



Investigation of the Relationship Between Polymorphism in miR-34b/c and Risk of Alzheimer's Disease

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Abstract

The significant role of microRNAs (miRNAs) in gene expression regulation has been demonstrated. Changes in the expression of miRNAs have been reported in a variety of neurological diseases. However, the role of miR-34b/c in the risk of Alzheimer's disease has been less studied. Therefore, the current study aims to investigate the relationship between miR-34b/c polymorphism and the risk of Alzheimer's disease. This study was performed with 40 Alzheimer's patients and 40 controls. Using the polymerase chain reaction technique to investigate polymorphisms in the miR-34b/c gene. The results of this study indicated an association between miR-34 b/c polymorphisms and the risk of Alzheimer's disease.

Keywords: Polymorphism, miR-34b/c, Alzheimer's Disease

1. Background

The most frequent and well-known cause of dementia is Alzheimer's disease (AD). Alzheimer's disease is an incurable and degenerative brain disease that affects cognitive function, personality, thought content, perception, and behavior. Alzheimer's disease is the fourth leading cause of death worldwide (1). One of the demanding disorders is Alzheimer's disease (AD) which is caused by the buildup of extracellular amyloid deposits (senile plaques; SP) and intracellular inclusions (neurofibrillary tangles; NFT) in separate areas of the basal forebrain, hippocampus, and association cortices (2, 3). Four Risk alleles have been identified for over 500 genes with susceptible genetic variations (4). The disease is estimated to affect 5 to 10 percent of people aged 65 and over (5). Dementia affects roughly 7.5 persons per 1,000 people each year (6). Dementia rates rise with age, from one in every 1,000 individuals per year in their 60s and 70s to more than 70 per 1,000 people a year in their 90s. Alzheimer's disease is a multifactorial disorder caused by a combination of genetic and environmental factors. The most significant determinant of Alzheimer's disease is age. However, gene mutations were observed in some patients. The high association between Alzheimer's disease and age may reflect the cumulative effect of life-threatening factors to some extent (7).

One of the main methods of regulating genetic processes is through mechanisms related to miRNA. MicroRNAs are small RNA (22 nucleotides), and single-strand molecules converted from Pri-miRNA to pre-miRNA, then eventually converted to microRNA. MicroRNA is a complement to mRNA, a protein-encoding gene, that can prevent the expression of a gene and the production of related proteins (8). It is estimated that these molecules regulate the expression of one-third of all genes (9, 10). One type of microRNA can regulate the expression of hundreds of proteins. Therefore, much research has been designed on the role of these molecules in various diseases including AD (11). Particular miRNAs are expressed in the central nervous system (CNS). Numerous studies have reported impaired regulation of the miRNAs in the brains of AD patients (12). Therefore, some microRNAs can be used as biomarkers in AD diagnosis.

MiR-34a, miR-34b, and miR-34c are members of the miRNA-34 family, and much research has been conducted on the miRNA-34 family (13). It has been discovered that this microRNA plays an important role in two signaling pathways: Bcl2 for cell survival/apoptosis and SIRT1 deacetylase for p53 or neuroprotection signaling (14, 15). Previous studies have shown changes in the expression of miRNAs and changes in the central nervous system during

aging (16). In animal models in which AD was induced, an increase in miR-34a expression levels was observed in the mice's brains (17). MiR-34c was also found in the hippocampal area of the animal models, which was associated with cognitive decline. It has also been shown that inhibition of this miRNA eliminates memory deficits in AD mice (18). Several studies have found that differing miR-34b/c (T-C replacement) alleles in the Pri-miR34b/c promoter area can impact miR-34b/c expression through genetic and epigenetic pathways, impacting a person's susceptibility to several disorders (19). However, according to the literature reviews, the relationship between miR-34b/c polymorphism and sensitivity to Alzheimer's has not been reported.

2. Objectives

The main aim of the present study is to investigate the polymorphism in miR-34b/c in Alzheimer's patients.

3. Methods

3.1. Subjects

In this study, 40 diagnosed Alzheimer's patients (ages 55 - 90), and 100 healthy people without cognitive impairment (ages 60 - 91), were registered. The latter group scored less than 4 on the test of Subjective Memory Scale of the Schmand and did not have any medical or neurological disease (20). Diagnosis of Alzheimer's disease was made using standardized NINCDS-ADRDS criteria (21) and this was matched with the recently corrected criteria for AD (22). The salting-out technique was also used to extract genomic DNA from patients' blood (23).

