

Short Communication



## Neurofilament heavy chain gene polymorphism and risk of multiple sclerosis

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### Abstract

**Background and aims:** Multiple sclerosis (MS) is a chronic disease characterized by inflammation and degeneration of the central nervous system (CNS). The high level of neurofilament (NF) heavy chain (NEFH) in cerebrospinal fluid (CSF) has been reported to be associated with MS. The aim of the present study was to determine the association between *NEFH* rs3815335 polymorphism and risk of MS.

**Materials and Methods:** A total of 40 MS patients and 40 controls were genotyped by polymerase chain reaction (PCR) and Sanger sequencing. Genotypic and allelic distributions were compared between cases and controls. Fisher's exact test was used to estimate the risk of MS based on genotypes in SPSS version 22.0.

**Results:** No significant difference was observed in the distribution of *NEFH* genotypes and allele frequencies between patients and controls ( $P=0.737$ ).

**Conclusion:** Our data indicated that *NEFH*, 1084-244G>A gene polymorphism, has no significant association with the susceptibility or severity of MS in Iranian patients. Further prospective studies are required for confirmation.

**Keywords:** Multiple sclerosis, *NEFH*, polymorphism

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### Introduction

Multiple sclerosis (MS), which is known as a chronic disease, leads to damage and ultimately loss of myelin in the central nervous system (CNS) and neurologic disability. It is more prevalent in women than in men and it is more common in younger people (20-40 years) (1). MS classification is as follows: progressive relapsing MS (PRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), and relapsing-remitting MS (RRMS) (2). MS symptoms include bladder and bowel dysfunction, dizziness, weakness, visual disorders, and memory problems (3).

Genetic susceptibility, some viral infections like Epstein-Barr infection, deficiency of vitamin D, lack of sunlight exposure, stress, and smoking are risk factors of MS (1). Vital elements of MS pathology include axonal loss and neurodegeneration; therefore, a valid biomarker to detect therapeutic responses would be valuable (4). An

established biomarker for neurodegeneration and early stages of MS is neurofilament (NF) level in body fluid which is a specific cytoskeletal protein and major structural element of neurons. Several previous studies have shown that damaged axon releases NF-H and NF level increases in the cerebrospinal fluid (CSF) of MS patients (5). For predicting, monitoring the process of disease, and assessing the efficacy or toxicity of future neuroprotective treatment strategies of diseases such as MS, blood and CSF levels of NF play important roles. Damage to axons of the CNS or peripheral nervous system was found to cause an increase in the level of NFs in the CSF and the bloodstream; therefore, the quantification of NF in body fluids can indicate the amount of neuroaxonal damage caused (6).

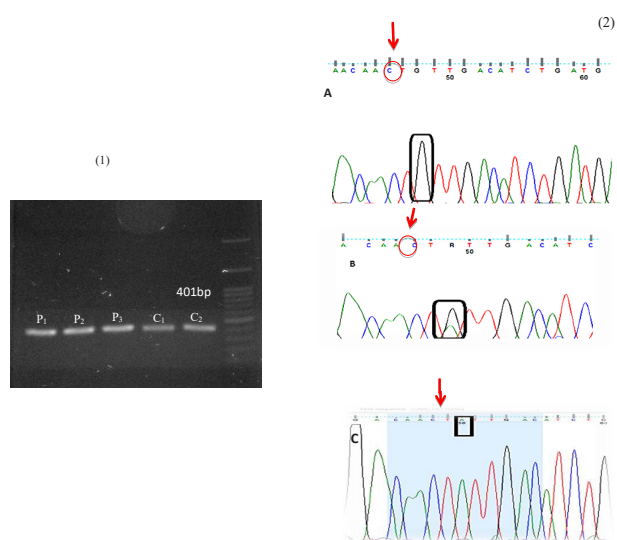
The aim of the present research was to investigate the association of *NEFH* 1084-244G>A polymorphism with the risk of MS.

