

Investigation of B2-AR, TLR2, PICALM, and BDNF Gene Variants in Iranian Alzheimer's Patients and Their Response to Rivastigmine

Parvin Mohabattalab¹, Fatemeh Rezaei Rad¹, Hamid Zamani¹, Fariba Shirvani², Mahdi Zamani^{1*}

¹Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

²Pediatric Infections Research Center, Research Institute for Children Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran

*Correspondence should be addressed to Mahdi Zamani; mzamani@tums.ac.ir

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Abstract

Alzheimer's disease is the most common form of dementia with polygenic disposition occurring within various populations. A series of molecular studies indicated that there are number of genes linked to late onset Alzheimer's disease (AD). In the current study, we examined the contribution of B2-AR (Gly16, Arg), TLR2 (-196TO-174del), PICALM (rs3851179) and BDNF (rs6265) alleles and genotypes, and their relevance in response to Rivastigmine in 150 Iranian AD patients and 150 controls. Genotyping was performed employing Tetra-primer ARMS-PCR and RFLP-PCR methods. Our statistical analysis suggests that A and G alleles of B2-AR show significantly negative ($P_c=0.02$, $RR=0.65$, $95\%CI=0.30-0.94$) and positive ($P_c=0.02$, $RR=1.21$, $95\%CI=1.06-3.29$) associations with familial Alzheimer's disease (FAD), respectively. Interestingly, after excluding APOE ϵ_4 allele, our results indicated that B2-AR A allele confers more significant protection ($P_c=0.006$, $RR=0.52$, $95\%CI=0.33-0.83$), while G allele ($P_c=0.006$, $RR=1.90$, $95\%CI=1.20-3.00$) and GG genotypes ($P_c=0.008$, $RR=2.25$, $95\%CI=1.26-4.06$) provide major susceptibility to AD. We also calculated the clinical relevance of the testing utilizing Prevalence-corrected Positive Predictive Value (PcPPV) formula. The PcPPV of B2-AR A and G alleles were 1.5% and 2.7% respectively in FAD patients. Moreover, the PcPPV for Apo E4 negative AD patients carrying B2-AR GG genotype was 3.5%, meaning that such patients have 3.5% absolute risk for developing AD. Pharmacogenetic analysis of AD patients in a two-year follow-up response to Rivastigmine was also performed. Analysis of Genotype-Related Drug Responses demonstrated that patients carrying B2-AR homozygous GG genotypes were the worst responders to Rivastigmine treatment when compared to total AD patients (baseline) ($\Delta CDR=0.54$). Interestingly, patients with B2-AR homozygous AA genotype, had a best response to the Rivastigmine therapy and decreasing trend in the disease severity and symptoms ($\Delta CDR=-0.12$). These findings suggest that B2-AR A allele acts as a recessive allele in positive response to Rivastigmine treatment. Furthermore, we examined the interactions of the genes related to this study with the genes studied previously in the same patients for response to Rivastigmine. The results indicated, patients, carrying B2-AR AX-A2M AX bigenic genotype show good response ($\Delta CDR=0.18$) and B2-AR GX-IL6 GX carriers were bad responder to Rivastigmine treatment ($\Delta CDR=0.97$).

Keywords: Alzheimer, B2-AR, TLR2, PICALM, BDNF, Rivastigmine

Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disorder with polygenic and multifactorial inheritance, determined by progressive loss of memory and other cognitive functions. AD is characterized by hallmark pathological changes such as extracellular aggregation of amyloid β ($A\beta$), intraneuronal

neurofibrillary tangles that lead to brain atrophy and loss of neural tissue [1,2]. Alzheimer's disease is categorized according to the age of onset as early-onset (EOAD) or late-onset AD (LOAD) [3]. And, based on family history, it is classified as sporadic (SAD) or familial Alzheimer's disease (FAD) [4]. There are various genetic and environmental factors involved in the pathogenesis of AD which makes the etiology of the disease complicated however, testing for

genetic markers and genes are useful tools to understand the pathogenesis of wide range of polygenic diseases such as cancers and AD [5-8]. In this regard, several studies were described the association of AD with some polymorphic genes such as Apolipoprotein E (ApoE4), Presenilin 2 (PS2), Alpha-2 macroglobulin (A2M), and Interleukin 6 (IL 6) [5,9,10]. In recent years various genome-wide associations and meta-analysis were performed to find inhibitory and predisposing genes involved in Alzheimer's. This search resulted in identification of the human genes for B-adrenergic receptor (B2-AR), Toll-like receptor 2 (TLR2), Phosphatidylinositol binding Clathrin assembly protein (PICALM) and brain-derived neurotrophic factor (BDNF) [11-14]. The B2-AR gene is encoded by an intronless gene that is located on chromosome 5q31-5q33. Accumulated convincing evidence from cell culture experiments and animal studies have suggested that β -2-contributes to the AD pathogenesis through its effects on amyloid- β (A β) production and complication of inflammation. Several single nucleotide polymorphisms (SNPs) have been described for this gene, notably codon 16 (Gly or Arg). Many studies observed that this SNP might alter cellular trafficking and receptor desensitization [15-17]. Abnormal activation of B2-AR protein might contribute to A β accumulation in AD pathogenesis [18,19].

Toll-like receptor 2 (TLR2) is suspected to be the most likely gene associated with the Alzheimer's disease, since it is localized on chromosome 4q, within the genetic region linked with AD, and that it plays a role in the microglial-mediated inflammatory response and amyloid- β (A β) clearance [20]. Numbers of studies have shown that TLR2 along with TLR4 and CD14 receptors on the surface of microglial cells are primary receptors for A β which upon their interactions stimulate neuronal inflammation and phagocytic initiation. Interestingly, the 196 to -174 polymorphic deletion is shown to change the promoter activity of the TLR2 gene which in turn might have an impact on the development of AD [21,22].