3.2. Genotyping

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to genotype the miR-34b/c polymorphism (24). The forward and reverse sequences for miR-34b/c are shown in Table 1:

Table 1. Forward and Reverse Sequences for miR-34b/c

Primers	Primer Sequence 5' → 3'	Length (nt)
Forward allele C	GGGAACCTTCTTGACCTATC	21
Reverse allele C	GTAGCCTCTGACATATGGGA	20
Forward allele T	GGGAACCTTCTTGACCTATT	21
Reverse allele T	GTTAGTTACATCAAAATCCGTGGT	22

3.3. Statistical Analysis

For statistical analysis, the MedCalc software (20.1) was used. χ^2 test was used to evaluate the association of candidate alleles with Alzheimer's disease. Also, the odds ratio (OR) and 95% confidence interval (CI) were determined. The significant level was considered to be $P < 0.05$.

4. Results

Polymorphisms of the miR-34b/c for 40 Alzheimer's patients (23 males, 17 females) and 100 controls (44 males and 56 females) were determined successfully. In the patient group, 15 samples had the homozygous TT genotype, 20 samples had the heterozygous CT genotype and 5 samples had the CC genotype. The control group had 51 samples with TT genotype, 43 samples with heterozygous CT genotype, and 6 samples with CC genotype. In general, in the patient group, CT genotype, and the control group, TT genotype had the highest frequency. The lowest frequency in both groups belonged to the CC genotype.

Applying MedCalc software (20.1), the amount of $\chi^2 = 6.40$ and $P = 0.05$ was determined in the chi-square test. In other words, there was a significant difference in genotypic distribution between the two groups. According to statistical analysis, people carrying the heterozygous genotype (CT) are approximately 2.29 times more likely to develop Alzheimer's disease than those with the TT genotype. Statistical analysis showed that the C allele increased the risk of Alzheimer's disease by 1.8 times. To identify allele C, amplification of 314 bp fragments, and to identify allele T, amplification of 170 bp fragments was considered in 2% agarose gel (Figure 1). The results of allelic and genotypic polymorphism frequencies in the miR-34b/c gene in Alzheimer's patients and control are given in Table 2. The results showed that the polymorphism in the miR-34b/c inheritance models had no relationship with the risk of Alzheimer's disease. In the current experiment, OR and 95% CI adjusted for genes and age were also calculated. The findings suggest that polymorphism in the miR-34b/c has no link to the risk of Alzheimer's disease (Table 2).

5. Discussion

Particular miRNAs have been reported to be expressed in the central nervous system (CNS), that they regulate differentiation, neurite outgrowth, and synaptic plasticity. Several studies have reported, that there is a dysregulation in miRNAs in the brains of Alzheimer's patients (12). Although the current study's findings indicated that polymorphisms in the miR-34b/c gene were not related to an increased risk of Alzheimer's disease. The significance of

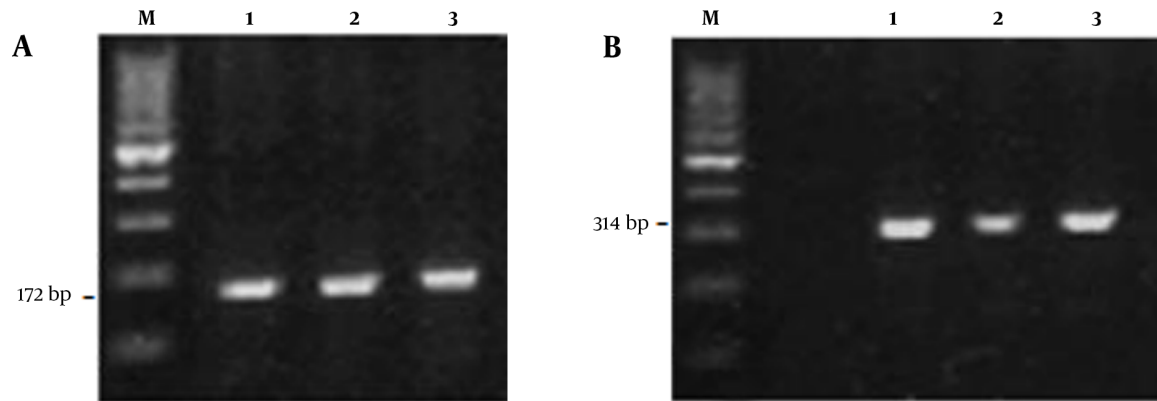


Figure 1. Genotypic polymorphism in the miR-34b/c gene in Alzheimer's patients. A, PCR product with T allele primers; B, PCR product with C allele primers.