For the present case-control study, 40 relapsing-remitting MS (RRMS) patients and 40 healthy individuals were selected. They were 20 to 47 years of age and did not suffer from other neurological disorders. The subjects were selected from the patients referred to Dr. Alaie's office from June to September 2017. The subjects included in the study were asked to provide their written informed consent and all of them were required to be Iranian. Genomic DNA was extracted from peripheral blood.

The selected gene polymorphism was analyzed by polymerase chain reaction (PCR) and direct sequencing technique. The forward and reverse primers were designed using PerlPrimer software. The sequences of primers were as follows: Forward: TCACTGAGCACTTTCTAAGCTC and Reverse: CAGGTCTGGTATTCTCGC. The thermal gradient was as follows: initial denaturation at 95°C for 3 minutes, followed by 38 cycles of denaturation at 95°C for 30 seconds, annealing at 56.5°C for 30 seconds, extension at 72°C for 1 minute, and final elongation at 72°C for 5 minutes. PCR product size was 401 bp and it was visualized on 0.8% agarose gel electrophoresis (Figure 1). Additionally, it was sequenced by the Sanger protocol and analyzed for genotypes using the FinchTV software. The differences in alleles and genotypes between cases and controls were compared by chi-square and Fisher's exact tests, using SPSS version 25.0 software. A *P* value of less than 0.05 was considered statistically significant. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated to evaluate the associations. Gender and age were included as covariates.

## Results

### 1084-244 G>A polymorphism (rs3815335) in RRMS



**Figure 1.** (1) Agarose gel Electrophoresis for PCR Amplification of *NEFH* Gene. (DNA ladder (100bp); lane P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub> represent case; lane C<sub>1</sub> and C<sub>2</sub> represent healthy control individuals), (2) Results of Direct Sequencing of *NEFH* Gene. (A: Homozygote healthy mode, B: Heterozygote state, C: Homozygous patients)

subjects (n=40) and healthy controls (n=40) was genotyped. The mean age of patients and control were 32.65 and 36.68 at the time of sample collection. Rs3815335 polymorphism and its association with MS was examined by PCR and Sanger sequencing method (Figure 1).

The genotype and allele frequencies of *NEFH* gene are shown in Table 1. The analysis illustrates that the most commonly observed genotype among case and control groups was GG, and the frequency of mutant genotype (AA) among the subjects was found to be low.

The frequencies of the AA, AG and GG genotypes were 2.5, 12.5, and 85%, respectively, among cases and 0, 10, and 90%, respectively, among controls. GG genotype is by far the most frequently observed genotype among the case and control groups. The results showed that there were no significant differences in the distribution of *NEFH* genotypes and allele frequencies between RRMS patients and controls, and the analysis showed that there was no significant association between RRMS and 1084-244G>A (rs3815335) polymorphism of *NEFH* gene in MS patients (*P*=0.737) (Table 1). Gender and age as covariates were analyzed and results illustrated that there was no significant association between the age (*P*=0.816) and gender (*P*=0.237) of subjects (cases and controls) and examined gene polymorphism (Table 1).

## Discussion

Finding reliable biomarkers and prognostic factors for MS, especially at the initiation of the disease, can facilitate therapy decision-making and timely diagnosis; therefore, valid biomarkers are very important and have a vital role. One of the sensitive and measurable biomarkers for some neurological diseases such as Alzheimer's, amyotrophic lateral sclerosis (ALS), and head and spinal cord trauma is the level of NF (NF-L and NF-H) (7), which is reported to be higher in both CSF and serum samples of MS patients compared to non-MS subjects (8). Therefore, we can use them as prognostic and predictive biomarkers in RRMS (9). Based on this information, in the present study, we examined the association between the polymorphism of the *NEFH* gene and the risk of RRMS for the first time in Iranian patients. The *NEFH* gene (11199 bp) is located at 22q12.2 and consists of 4 exons and 3 introns. rs3815335 which is located at the intronic region leads to the conversion of guanine to adenine.

Several previous studies indicate that Neurofilament Heavy-Chain NfH<sup>SMI35</sup> in CSF supports the differential diagnosis of Parkinson Syndromes (10). A previous study by Dujmovic et al showed that CSF NFH could be a prognostic biomarker of Guillain-Barre syndrome (11). In another study, increased levels of CSF neurofilament heavy chain in neuromyelitis Optica (12) and *NEFH* protein levels in schizophrenia (13) have been shown.