PICALM gene is also known as one of the important candidate genes associated with AD. A strong association of a particular allele of this gene, *rs3851179* polymorphism, with AD is demonstrated in number of studies [23,24]. This gene, located on 11qarm of chromosome 14, encodes for 23 different transcripts that are mainly expressed in neurons and shown to play key roles in endocytosis, Iron metabolism and cellular proliferation [25]. PICALM gene contributes to the pathogenesis of AD through intervention in production, transportation, cleaning and other pathways related to A β [26].

One of the notable events in the pathogenesis of AD is reduced expression of neurotrophic factors and their receptors, a good example of such factor is Brain-derived neurotrophic factor (BDNF) [27]. The most well-known

SNP of BDNF gene is rs6265 that is a conversion of Guanine to Adenine in the 196 nucleotide position (G 196 A), which replaces a Valine for Methionine in prodomain part of the protein structure (Val 66 Met) [28]. This amino acid replacement affects neuronal activity by impairing the secretion of BDNF which is shown to be associated with the establishment of Alzheimer's disease [29].

Since the first cholinesterase inhibitor production in 1997, cholinergic drugs, such as Rivastigmine, were considered as the initial line of treatment for mild to moderate AD [2,30]. By blocking the acetylcholinesterase enzyme, this drug helps to maintain high levels of acetylcholine in AD patients, a neurotransmitter that plays an important role in human memory and cognitive functions [31]. Innovative studies in the field of pharmacogenetic have shown that differential response of the patients with Alzheimer's disease to Rivastigmine may be dependent on a particular genotype, hence strengthening the genotype-specific response hypothesis in AD [32]. Therefore, considering the heterogeneity of the disease, and also the importance of the ethnic origin and geographical location of the patients, this study is aimed at investigating the association of B2-AR, TLR2, PICALM, and BDNF alleles and genotypes with AD in Iranian population. In addition, we have investigated the genotype-specific response of Iranian AD patients, with the mentioned polymorphic genotypes to Rivastigmine treatment.

Experimental Procedure

Patients and controls

In the current study, a total of 150 blood samples from unrelated patients with AD (112 SAD and 38 FAD patients, age=7.6 \pm 77.73 years) were accumulated from hospitals in Tehran and the Dementia Out-patients Clinic of the Iranian Alzheimer Association (IAA). All these Alzheimer's disease were genetically independent and diagnosed with following Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) [33]. Criteria and other psychological scales and severity of AD were determined with the Clinical Dementia Rating Sum of Boxes (CDR-SB scale) at the start of treatment by the neurologist. The control group included 150 age, gender, and ethnic background matched healthy individuals, with no family history of dementia or AD that were randomly picked after assessing their cognitive function using the Mini-Mental State Examination [34]. To eliminate possible errors in sample selection and genotyping, the Hardy-Weinberg equilibrium (HWE) test was performed in control group for each polymorphic gene under study. The cognitive functions of participants were assessed by a standard questionnaire form for each patient and controls. Before participating in the research, all individuals gave informed consent. This study was authorized by the ethics

committee and review board of Tehran University of Medical Sciences. The severity of AD was measured with CDR-SB after two years, and statistical analysis for the drug response was calculated by the SPSS software version 25 for WINDOWS (Chicago, IL, USA).

B2-AR, TLR2, PICALM, and BDNF genotyping

Blood specimens were gathered in sterile tubes containing EDTA and genomic DNA was extracted using a modified salting-out method. B2-AR polymorphisms (alleles A and G) were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described previously [35] according to the following protocol: 5 min denaturation at 94°C then 33 cycles of polymerization each 30 s at 94°C, 30 s at 57°C and 60 s at 72°C, followed by final extension for 5 min at 72°C. The (-196TO-174 del) polymorphism in 5'UTR region of TLR2 gene was determined by Allele-Specific-PCR method as described previously [21]. Briefly, the PCR was performed according to the following protocol: 5 min denaturation at 94°C then 33 cycles of polymerization each for 30 s at 94°C, 30 s at 57°C and 60 s at 72°C followed by 5 min final extension at 72°C. PICALM (rs3851179) genotyping was performed by Allele-Specific-PCR method according to the process reported previously [36]. In order to reduce the cost of BDNF and PICALM genotyping, we designed and employed three and tetra-primer amplification refractory mutation system-polymerase chain (Tetra-primer ARMS-PCR) which are offer fast detection and extreme simplicity at a negligible cost for genotyping, respectively (Table 3). Tetra-primer ARMS-PCR profile of PICALM

gene for amplification was 5 min denaturation at 94°C followed by polymerization for 30 s at 94°C, 30 s at 59°C, and 60 s at 72°C for 35 cycles, and 10 min at 72°C for final extension. Genotyping of (rs6265) SNP of BDNF gene by ARMS-PCR was performed with the modified methods reported already [37] which follows 5 min denaturation at 94°C then 33 cycles of polymerization for 30 s at 94°C, 30 s at 57°C, 30 s at 72°C followed by 5 min final extension at 72°C. The primer designs were carried out employing Oligo7 program and Ensemble Genome Browser for blasting.

The PCR products were separated by electrophoresis on 6% polyacrylamide gels alongside a 50 bp DNA ladder. The PCR bands were visualized by Ethidium Bromide staining, and the proper size bands for particular alleles were estimated with the corresponding band fragments of the running DNA markers.

Statistical analysis

All data, including clinical and genotyping of the TLR2, PICALM, B2-AR, and BDNF genes, were recorded into a database, and statistical analysis was done using the SPSS version 25 for Windows (Chicago, IL, USA). Genotype and allele frequencies of the TLR2, PICALM, B2-AR, and BDNF were compared between the patients and the controls, and fisher exact tests was used to for statistical analysis of data, and p values were corrected for multiple testing by Bonferoni's correction. Likewise, Relative risk (odds ratio) was calculated using the Woolf formula: (number of patients with the specific allele/number of patients

Table 3: Primers for genotyping of B2-AR, TLR2, PICALM and BDNF genes.

