Analysis of Association Between Genetic Heterogeneity of Interferon Beta Receptor Promoter and Therapeutic Response to Interferon-beta in a Patient with Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is an autoimmune disease characterized by inflammatory neuronal damages and consequent disabilities. Episodic relapses of the disease could be decreased by the interferon-beta (IFN-β) therapy in most MS patients. However, the drug response's efficiency is variable among patients, and the precise mechanism of action of the IFN-β is not clear. This study aimed to investigate the interferon beta-receptor (IFNAR) promoter polymorphisms on response to interferon beta in MS patients. Patients were divided into either responding (n = 57) or non-responding (n = 43) groups according to interferon beta treatment and Expanded Disability Status Scale score. The Sanger sequencing method is used for genotyping. Here, we found a significant association between 65 SNP in responders and non-responders to interferon beta (p-value < 0.05). The results also showed a significant difference between the two groups of responders and non-responders to the treatment in the presence or absence of insertion before GT repeat dinucleotide microsatellite (p-value < 0.02). The present study's obtained results suggested the genetic heterogeneity in the promoter region of IFNAR can affect response to IFN-β. However, more studies with a larger sample size are needed to demonstrate this relationship further.

1. Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the white matter of the central nervous system (CNS), affecting about 2.5 million people worldwide (1). This incapacitating disease of the CNS usually follows a waxing and waning course over many years before advanced disability supervenes (2). It is hypothesized that loss of axons is the main mechanism underlying progressive disability (3). MS is more frequent in women than men in a 3:1 ratio, but the disease's severity is higher in men (4). It clinically manifests itself with multiple neurological dysfunctions (e.g., visual and sensory disturbances, bilateral Babinski signs, limb weakness, gait problems and bladder, and bowel symptoms) followed by recovery or growing disability because of irreversible functional disability over time (5). It is assumed that environmental and genetic factors are effective in multiple sclerosis, although in genetic factors, immunological factors have been implicated in the pathophysiology (5). Hence, there is a rationale for considering immunotherapy by using disease-modifying drugs (e.g., IFN-β) as the first-line therapy for relapsing-remitting MS (RRMS), the most common form of MS (6).

Various studies showed that IFN-β production decreases in people with MS as the disease progresses. Hence, the production of the IFN-β-stimulating gene is limited in these people (7). The evidence also shows the mechanism of action and treatment by IFN-β in these individuals may be complex and related to various factors. Clinical trials show that treatment with IFN-β is associated with the prevention of relapse compared with placebo across all relapsing subtypes of MS; however, up to 30–50% of patients show suboptimal responses to therapy (8). Therefore, it is crucial to determine reliable predictors of response to IFN-β and reduce the risk of disease progression in MS patients.

The biological effects of IFN-β as a pleiotropic cytokine occurred through its binding to the IFN-β receptor (IFNAR)1 and 2 subunits of heterodimeric cell surface receptor complex (9). The interaction triggers the

JAK-STAT signaling cascade, which finally principals to transcriptional activation of various IFN-stimulated genes (10). Specific transcription players partly mediate the IFN-β mechanism with the so-called IFN-stimulated response elements (ISREs), which frequently originate in the promoter regions of IFN-inducible genes (11).

Pharmacogenomics identifies gene variants responsible for differences in response to the drug in different patients. With the ongoing innovations in genomic technologies, personalized medicine provides the opportunity for optimizing individual treatment outcomes. Single Nucleotide Polymorphisms (SNPs) can affect pharmacodynamics, metabolism, or the mechanism of action of IFN-β in MS patients (12).

In the present study, As far as we know, we evaluated the role of the promoter sequence of the IFNAR gene for the first time in the therapeutic response to IFN-β in MS patients. Our study provides an insight into the pharmacogenetics study in MS patients and offers a promising approach to predicting therapeutic response to IFN-β.

2. Material & Methods

2.1. Patients

The present study consisted of 100 Iranian RRMS patients who lived in Hamadan Province (IRSN). The patients were followed prospectively for 2 years (from July 2018 to June 2020) after initiation of treatment with IFNβ-1a (intramuscular injection of CinnoVex [CinnaGen Co, Tehran, Iran]) in Emam Khomeini clinic (Hamadan, Hamadan, Iran).