Table 2. Allelic and Genotypic Polymorphism Frequencies in the miR-34b/c Gene in Alzheimer's Patients and Control

Polymorphism	Case (%)	Control (%)	OR (95%CI)	P ^a	OR ^b	P ^c
Co-dominant						
TT	15 (38)	51 (51)	1.00	-	1.00	-
TC	20 (50)	43 (43)	1.04 (0.64 - 1.76)	0.815	1.03 (0.62 - 1.73)	0.885
CC	5 (12)	6 (6)	2.10 (0.89 - 4.96)	0.112	2.04 (0.82 - 4.89)	0.106
Dominant						
TT	15 (38)	51 (51)	1.00	-	1.00	-
TC + CC	25 (65)	49 (49)	1.28 (0.68 - 2.05)	0.385	1.26 (0.69 - 2.04)	0.455
Recessive						
TT + TC	35 (89)	38 (95)	1.00	-	1.00	0
CC	5 (12)	6 (6)	0.88 (0.52 - 0.142)	0.099	1.99 (0.93 - 4.53)	0.112
Over dominant						
TT + CC	20 (50)	19 (47.5)	1.00	-	1.00	-
TC	20 (50)	21 (52.5)	0.91 (0.55 - 1.47)	0.775	1.12 (0.71 - 1.82)	0.845
Allele						
T	50 (62.5)	145 (70)	1.00	-	-	-
C	30 (37)	55 (30)	1.22 (0.85 - 1.75)	0.168	-	-

^a Significance level for allele and genotype frequencies in cases and controls.

^b Adjusted for sex and age.

^c Allele and genotype frequencies in cases and controls were compared using χ^2 test.

miR-34 family members in a variety of physiological processes has been established. In AD, the miR-34 appears to regulate p53 expression, and this is related to tau phosphorylation (25). This microRNA has also been detected in the hippocampus of AD patients, as well as in AD animal models. Interestingly, it has been shown that reducing the expression of miR-34 rescued some cognitive abilities in these patients (18). These studies indicate the role of miR-34 in Alzheimer's disease, which has been shown

to increase expression in these patients. According to the current research, an association was observed between different variants of the miR-34b/c and AD, which indicates a direct relationship between miR-34 b/c polymorphism and the risk of Alzheimer's disease. Important targets for miR-34 are NDUFC2, SDHC, UQCRB, UQCRCQ, and COX10 genes, which are components of the electron transport chain and have been reported to be induced in AD patients. Decreased expression of these genes is also associated with

decreased mitochondrial function in AD patients (26).

The polymorphism of miR-34b/c has not been related to an enhanced risk of breast cancer (22) or retinoblastoma (25). In addition, other research has related this polymorphism to an elevated risk of papillary thyroid carcinoma (PTC) (26) and nasopharyngeal carcinoma (NC) (19). However, the current study show association between the miR-34b/c polymorphism and Alzheimer's disease. Although, the risk of Alzheimer's disease can be elevated depending on race, genetics, environmental factors, and the gene's response to diet. In general, the results of this case-control study suggest miR-34b/c Alzheimer's disease polymorphism. Accordingly, it can be considered a risk factor for Alzheimer's disease in the study population. Although this polymorphism is critical in Alzheimer's disease, more extensive studies in different communities are needed.

Footnotes

Authors' Contribution: Z. S. conceived and designed the evaluation of the manuscript. Z. S. participated in designing the evaluation, M. D.A.P. and H. B. performed the statistical analysis and helped to draft the manuscript. Z. S. re-evaluated the clinical data, A. G. collected the clinical data, interpreted them, and revised the manuscript. M. D.A.P. and H. B. re-analyzed the clinical and statistical data and revised the manuscript. All authors read and approved the final manuscript.

Conflict of Interests: The authors declare no conflict of interest in this study.

Data Reproducibility: The data presented in this study are uploaded during submission as a supplementary file and are openly available for readers upon request.

Ethical Approval: This study was conducted in 2014 and at that time the code of ethics was not issued

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