Gene	RFLP primers
B2-AR	B2-AR G:5'-CTT CTT GCT GGC ACC CTA TG-3'
	B2-AR A:5'-CTT CTT GCT GGC ACC CTA TA-3'
	PCR primer
TLR2	TLR2 F:5'-CTCGGAGGCAGCGAGAAA-3'
	TLR2 R:5'-CTGGGCCGTGCAAAGAAG-3'
	Tetra-primer ARMS-PCR
PICALM	PICALM NF: 5'-GCAAACAATACACACTTCAGTAAAC-3'
	PICALM NR:5'-GCTAATTTTGGTCTTGGTAGAG-3'
	PICALM MF:5'-CTATTCAGCTTGGGCGAATTACTAAA-3'
	PICALM MR: 5'-CACCATATAATAGTTGTGATAGATACC-3'
	ARMS-PCR
BDNF	BDNF M: CTTTCTCCCTACAGTTCCACCA
	BDNF N: CATCCAACAGCTCTTCTATCAC
	BDNF C: CATCCAACAGCTCTTCTATCAT

SSP: Specific Sequence Primers; RFLP: Restriction Fragment Length Polymorphism; PCR: Polymerase Chain Reaction; ARMS: Amplification Refractory Mutation System

without this allele)/(number of controls with the specific allele/number of controls without this allele). Prevalence corrected Positive predictive value (PcPPV) evaluates the value of a diagnostic test and indicates the likelihood for a person with a positive test of having or developing the disease, indeed this formula calculate the absolute risk for developing disease. In this case-control study, the following formula was used to determine Prevalence corrected Positive Predictive Value (PcPPV): $PcPPV = a / (a + b) = (P_{DT+} \times P_D) / (P_{DT+} \times P_D) + [P_{CT+} \times (1 - P_D)]$.

Where P_D is the prevalence of the disease in the general population, P_{DT+} is the ratio of the patients with a positive test, and P_{CT+} is the ratio of the controls with a positive test [38].

Results

Distribution of B2-AR, TLR2, PICALM, and BDNF alleles and genotypes in AD Patients

The distribution of B2-AR, TLR2, PICALM and BDNF alleles and genotypes frequencies in the total AD patients and healthy controls are presented in Table 1. The initial data analysis did not show any significant association between the B2-AR alleles or genotypes with total AD patients as compared with the control group (Table 1). But further analysis revealed that the B2-AR G allele is more frequent in the FAD patients (75%) compared to the controls (61.6%) ($P_c=0.02$), yielding an increased absolute risk of 2.7% ($PcPPV=2.7$, $RR=1.21$, $95\%CL=1.06-3.29$) and On the other hand, the frequency of B2-AR A allele was significantly higher in the control group (38.3%) than in the FAD patients (25%) ($P_c=0.02$, $RR=0.65$, $95\%CL = 0.30-0.94$). Therefore, these results suggest that the B2-AR G and A alleles provided susceptibility for and protection against FAD, respectively.

Statistical analysis of TLR2, PICALM (rs3851179), and BDNF alleles and genotypes did not display any significant association with Alzheimer's disease in total AD, SAD, and FAD patients when compared to controls.

Possible interaction of B2-AR, TLR2, PICALM, and BDNF alleles or genotypes with APOE ε4 allele.

In our preparatory studies, we examined the association of the APOE ε4 allele and AD in a similar patient population [10]. Data from this study and the previously mentioned studies were collected for meta-analysis to achieve more valid conclusions. The distribution of B2-AR, TLR2, PICALM, and BDNF alleles and genotypes frequencies in AD patients and healthy controls, after excluding APOE ε4 genotype, are presented in Table 2. After stratification for the APOE ε4 genotype, it was observed that the frequency of TLR2 and BDNF alleles between APOE ε4-negative AD

patients and the controls were not considerably different. While, the frequency of the B2-AR GG homozygote genotype in AD patients who did not carry APOE ε4 allele was significantly increased ($P_c=0.008$, $RR=2.25$, $95\%CL=0.33-0.83$). Since B2-AR A allele is protective and G allele present susceptibility and on the other hand, B2-AR AA and AG genotypes do not show any significant association with AD while, B2-AR GG homozygote genotype indicates significant association with AD ($P_c=0.008$, $RR=2.25$, $95\%CL=0.33-0.83$) therefore, based on Mendelian inheritance B2-AR G allelic gene is a recessive gene and provide susceptibility in homozygote form for AD.

Furthermore, statistical analysis at the allelic level demonstrated a significant association between B2-AR A and G alleles and AD as compared with controls, in that the G allele imposes susceptibility for ($P_c=0.006$, $RR=1.90$, $95\%CL=1.21-3.0$) and A allele provides protection against AD ($P_c=0.006$, $RR=0.52$, $95\%CL=0.33-0.83$).

Also, after eliminating the effect of APOE ε4, statistical analysis indicated that the frequency of PICALM alleles of A and G in AD patients were increased and decreased, respectively when compared with the controls, but it was not significant after Bonferoni's correction for multiple testing (Table 1).

Clinical significance of testing for B2-AR A and G alleles and B2-AR GG genotype

The individual absolute risk in the case-control studies can be calculated by PcPPV formula [38]. We employed this formula, which considers the prevalence of AD disease in the general Iranian population, to calculate the PcPPV of testing for B2-AR A and G alleles and B2-AR GG genotype (Table 1). The prevalence of AD for evaluating the predictive values was taken as 2.3% for the Iranian population [39]. The PcPPV of B2-AR A and G alleles were 1.5% and 2.7% respectively in the Iranian FAD patients. Also, the PcPPV of B2-AR GG genotype was the highest among other alleles ($PcPPV = 3.5\%$), after excluding the patients carrying APOE ε4 genotype (Table 2). This indicated that non-carriers of APOE ε4 who carry the B2-AR GG genotype had 3.5% absolute risk of developing AD. These results reinforce the susceptibility role for B2-AR G and the protective role for B2-AR A alleles in the Iranian AD patients.