All patients recruited in this study had clinically definite MS and specifically RRMS according to Poser's criteria (13). After 2 years of follow-up, the patients were classified as IFN-β responders (n = 57) when there was no sustained progression in the Expanded Disability Status Scale (EDSS) and no relapse during the follow-up period. Moreover, the patients were considered -non-responders (n = 43) when at least one relapse occurred during follow-up plus an increase of at least one point in the EDSS that continued for two consecutive visits separated by a 6-month interval (8).Hamadan University of Medical Sciences' institutional ethics committee approved this study (IR.UMSHA.REC.1397.258).All patients gave their informed consent to be included in the study.

2.2. DNA extraction and PCR analysis

According to the manufacturers' protocol, genetic DNA was extracted from venous peripheral blood samples by applying BioFACT Genomic DNA Prep Kit for Blood (Daejeon, Korea). Then, IFN-β promoter sequence DNA was amplified by PCR using forward primer (Plus): CAAGTCGCCCGGAAAACGAG and reverse primer (Minus): GCTGCGTGCCCTACCTC. Following PCR amplification, the PCR products were analyzed using 2.0% agarose gel. Sequencing and sequence analysis of PCR products

the PCR products were sequenced using Plus and Minus primers by the Sanger sequencing method. High-quality sequence data were generated, which was reflected by a long contiguous read length (CRL) (up to

850 bp), and a high Phred quality value (QV20 + for over 95% of the bases) Sequencing. Results were analyzed using the Sequencher 5.4.6 (Gene Codes Corporation).

2.3. Statistical analysis

Statistical analysis was performed using SPSS version 20 (SPSS Inc., Chicago, IL) using a chi-square test with Yates' correction or Fisher's exact test, where appropriate. The risks contributed by the haplotype and genotype were assessed by calculation of odds ratio (OR) with 95% CI. Also, an independent t-test was performed to analyze the quantitative data compared to two groups.

3. Results

Baseline and clinical characteristics of 100 RRMS patients according to the responsiveness to IFN-β therapy are presented in Table 1. The percent of mean age of respondents (in < 30) was 57.9, and respondents (in > 30) was 42.1. The percent of mean age of non-respondents (in < 30) was 37.2 years, and non-respondents (in > 30) was 62.8. 78.9% of the respondents were women, and 21.1% were men. In the non-respondents, 86% were women, and 14% were men, were included in this study for further comparison of SNPs and haplotypes distributions between the patients and population-stratified control group.

Table 1
Demographics and clinical characteristics of MS patients

variables	All patients n = 100	Responders n = 57(57%)	Non-responders n = 43(43%)	p-value
sex	82(82%)	45(78.9%)	37(86%)	0.36
Female,n(%)	18(18%)	12(21.1%)	6(14%)	
Male,n(%)				
Age	49 49	33 57.9	16 37.2	0.04
< 30	51 51	24 42.1	27 62.8	
≥ 30				
MS course (RRMS/SPMS)	100.0	57/0	43/0	0.43
At the baseline	91/9	53/4	38/5	
At the study endpoint				
Mean EDSS ± SD (range)		2.04 ± 1.1	2.33 ± 1.25	0.23
At the baseline		1.97 ± 1.05	3.44 ± 1.73	< 0.001
At the study endpoint				
adverse drug reactions	30 30	29 50.9	1 2.3	< 0.001
No reaction	30 30	18 31.6	12 27.9	
Moderate	40 40	10 17.5	30 69.8	
Severe				

As shown in Table 1, the mean age of the IFN-β‴ ’responder’s group of the patients (> 30) was 42.1 versus 62.8 years in non-responders (p = 0.04). The mean number of relapses after treatment for the disease was higher in responders than non-responders (p < 0.001). The disease’s adverse drug reactions were significantly lower in responders than non-responder groups of the patients (p < 0.001). The mean points for EDSS at the baseline (2.04 ± 1.1 vs 2.33 ± 1.25; p = 0.23) and at the study endpoint (1.97 ± 1.05 vs 3.44 ± 1.73; p < 0.001) were also significantly different between responders and nonresponders.

Bioinformatics analysis revealed 11 SNPs within the promoter region that altered putative transcription factor binding sites.

Sequencing analysis revealed 6 SNPs within the promoter region of the IFNAR gene, which is different between responders and non-responders. These SNPs are located in 208, 343, 378, 420, 537, and 608 bp from the start codon. The results of the statistical analysis are shown in Table 2.

Table 2
Evaluation of SNPs in the promoter region of the IFNAR gene.

