Relationship between Bleomycin hydrolase and Apolipoprptein E genes in Alzheimer patients in Northwest of Iran

Seyed Mahmoud Tabatabaei

Department of Physiology, Tabriz Branch, Islamic Azad University, Tabriz, Iran
E-mail: smt1351@yahoo.com; Tel: +98-914 116 53 19

Abstract: Background and Aims: Alzheimer's disease (AD) is the most common and devastating neurodegenerative disease of the elderly. Several studies indicated a relationship between different genes and AD. Bleomycin hydrolase (BLMH) and the Apolipoprotein E (ApoE) gene allele epsilon4 (E4) known as genetic risk factors for late-onset AD in sporadic cases. We have analyzed the association between the Apo E and BLMH polymorphisms in AD and control group in Northwest of Iran. **Methods:** EDTA blood from 62 AD and 65 controls were collected and DNA was extracted. The genes were amplified with SSCP-PCR for BLMH and RFLP-PCR for ApoE and allelic frequencies were performed. Then the results compared between AD cases and control group by chi- squared test. **Results:** Apo E4 and BLMH alleles frequencies are %8.33 Control, %18.33 AD and %20 Control, %36 AD respectively. **Conclusion**: According with our result, no association was observed between carrying the G allele of BLMH gene and AD in epsilon4 negative groups but carrying the epsilon4 allele is a dose-dependent risk factor for the AD and decrease the age of symptom onset ($p < 0.05$). Finally when considering the ApoE and BLMH polymorphisms alone, ApoE4 status is the best predictor of AD.
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1. Introduction

Alzheimer's disease (AD) the most common form of dementia is a disease of the brain that is characterized by the deposition of β-amyloid plaques and neurofibrillary tangles in selected regions of the brain (Braak et al 1998 and Price et al 1999). It destroys different brain cells, causing problems with memory, thinking and behavior. Major risk factors for the development of AD include age, gender, nutrition and genetic factors such as Bleomycin hydrolase (BLMH) and Apolipoprotein E gene allele E4(epsilon4) (Morris et al 2003).

It is well known that BLMH a cysteine protease belonging to the Papain super family.This protein plays an important role in production and secretion of beta-amyloid proteins. BLMH encoding gene contains 12 exons with different sizes ranged from 69 to 198 base pairs that are separated by introns about 1 kb. This gene locus is located on the long arm of chromosome 17 (17q11.1-11.2) (Namba et al 1999).

1430 A>G nucleotide polymorphism, which finally leads to the displacement of I443V amino acid, is affected at the end of the c-terminal of this enzyme. Bromme et al. in 1996 using the technique of PCR-SSCP, identified a polymorphism at 1430 BLMH gene locus that alter A>G which would change isoleucine to valine (I443V) in the sequence of amino acids at 443 residue. It is said that BLMH is a candidate for beta secretase that may be involved in analyzing beta amyloid from amyloid beta precursor protein (APP), the same substance that is associated with AD (Bromme et al 1996).

Also Apolipoprotein E as a cholesterol transport protein in cell biology (Hooijmans et al 2008 and Wahrle et al 2004), it plays a prominent role in the redistribution of cholesterol to the neurites for membrane biosynthesis during the axon and to the Schwann cells for myelin formation. ApoE is a multifunctional lipoprotein consisting of 299 amino acids, synthesized in various organs, including liver, spleen, kidney and brain (Mahley et al 1988).

Existence of Apolipoprotein E4 allele into other forms of alleles (E2 and E3) increased the possibility of getting Alzheimer's disease and also decreased the age of getting the disease.The risk of getting Alzheimer's disease for individuals that don't carry E4 allele is about 10%, while individuals carrying one allele from E4 is approximately 30% (Seshadri et al 2005).On the other hand people with ApoE2 allele, have the late-onset of disease (Corder et al 1994); however, the protective role of ApoE2 allele is not very clear yet. It has been shown that one of the two isoforms of BLMH (BLMH-val443) is associated with an increased risk of sporadic AD in non-ApoE4 patients (Montoya et al 1998).

In this study we try to show the relationship between ApoE gene polymorphism and BLMH; which two candidate genes for AD. So we compared 62 affected people of AD, with 65 control cases in Northwest of country.

2. MATERIALS AND METHODS

2.1. Participants

The patient group consisted of 62 patients (29 women and 33 men) from Northwest area of Iran who were referred from Neurologists with DSM IV criteria of AD. Also the control group consisted of 65 (30 women and 35 men) with no personal history of psychiatric or neurological abnormalities from this area.

2.2. Blood samples

Peripheral blood obtained with vacuum system and DNA extracted from White Blood Cells with using QIAamp DNA Blood Mini Kit (Qiagen; Catalog number: 56304).

The used method for amplification and study of BLMH gene is SSCP-PCR and RFLP-PCR for Apo E gene.

2.3. The primers used

The characteristics of primers used for PCR to examine BLMH-I344V and ApoE genes polymorphism are shown in Table1.