Response to Rivastigmine

The severity of AD in patients was calculated by CDR-SB scale. This calculation was first done at the time of sampling and then after two-year patient follow-up under Rivastigmine treatment. The calculations for the two regimens in this study are signified as CDR1 (initial treatment) and CDR2 (after 24-month treatment), respectively, and $\Delta CDR=CDR2-CDR1$. In this way, the genotype-specific drug response in AD patients

Table 1: The distribution of alleles and genotype frequencies in 150 Iranian AD patients and 150 healthy controls.											
	All AD Patients	Control	SAD Patients	FAD Patients	All AD vs. controls		SAD vs. controls		FAD vs controls		PcPPV%
					Pc	RR	Pc	RR	Pc	RR*	
Alleles											
B2AR	n=300	n=300	n=224	n=76							
A	105	115	87	19	NS		NS		0.02	0.65	1.5
G	195	185	137	57	NS		NS		0.02	1.21	2.7
TLR-2	n=268	n=260	n=194	n=74							
D	64	61	45	19	NS		NS		NS		
I	204	199	149	55	NS		NS		NS		
PICALM	n=268	n=260	n=194	n=74							
A	153	136	110	43	NS		NS		NS		
G	115	124	84	31	NS		NS		NS		
BDNF	n=224	n=196	n=162	n=62							
A	36	32	25	11	NS		NS		NS		
G	188	164	137	51	NS		NS		NS		
Genotype											
B2AR	N=150	N=150	N=112	N=38							
A/A	17	18	15	2	NS		NS		NS		
A/G	71	79	57	15	NS		NS		NS		
G/G	62	53	40	21	NS		NS		NS		
TLR-2	N=134	N=130	N=97	N=37							
D/D	5	5	4	2	NS		NS		NS		
D/I	52	50	37	15	NS		NS		NS		
I/I	77	75	56	20	NS		NS		NS		
PICALM	N=134	N=130	N=97	N=37							
A/A	40	33	30	11	NS		NS		NS		
A/G	72	67	50	21	NS		NS		NS		
G/G	22	30	17	5	NS		NS		NS		
BDNF	N=112	N=98	N=81	N=31							
A/A	3	4	2	1	NS		NS		NS		
A/G	30	24	21	9	NS		NS		NS		
A/G	79	70	58	21	NS		NS		NS		

Pc: P-value of Fisher's exact test with Bonferroni-correction for multiple testing; RR: Relative Risks, CL: 95% Confidence Limits of RR; n: number of chromosomes; N: Number of patients, PcPPV: Prevalence corrected Positive Predictive Value; NS: Not Significant, *= 95%CL for B2AR G allele (1.06-3.29) and for A allele (0.30-0.94)

Table 2: The distribution of alleles and genotypes frequencies after excluding APOE ε4.					
			All AD vs. controls		
	All AD patients	Control	Pc	RR*	PcPPV%
Alleles					
B2AR	N=134	N=300			
A	33	115	0.006	0.52	1.4
G	101	185	0.006	1.9	2.7
TLR2	N=196	N=260			
D	40	60	NS		
I	156	200	NS		
PICALM	N=82	N=260			
A	51	133	0.1	1.49	
G	31	127	0.1	1.04	
BDNF	N=108	N=196			
A	18	32	NS		
G	90	164	NS		
Genotype					
B2AR	N=67	N=150			
AA	3	18	0.12	1.22	
AG	27	79	0.12	1.06	
GG	37	53	0.008	2.25	3.5
TLR2	N=66	N=130			
DD	2	5	NS		
DI	27	50	NS		
II	37	75	NS		
PICALM	N=41	N=130			
AA	15	33	NS		
AG	21	67	NS		
GG	5	30	NS		
BDNF	N=54	N=98			
AA	2	4	NS		
AG	14	24	NS		
GG	38	70	NS		
Pc: P-value of Fisher's exact test with Bonferroni-correction for multiple testing; RR: Relative Risks; CL 95%: Confidence Limits of RR, n: number of chromosomes; N: Number of patients; NS: Not Significant; PcPPV: Prevalence-corrected Positive Predictive Value; *= 95%CL for B2AR G allele (1.21-3.00), for A allele (0.33-0.83) and GG genotype (1.26-4.06)					

was examined for B2-AR, TLR2, PICALM, and BDNF genotypes. Analysis of Genotype-Related Drug Responses to Rivastigmine therapy during 24-month follow-up indicated that patients with B2-AR homozygous GG or heterozygous GA genotypes were the worst responders to drug therapy ($\Delta\text{CDR}=1.65$ and 1.97 respectively) when compared to total AD patients (baseline) ($\Delta\text{CDR}=0.54$) (Figure 1). Interestingly, patients with B2-AR homozygous AA genotype, had a positive response to the drug and decreasing trend in the disease severity and symptoms

($\Delta\text{CDR}=-0.12$) (Figure 1).

The patients with TLR2 DD genotype exhibited $\text{CDR}_1=5.9$ at the start of the drug treatment which was lower than what was calculated for the total AD patients ($\text{CDR}_1=8.81$). After 24 months of treatment, these calculated values were $\Delta\text{CDR}_2=0.2$ for the patients carrying TLR2 DD genotype and $\Delta\text{CDR}_2=0.54$ for the total AD patients (Figure 2). Note that patients with TLR2 DD genotype have lower ΔCDR_2 value than that of the patients with B2-AR AX genotype.

