15(47)	17(53)	
Mild	8(53)	7(47)	0		23(77)	7(23)	
Moderate	12(67)	6(33)	0		30(83)	6(17)	
Severe	4(50)	4(50)	0		12(75)	4(25)	

Table 2: BDNF Val66Met (rs6265/G196A) genotype frequency (N=180) and allele distributions (N=360) in all participants

Groups		Genotype distribution (%)				Allele distribution (%)			
	N	AA	AG	GG	*P*	N	A	G	*P*
Elderly	65	27(41.5)	23(35.3)	15(23.2)	0.23	130	77(59.3)	53 (40.7)	0.54
AD	40	21(52.5)	12(30)	7(17.5)		80	54(67.5)	26 (32.5)	

Table 3: Distribution of Val66Met (rs6265/G196A) genotype frequency and allele distribution for control elderly participants (N=65) and AD patients (N=40)

		Genotype distribution (%)				Allele distribution (%)			
	N	AA	AG	GG	P	N	A	G	P
Elderly without SSRI	48	18(37.2)	15(31.2)	15(31.2)	0.031	96	51(53.2)	45(46.8)	0.017
Elderly with SSRI	17	9(53)	8(47%)	-		34	26(76.5)	8(23.5)	
AD without SSRI	27	15(55.5)	11(40.8)	1(3.7)	0.32	54	41(75.9)	13(24.1)	0.950
AD with SSRI	13	6(46.2)	1(7.6)	6(46.2)		26	13(50)	13(50)	
Total	105	48(46)	35(33)	22(21)		210	131(62)	79(38)	

Table 4: Distribution of Val66Met (rs6265/G196A) genotype and allele in the elderly group (N=65) and AD patients (N=40) with/without the use of SSRIs

	Genotype distribution (%)			Allele distribution (%)		
	GG With SSRIs	GG Without SSRIs	P-value	G With SSRIs	G Without SSRIs	P-value
Elderly	-	15	<0.001	8	45	0.001
AD	6	1		13	13	

Table 5: Distribution of Val66Met (rs6265/G196A) GG genotype and G allele in the elderly group and AD patients

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Genotype	2	121.1	60.53	1.21	0.301
Use of SSRIs	1	5015.1	5015.08	100.65	<0.001
Error	101	5032.4	49.83		
Lack-of-Fit	2	546.8	273.38	6.03	0.003
Pure Error	99	4485.7	45.31		
Total	104	10320.1			

Table 6: Two-way ANOVA test between the use of SSRIs and genotype distributions in response to CDR scores for AD patients and the elderly group.