Table 1 - Primers used for PCR to examine BLMH and APOE genes polymorphism

Primer's name	Sequence
BLMH- F	5`CCTGGATCTGTCCTTTGCAGCTACG3`
BLMH- R	3`GGAAGCATGTCCCTGAAGAGGTGC5`
APOE- F	5`GGCACGGCTGTCCAAGGAGCTGCAG3`
APOE-R	5`CCCCGGCCTGGTACACTGCCAGGC3`

2.4. PCR condition

The steps of PCR reaction for amplification of BLMH and APOE genes are shown in Table 2 (Montoya et al 1998).

Table 2- Steps of PCR, temperatures and durations for amplification of BLMH and APOE genes

APOE GENE			
Step	$^{\circ}C$ Temperature	Time	Cycle No.
Initial Denaturation	95	10 Min.	1
Denaturation	95	30 Sec.	32
Annealing	56	30 Sec.	
Extension	72	1 Min.	
Final Extension	72	5 Min.	1
BLMH GENE			
Step	($^{\circ}C$) Temperature	Time	Cycle No.
Initial Denaturation	95	10 Min.	1
Denaturation	95	30 Sec.	35
Annealing	67	30 Sec.	
Extension	72	1 Min.	
Final Extension	72	10 Min.	1

2.5. Statistical analysis

We studied this hypothesis that, "what is the relationship between ApoE4 and BLMH genes and existence of this two gene mutations in Alzheimer's disease?" Chi-Squared test is used in order to analysis data related to this hypothesis.

3. Results

The sequence of BLMH gene 1430 A> G Polymorphism has different structures in AD compared with normal individuals. With using the Chi-Squared test and according to Table 3 significant differences is observed between groups and sequence of BLMH gene 1430 A> G polymorphism has different structures in AD compared with normal individuals.

Table 3- Relationship between presence of BLMH and ApoE ε4 in AD and control cases.

		ApoE 4-carriers		ApoE 4 non-carriers		All Participants
		AD	Control	AD	Control	
BLMH Genotype	AA (%)	(10%)	(6.67%)	(40%)	(53%)	(55%)
	GG (%)	(10%)	0	(13.33%)	0	(11.67%)
	AG (%)	(6.67%)	(3.33%)	(20%)	33.33%)	(33.34%)
Allele Frequencies	A	16.67%)	(8.33%)	(50%)	71.67%)	(71.66%)
	G	(13.33%)	(1.67%)	(23.33%)	18.33%)	(28.33%)

There was significant relationship between this two gene mutations in people with Alzheimer's disease and this indicated that a combination of ApoE4 and BLMH in this group is not identical according to its variants, as existence of E4 allele along with a low incidence of G allele($P<0.01$).

4. Discussion:

Findings of our study showed that both Alzheimer's disease group and control group have a different structure in BLMH gene 1430 A>G and ApoE gene polymorphism. In explaining of these findings it can be stated that BLMH is a candidate for beta secretase that may be involved in analyzing beta amyloid fragment from amyloid precursor protein (APP), the same substance that is associated with Alzheimer's disease. G/G genotype of A1430G polymorphism in this gene have a great effect on the progression of Alzheimer's disease. Increased risk due to homozygosis for the genotype G/G is limited to people who do not carry the ApoE4 allele.

Another interesting point is that the various studies, including results of Montoya et al. in 1998 and Ferrando et al. in1996 reported the inhibitory effect of genotype G/G from 1430 A>G polymorphism in individuals who are carriers of ApoE4 (Ferrando et al 1996). These findings are

conformed to the present findings. It seems that the absences of the ApoE4 allele and BLMH in a person deprive him/her from living and now during the evolution just the people can survive that have at least one of two alleles. Another important point in the presence of G allele in Alzheimer's patients with ApoE4 genotype is that, maybe somehow during the genetic evolution this issue is missed that the presence of two genes, which predisposing Alzheimer's disease, causes rapid death of individuals carrying these two genes.

Other findings indicated that there is a relationship between ApoE4, BLMH genes and the mutations of them. In this regard, several studies such as of Montoya et al. in 1998 showed that the frequency of G/G homozygous in Alzheimer patients than control subjects is significantly higher in the group without ApoE4 (15.9% of Alzheimer cases versus 4.7 % of control cases) (Montoya et al 1998). These findings are aligned with the findings of present research, so that the most frequent genotype among Alzheimer subjects is related to the G/G genotype but without ApoE4 allele. Also findings related to the states of cognitive functions is different in Alzheimer's disease according to the BLMH gene1430 A>G polymorphism showed that the cognitive status of Alzheimer's disease according to levels of BLMH gene1430 A>G polymorphism was not significantly different and on the other hand there is no significant correlation between the BLMH genotype and cognitive states.

In the explanation of these findings it can be pronounced that BLMH gene isn't directly involved in causing Alzheimer's disease and the protein production of this gene causes the Alzheimer's disease by involving in the many cascade pathways of amyloid precursor protein (APP) production and without beta-amyloid degradation (βA).

Conflict of interests

Authors declare no conflict of interests.

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