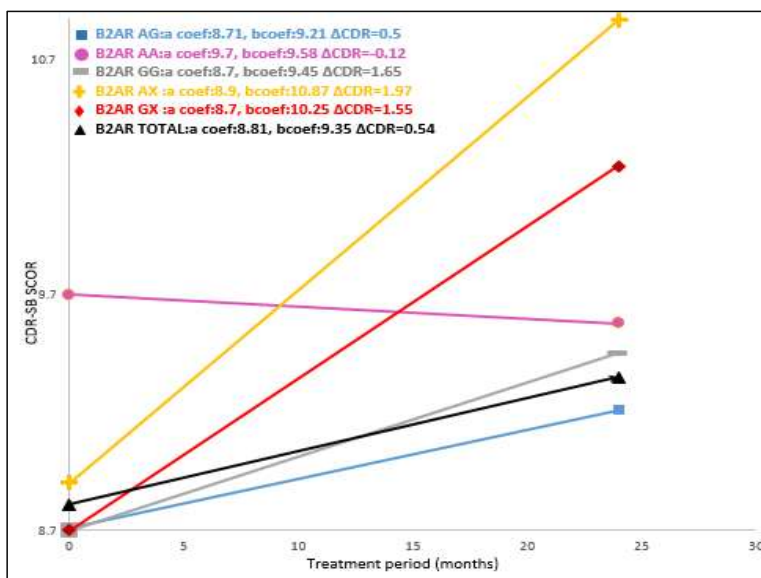


Figure 1: B2AR genotype-related therapeutic response to Rivastigmine therapy in 150 Iranian Alzheimer's disease patients. a coef. = y-intercept of the line; b coef = x-intercept of the line.

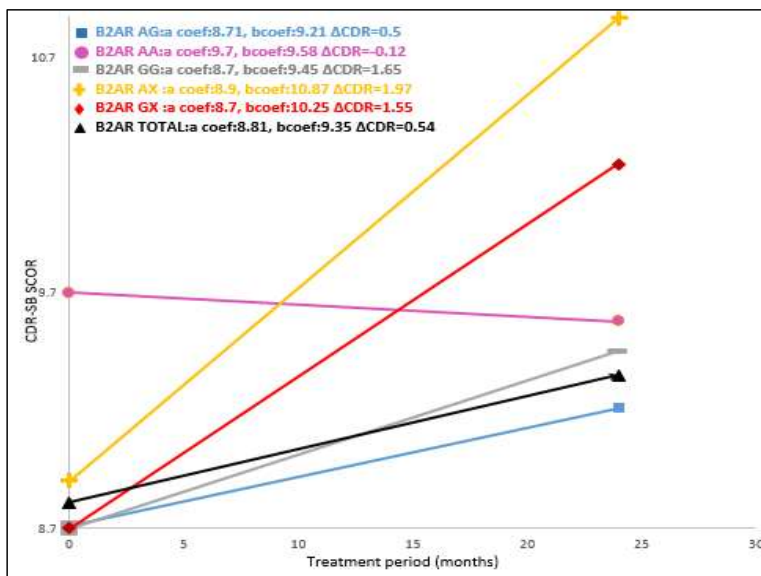


Figure 2: TLR2 genotype-related therapeutic response to Rivastigmine therapy in 140 Iranian Alzheimer's disease patients. a coef. = y-intercept of the line; b coef. = x-intercept of the line.

The two-year drug response was also investigated for the patients who were carriers of BDNF genotypes. These results indicated that AD patients with BDNF AX genotype as compared with the total AD patients, exhibited downward trend in the disease severity and symptoms ($\Delta\text{CDR}=-0.25$). This finding indicates that AD Patients carrying the BDNF AX genotype present the best response to Rivastigmine therapy (Figure 3).

Bigenic genotype interaction in response to Rivastigmine

The bigenic genotype-related response of Alzheimer's patients to Rivastigmine therapy for TLR2 and PICALM genotypes is shown in Figure 4. The calculated ΔCDR during the two-year Rivastigmine follow-up indicated that patients with TLR2 DI-PICALM AG bigenic genotype were

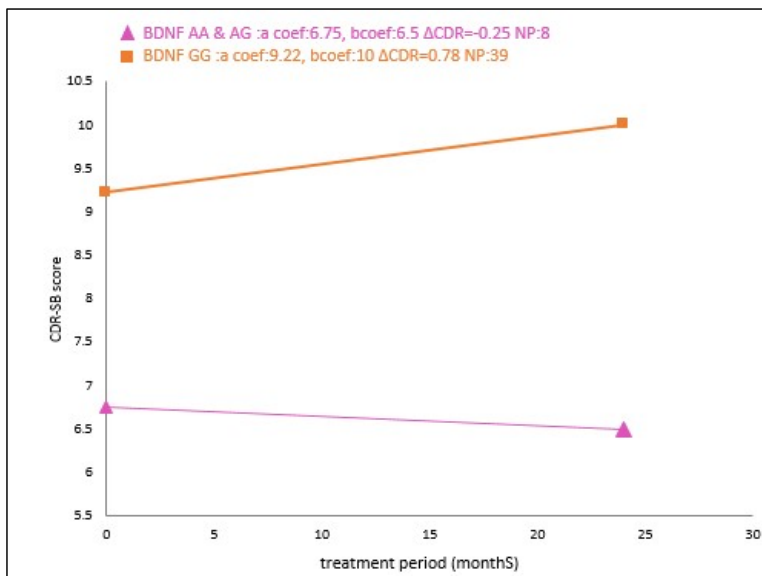


Figure 3: BDNF genotype-related therapeutic response to Rivastigmine therapy in 112 Iranian Alzheimer's disease patients. a coef.=y-intercept of the line; b coef.= x-intercept of the line.