Genotype	N	%	P-value
208 SNP	49	86	0.26
CC	40	93.02	0.18
Responder	5	8.8	0.89
Non-Responder	1	2.5	
CT	3	5.3	
Responder	2	4.7	
Non-Responder			
TT			
Responder			
Non-Responder			
343 SNP	56	98.2	0.84
TT	42	97.7	0.84
Responder	1	1.8	-
Non-Responder	1	2.3	
TA	0	0	
Responder	0	0	
Non-Responder			
AA			
Responder			
Non-Responder			

Genotype	N	%	P-value
378 SNP	56	98.2	0.4
AA	41	95.3	0.4
Responder	1	1.8	-
Non-Responder	2	4.7	
AT	0	0	
Responder	0	0	
Non-Responder			
TT			
Responder			
Non-Responder			
420 SNP	51	89.5	0.28
CC	41	95.3	0.28
Responder	6	10.5	-
Non-Responder	2	4.7	
CT	0	0	
Responder	0	0	
Non-Responder			
TT			
Responder			
Non-Responder			

Genotype	N	%	P-value
537 SNP	53	93	0.62
CC	41	95.3	-
Responder	0	0	0.62
Non-Responder	0	0	
CT	4	7	
Responder	2	4.7	
Non-Responder			
TT			
Responder			
Non-Responder			
605 SNP	33	60	0.14
TT	22	40	0.01
Responder	12	80	0.27
Non-Responder	3	20	
TC	12	40	
Responder	18	60	
Non-Responder			
CC			
Responder			
Non-Responder			

According to the results, in 208, 344, 378, 420, and 537 SNPs, there was no statistically significant difference between the responder and non-responder groups. However, 605 SNP indicates a statistically significant relationship between responder and non-responder. (p-value < 0.05).

According to the results of Table 3, the frequency of SNP TT (605 SNP) genotype of IFNAR promoter is 33 (60%) in respondents and 22 (40%) in non-responders. The frequency of SNP TC (605 SNP) IFNAR promoter genotype is 12 (80%) in responders and 3 (20%) in non-responders. The frequency of CC SNP (605 SNP) IFNAR promoter genotype is 12 (40%) in responders and 18 (60%) in non-responders. There was a significant difference between responders and non-responders in the TC genotype (p-value < 0.05). In contrast, the frequency of TT and CC (605 SNP) genotype of the IFNAR promoter was not significantly different between the two groups.

Table 3
Determination and comparison of the frequency of 605 SNP genotypes of IFNAR gene promoter in responders and non-responders to treatment.

Genotype	group	N	%	P-value	P-value
TT	Responders	33	60	0.14	
	Non-responders	22	40		
TC	Responders	12	80	0.01	0.03
	Non-responders	3	20		
CC	Responders	12	40	0.27	
	Non-responders	18	60		

The results show that compared to the CC genotype, the probability of response to treatment is higher in the TC genotype (OR = 6.95% CI = 1.4–25.8). There is a statistically significant relationship in terms of probability or Less response to treatment was found in individuals carrying the TT genotype than in those carrying the CC genotype. (OR = 2.25,95% CI = 0.91–5.6) Compared to the CC genotype, the probability of response to treatment is higher in the dominant model. (OR = 2.22,95% CI = 0.92–5.4) (Table 4). Also, in the results of allele frequency, it was observed that a significant difference was observed between the T allele in responding and non-responding individuals (p-value < 0.08) (Table 5). This was while no significant difference was observed in the C allele (Table 5).

Table 4
Association between allele and genotype frequencies of SNPs

Variable	Responders N %	non-responders N %	OR	95% CI	P-value
TT	33 60	22 40	2.25	0.91–5.6	0.08
TC	12 80	3 20	6	1.4–25.8	0.02
CC	12 40	18 60	Ref.		
Recessive model	TC + CC vs. TT		0.76	0.34–1.7	0.52
Dominant model	TC + TT vs. CC		2.22	0.92–5.4	0.08

Table 5
Frequency of alleles in responders and non-responders to treatment

Allele	Responder N %	Non-responder N %	p-value	p-value[a]
T	45 64.3	25 35.7	0.03	0.08
C	24 53.3	21 46.7	0.8	

The second marker was GTn repeat element ranging from 542–551 bp from the start codon. According to the results (Table 6), there is a significant difference between the two groups of responders and non-responders to the treatment in terms of presence or absence of insertion before GT repeat dinucleotide microsatellite (P-value = 0.02).