A previous study suggested that NfL is superior to NfH (SMI) as therapeutic biomarker and it is a

Table 1. Allele distribution, genotype frequency, and statistical analysis of *NEFH* rs3815335 polymorphism among RRMS patients versus control group

| 1084-244G>A |            | Group      |            | P value <sup>a</sup> | P value <sup>b</sup>         | OR    | 95% CI for OR |       |
|-------------|------------|------------|------------|----------------------|------------------------------|-------|---------------|-------|
|             |            | MS patient | Control    |                      |                              |       | Lower         | Upper |
| Genotype    | GG         | 34 (85%)   | 36 (90%)   |                      | Reference                    | 1     | -             | -     |
|             | GA         | 5 (12.5%)  | 4 (10%)    | 0.737                | 0.737                        | 1.324 | 0.328         | 5.345 |
|             | AA         | 1 (2.5%)   | 0 (0%)     |                      | 0.493                        | 1.029 | 0.973         | 1.090 |
| Allele      | G (ref)    | 73(91.25%) | 76(95%)    |                      | ( $\chi^2$ test)<br>0.349    | 1.822 | 0.512         | 6.486 |
|             | A          | 7(8.75%)   | 4(5%)      |                      |                              |       |               |       |
| Age         | <35        | 26 (65%)   | 25 (62.5%) |                      | (Binary logistic regression) | 1.114 | 0.448         | 2.774 |
|             | >35 (ref)  | 14 (35%)   | 15 (37.5%) |                      | 0.816                        |       |               |       |
| Gender      | Male (ref) | 11 (27.5%) | 16 (40%)   |                      | ( $\chi^2$ test)<br>0.237    | 1.758 | 0.687         | 4.495 |
|             | Female     | 29 (72.5%) | 24 (60%)   |                      |                              |       |               |       |

<sup>a</sup> Fisher's exact test.

<sup>b</sup> Chi-square test.

promising candidate for measuring neuroaxonal damage in MS treatment trials (7). Another study showed that routine measurement of serum pNF-H should be further investigated for monitoring axonal injury in MS (14).

In addition, Shehab et al reported that pNF-H is a promising marker of MS disease activity and disability assessment (15). Another study has shown that the evaluation of NfH (SMI35) levels is likely to provide a useful tool for measuring the rate of neurodegeneration in MS (9). However, in our recent study, we have identified that *NEFH* gene polymorphism was not associated with susceptibility to MS in our patients. These differences may be due to the ethnic variations of *NEFH* gene polymorphisms. Although the A/G ratio at rs3815335 appeared to be too low compared with other studies, the present data may provide useful information about this SNP. In our study, we have illustrated that rs3815335 of *NEFH* gene has no association with susceptibility to RRMS in MS patients. In addition, no significant association between *NEFH* genotype or allele frequencies and MS risk was observed. However, further prospective studies are required to confirm the findings of this study and as it is a preliminary study and is presenting data for future comprehensive study for making a clinical conclusion, further analysis of other SNPs in the *NF* gene would be needed in future studies.

### Conclusion

We concluded that rs3815335 polymorphism of *NEFH* gene in position 1084-244G>A did not show a statistically significant association with the risk of MS and no differences were found in allele frequency between cases and controls for this SNP.

### Conflict of Interests

There is not any conflict of interests.

### Ethical Approval

This study was conducted in accordance with the principles of the

Declaration of Helsinki and then its protocol was approved by the Ethics Committee of Royan Institute for Reproductive Biomedicine (IR.ACECR.ROYAN.REC.1396.97).

### Authors Contribution

SASF; Contributed to conception and design, responsible for overall supervision. EGJ; Contributed to all experimental works, and interpretation of data. SA; Selected MS patients for this study. SPCT; Collaborated on interpretation of data, Date analysis, Drafted revised and submitted manuscript. ZS; Statistical and data analysis. All authors read and approved the final manuscript.

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