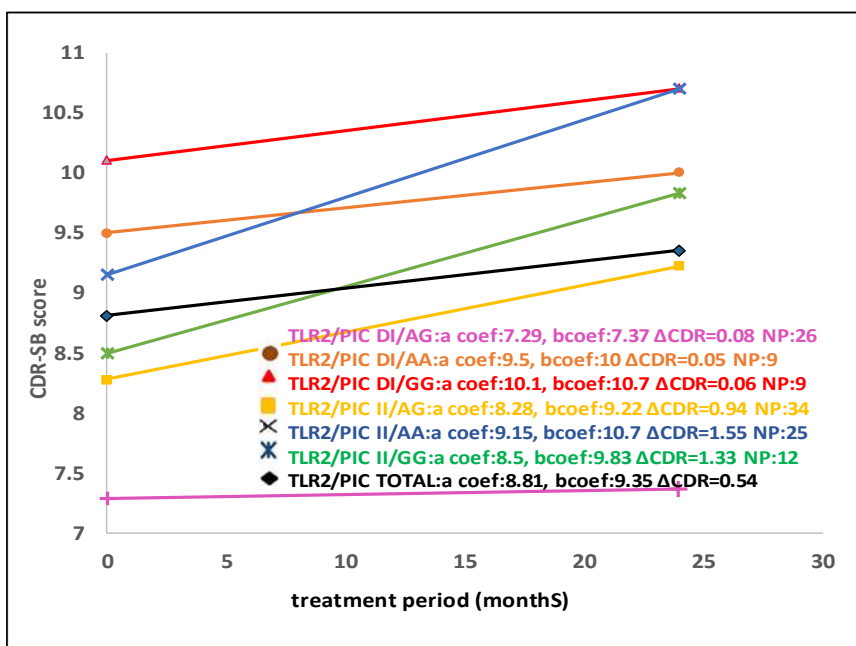


Figure 4: PICALM and TLR2 genotype-related therapeutic response to Rivastigmine therapy in 140 Iranian Alzheimer's disease patients. a coef.=y-intercept of the line; b coef.= x-intercept of the line.

good responders ($\Delta\text{CDR}=0.08$) and TLR2 II- PICALM AA genotype carriers were bad responders to Rivastigmine ($\Delta\text{CDR}=1.55$) (Figure 4). Moreover, we check out interactions between the genes related to this study and the genes studied previously in the same patients for response to Rivastigmine (43). The results of ΔCDR calculation show patients with B2-AR AX- A2M AX bigenic genotype indicated good response to Rivastigmine ($\Delta\text{CDR}=0.18$) and B2-AR GX- IL6 GX carriers were bad responder to Rivastigmine treatment ($\Delta\text{CDR}= 0.97$) (Figures 5 and 6).

Discussion

Alzheimer's disease is a complex and multifactorial disease with diverse genetic and environmental contributing factors [1,2]. Many studies indicate that this disease has genetic essence and having a first-degree relative with AD strengthens the risk of developing the disease as much as four times [40]. AD etiology was studied by many researchers who suggested that number of genes contributed to AD risk [5,9,10]. With regard to

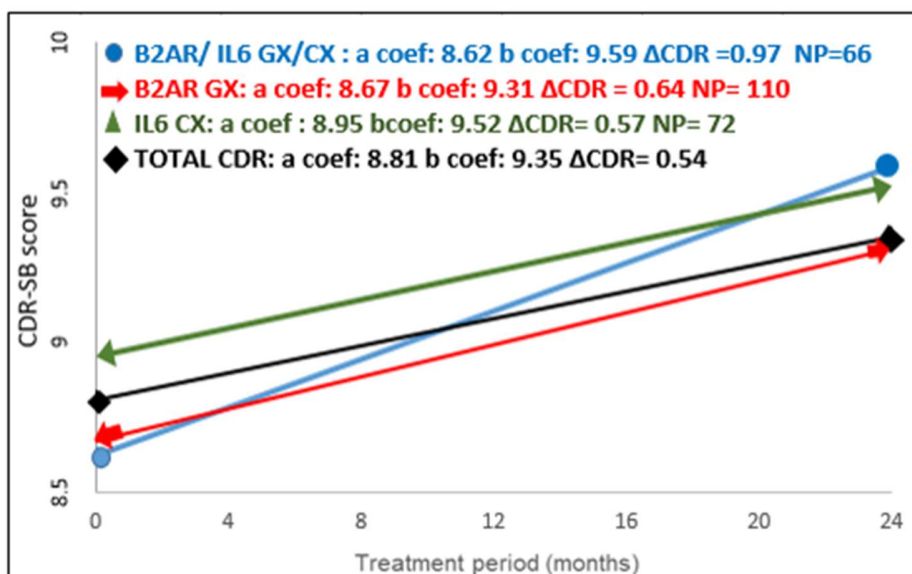


Figure 5: B2-AR and IL6 genotype-related therapeutic response to Rivastigmine therapy in 150 Iranian Alzheimer's disease patients. a coef.=y-intercept of the line; b coef. = x-intercept of the line.

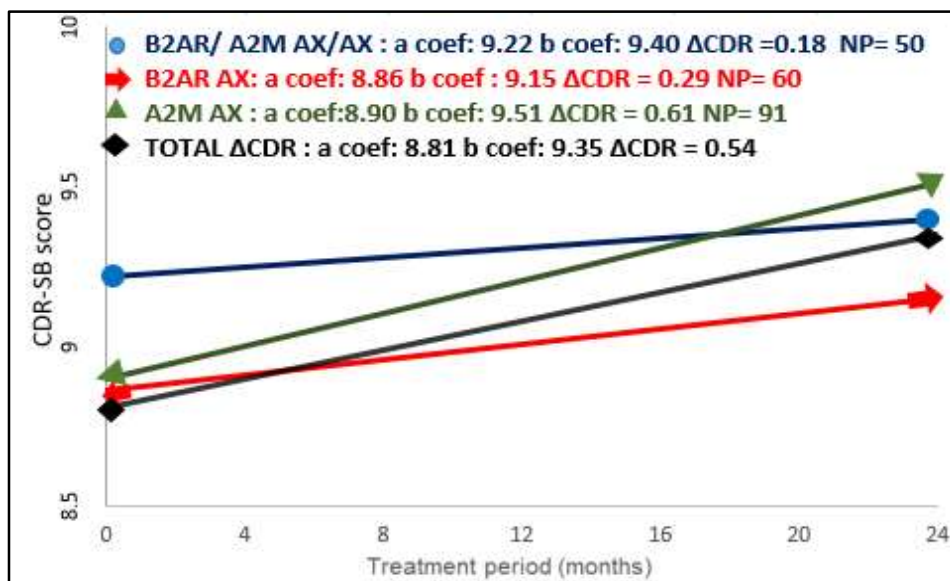


Figure 6: B2-AR and A2M genotype-related therapeutic response to Rivastigmine therapy in 150 Iranian Alzheimer's disease patients. a coef.=y-intercept of the line; b coef. = x-intercept of the line.