Table 6
Investigation of insertion before GT repeat dinucleotide microsatellite in the promoter region of IFNAR gene

Variable	All patients N %	Responder N %	Non-responder N %	P-value
Insertions before GT	89 89	47 82.5	42 97.5	0.02
no	11 11	10 17.5	1 2.3	
yes				

The results of Table 7 indicate that numbers of GT sequences tended to be positively associated with the therapeutic response but were not significant (P-value = 0.3)

Table 7
Investigation of $(GT)_n$ repeat dinucleotide microsatellite in the promoter region of IFNAR gene

variable	All patients N %	Responder N %	Non-responder N %	P-value
Number of GT repeat	91 91	51 89.5	40 93	0.3
5	6 6	5 8.8	1 2.3	
8	3 3	1 1.8	2 4.7	
9				

4. Discussion

The beneficial properties of IFN-β therapy in MS have been shown in various studies, lessening the relapse rate by approximately 30%. Though, a long-term change in the normal history of the disease has not been established. Furthermore, many patients are refractory or display adverse responses following IFN-β therapy (14). IFN-β binds to type I interferon receptors and induces a complex transcriptional response, leading to multiple immunomodulatory proteins expression. So, up or down-regulation of the IFNAR receptor can affect the IFN-β therapy. Genome-wide pharmacogenomics analyses have shown a relation between response to immunotherapy and genetic heterogeneity (15).

In the present study, we investigated the IFNAR promoter's genetic heterogeneity and its relation to immunotherapy response. Our results were indicated a significant association between the 605 SNP genotypes and the responses to IFN-β therapy. SNPs in non-coding regions, such as a promoter, can alter transcription factor binding sites resulting in a change in the protein expression. Various studies have shown that SNP in the promoter can impact protein expression. Liu et al. found that polymorphism in the promoter region of interleukin-12B can upregulate the gene expression and increase colon cancer probability (16). Russa et al. demonstrated that polymorphism in the promoter region was associate with MS in Italian patients (17). In line with these results, Ibayyan et al. found that the SNP in the promoter of interleukin-7 receptor alpha was linked to MS in Jordanian patients (18).

The results also showed a significant difference between the two groups in terms of presence or absence of insertion before GT repeats dinucleotide microsatellite. Insertion in the promoter region can impact the protein expression. Moshynska et al. found that short sequence insertion in the MCL-1 promoter leads to higher protein expression and inadequate chemotherapy response (19).

The present study revealed that the promoter GT repeat dinucleotide microsatellite polymorphism of the IFN-β gene might be associated with the response to IFN-β. Promoter microsatellite polymorphism has been investigated in various studies. For example, Fiotti et al. 39 reported that the microsatellite of the promoter region of matrix metalloproteinase 9 (MMP-9) was higher in the MS than in the control group and may play a role in susceptibility to multiple sclerosis (MS) (20). Matsuyama et al. 40 reported that the 5/5 or 5/14 genotype of the GT repeat dinucleotide microsatellite polymorphism was related to the superior antiviral activity IFN-α (21). In our results, the promoter GT repeat dinucleotide microsatellite polymorphism in IFNAR might affect the transcriptional efficiency.

Conclusions

In conclusion, the present study investigates the genetic heterogeneity in the IFNAR promoter and its relation to IFN-β therapy in MS patients. Here, we found a significant association between 605 SNP in responders and non-responders to IFN-β therapy. The results also showed a significant difference between the two groups in the presence or absence of insertion before GT repeats dinucleotide microsatellite. However, more studies with a larger sample size are needed to demonstrate this relationship further. Furthermore, the potential impact of mentioned SNP, insertion, and microsatellite

polymorphism in protein expression must be investigated *in vitro* and *in vivo*. These studies can help to choose optimal therapy for MS patients.

Declarations

Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest.

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Author contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mehrdokht Mazdeh, Fatemeh Nouri and Meysam Soleimani. The first draft of the manuscript was written by Samin Hajian and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Consent to participate

This study was approved by the Ethics Committee of Hamadan University of Medical Sciences IR.UMSHA.REC.1397.258). Informed consent was obtained from all study participants before the project began.

Consent to publish

Neither the article nor portions of it have been previously published elsewhere. The manuscript is not under consideration for publication in another journal, and will not be submitted elsewhere until the Molecular Biology Report editorial process is completed. All authors consent to the publication of the manuscript in Molecular Biology Report.

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