the complexity of the disease, with numerous possible associated gene mutations, it is highly probable that the underlying genetic predisposition to Alzheimer's disease could differ among various populations [5-7]. With the purpose of establishing the genetic profile of AD in the Iranian patients, we previously investigated the association of AD with several genes [10,41-43]. In the present study, the association of B2-AR, TLR2, PICALM and BDNF genotypes with the development of AD were examined. Furthermore, the effect of Rivastigmine therapy on Alzheimer's disease in the Iranian population in B2-AR, TLR2, PICALM and BDNF polymorphic genes was assessed.

Our statistical analyses indicate that between the total AD patients and the control group, there are no significant differences in the frequency of B2-AR alleles. But after stratifying the AD patients into FAD and SAD groups, a significantly positive association of B2-AR G allele ($P_c=0.02$, $RR=1.21$, $95\%CI=1.06-3.29$) and negative association of allele A ($P_c=0.02$, $RR=0.65$, $95\%CI=0.30-0.94$) with FAD was observed. After eliminating the effect of APOE ϵ_4 , those patients associated only with APOE ϵ_4 and not associated with second gene are deleted, by eliminating patients carrying APOE ϵ_4 the frequency of second associated gene (e.g. B2-AR GG) is increased and consequently association of second gene becomes more significant. In this study, after adjusting for the effect of APOE ϵ_4 , a significantly positive association was identified between B2-AR GG homozygote genotype and AD ($P_c=0.008$, $RR=2.25$, $95\%CI= (1.26-4.06)$). While B2-AR homozygote AA and heterozygote AG genotypes did not demonstrate any significant association with AD, this finding suggest that B2-AR G allelic gene is a recessive gene and predispose to AD in homozygote form of B2-AR GG genotype. Furthermore, association of B2-AR A and G alleles with AD become more significant when APOE ϵ_4 allele is excluded (G allele: $P_c=0.006$, $RR=1.90$, $95\%CI= 1.20-3.00$, and A allele: $P_c=0.006$, $RR=0.52$, $95\%CI= (0.33-0.83)$) (Table 2). Based on these results, therefore, we can conclude that the G allele of B2-AR impose predisposition while the A allele confers protection to AD. This finding is in line with the investigation performed in Chinese populations [14]. Increasing evidence has suggested that activation of B2-adrenergic receptors (B2-AR) enhances the gamma-secretase activity and in turn A β production. Several single nucleotide polymorphisms (SNPs) have been detected in B2-AR gene; one of them occurring at codons 16 (Gly or Arg). Many studies have observed that this SNP might alter cellular trafficking and receptor desensitization [15-17]. Abnormal activation of B2-AR gene might contribute to A β accumulation, one of the hallmarks of AD pathogenesis. Therefore, it can be hypothesized that A allele as a protective effect can enhance, while the G allele as a susceptibility force can reduce the agonist-mediated desensitization of B2-AR,

and hence potentially contribute to the pathogenesis of AD [44]. The patients carrying the Gly16 allele of B2-AR (the G allele) have enhanced responsiveness to endogenous norepinephrine which is progressively synthesized by the impact of environmental factors such as stress. Higher levels of norepinephrine provoke increased endocytosis particularly that of presenilin-1(PS1). Subsequently, the increased traffic of PS1 to the late endosomes and lysosomes will provide an optimal environment for γ -secretase activity which leads to more A β production. Therefore, individuals with Gly16 allele of B2-AR are more susceptible to AD [45-47].

Growing evidence shows that microglia, which are activated by Amyloid β peptides, play a key role in pathogenesis of AD [48,49]. Chen et al., in 2005 showed that activation of toll-like receptors (TLRs) on microglia promote cellular uptake of A β peptides. Also, TLR2 plays a potential role in stimulating the cellular response of microglia to the neurotrophic factors secreted in inflammatory lesions of the neuronal tissue. Interestingly, the results collected here show that none of the TLR2 alleles and genotypes have any impact on the pathogenesis of AD in the Iranian patient population. In contrast to our findings, several studies have shown that TLR2 genotype imposed susceptibility to AD [21,22]. While in other studies and in line with our findings, the authors did not find any association between TLR2 (-196 TO -174 del) polymorphism and AD [50]. Phosphatidylinositol binding Clathrin assembly protein (PICALM) encodes a protein that plays a role in collecting Clathrin. This gene contributes to the pathogenesis of AD through intervening the production, transportation, cleaning, and other pathways related to A β [26]. Our observations show no indications of significant differences in infrequencies of PICALM genotypes in total AD patients when compared to controls.

After eliminating the effect of APOE ϵ_4 gene, as compared with controls, the frequency of PICALM allele A and G in the Iranian AD patients was increased and decreased, respectively, but no statistically significant differences were observed. Opposite to this finding however, evidence of association between PICALM GG genotype and increased risk of AD in two studies on Brazilian (P -value=0.007) and Chins (P -value<0.05) patients were reported [51,52]. Interestingly, in another study conducted by Jiang et al. in Han Chinese, the association between the PICALM gene and Alzheimer's disease was not observed [53]. One of the notable events in the pathogenesis of AD is reduced expression of neurotrophic factors and their receptors, such as Brain-derived neurotrophic factor (BDNF) [27]. This factor promotes neuronal survival and regulates the proliferation and differentiation of nerve cells in the peripheral and central nervous systems. BDNF also facilitates the release of acetylcholine from the end of

hippocampal cholinergic neurons [54]. In agreement with the studies of Desai et al. [55] and He et al. [12], our results demonstrated that between the FAD, or SAD patients and controls, there are no significant differences in the frequencies of BDNF genotypes (Table 1 and 2); this observation is contrary to the study presented by Huang et al. [12]. Expectedly, in case-control studies with diverse populations, and small sample sizes, different and sometimes contradictory results were reported. Differences in ethnic origins in addition to the heterogeneity of AD disease may justify the discordance in results obtained from different populations in the world. For instance, Zamani et al. reported a significant difference in frequency and distribution of cystic fibrosis (CF) gene mutations between the population of Iran and those of its neighboring countries such as Pakistan, Turkey, and the Arab countries [56].

The effectiveness of testing for particular allele or genotype can be assessed by employing PcPPV formula [38]. The lifelong prevalence of a disease in a population is needed to measure the PcPPV in that population; the prevalence of AD is 2.3% in the general Iranian population [41]. The PcPPV of B2-AR G allele was 2.7% in FAD patients (Table 1), while after excluding APOE ϵ 4 allele, this number for the homozygous B2-AR GG increased to 3.5% (Table 2). This means that a person who carries the B2-AR GG genotype has a 3.5% absolute risk for developing AD, increasing the risk of AD to 1.5 times higher than what is calculated for the general Iranian population (2.23%). On the other hand, the PcPPV of testing for B2-AR A allele was 1.5% for all FAD patients in the Iranian population (Table 1). This result, therefore, strengthens the notion that B2-AR A allele plays a protective role in developing AD in the Iranian population.

In recent years, numbers of studies have investigated the response of Alzheimer's patients to Rivastigmine drug, in the context of certain allelic forms of APOE, IL6, A2M and TOMM40 genes. These studies suggested a genotype-specific response to the drug, facilitating a targeted and advantageously economical treatment for AD [10,36]. Interestingly, it has also been reported that only up to 50% of Alzheimer's patients responded in acceptable levels to acetylcholine esterase inhibitors [57] which is in line with the reported differential allelic effects on such response. Furthermore, research conducted in 2015 indicated that interactions between or independent combinations of different genes could also affect drug responses in AD patients; the presence of certain combined genotypes elicited the best responses, while others elicited the worst responses to Rivastigmine treatment [32]. In this study, we used the CDR-SB scale for determining the effectiveness of Rivastigmine in 140 Iranian Alzheimer's patients. The results after 24 months of follow-up treatment indicate that, as compared with total patients (Δ CDR=0.54), the patients carrying BDNF AA and AG genotypes had the

best response (Δ CDR=-.025), while the patients carrying genotype B2-AR AX had the worst response (Δ CDR=1.97) to Rivastigmine. From this finding, it can be speculated that impaired secretion of BDNF in carriers of AA allele could induce the levels of cholinesterases, hence reduced acetylcholine levels. Therefore, anticholinesterase drugs such as Rivastigmine could bring improvements in the memory and cognitive conditions of such patients. Likewise, considering the role of PICALM protein in endocytosis of acetylcholine in the synaptic regions, the positive response of PICALM AG carriers may have been reinforced by the impact of Rivastigmine. Finally, our data indicate that Alzheimer's patients with bigenic genotype TLR2 DI-PICALM AG (Δ CDR=0.08) respond positively, while carriers of TLR2 II-PICALM AA combined genotypes respond negatively to Rivastigmine therapy (Δ CDR=1.55). Also, according to the bigenic genotype response to Rivastigmine in relation to interaction between these genes and other genes studied in the same population before, patients with B2-AR AX- A2M AX genotype were the good responder (Δ CDR=0.18) and B2-AR GX- IL6 GX carriers were bad responder to Rivastigmine therapy (Δ CDR= 0.97) (Figures 5 and 6).

These findings suggest that in addition to the effect of single gene polymorphisms, gene-gene interactions or none interacting combination of number of genes might also impact the response of the Alzheimer patients to Rivastigmine therapy.

In Conclusion, our findings suggest that the B2-AR A allele plays a protective role in AD and patients with this allele respond positively to Rivastigmine treatment (Δ CDR=0.24). In contrast, B2-AR G allele imposes susceptibility for AD and the patients carrying this allele exhibited the worst response to Rivastigmine therapy (Δ CDR=0.81). Furthermore, after eliminating the effect of APOE ϵ 4 gene, as compared with controls, PICALM A allele exhibited positive ($P=0.05$, $RR=1.49$), while the G allele exhibited negative ($P=0.05$, $RR=1.05$) associations with AD in our selected Iranian patients. Also, AD Patients carrying the BDNF AX genotype revealed the best response to Rivastigmine therapy (Δ CDR=-0.25). Studies with larger and more diverse patient populations are required to be determined the exact role of B2-AR, TLR2, PICALM, and BDNF polymorphic genes and their possible contributing factors in Alzheimer's disease.

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Conflicts of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Author Contributions

Parvin Mohabattalab: Conceptualization, Methodology, Formal analysis, Writing - original draft, Visualization. Fatemeh Rezaei Rad: Writing - review & editing, Software, Form analysis. Hamid Zamani: Conceptualization, Methodology, Software, Form analysis, Resources, Writing. Fariba Shirvani: Writing - review & editing. Mahdi Zamani: Conceptualization, Methodology, Resources, Investigation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Compliance with Ethical Standards

This study was certified by the ethics committee and review board of the Tehran University of Medical Sciences.

Research Involving Human Participants and Informed consent

Questionnaire forms were prepared for each patient and control individuals. Before being included in the study, informed consent was taken from all the patients, or their legal guardians and control subjects.

Consent for Publication

All study participants, or their legal guardian, provided informed written consent for publication.

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