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ORIGINAL ARTICLE

Myxovirus resistance protein A (MxA) polymorphism is associated with IFNβ response in Iranian multiple sclerosis patients

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Abstract Multiple sclerosis (MS) is a heterogeneous immune-related demyelinating disorder of central nervous system with several genetic and environmental factors contributing in its pathogenesis or patients' response to therapies. Myxovirus resistance protein A (*MxA*) is among the genes which are induced by IFNβ and are involved in the MS pathogenesis and/or response to IFNβ. In the present case-control study, we evaluated the association between three SNPs at nt −123 (A or C, rs17000900), nt −88 (G or T, rs2071430), and nt +20 (A or C, rs464138) and MS risk as well as treatment response in a population of Iranian MS patients including 146 IFNβ responders and 85 non-responders as well as 180 healthy controls. The AGA (−123, −88, +20) haplotype was more frequent in controls compared with MS cases ($P = 0.038$, OR (95% CI) = 1.77 (1.03–3.02)). Of particular note, the frequency of rs464138 AA genotype was significantly higher in responders compared with non-responders. However, the allele and genotype frequencies of other SNPs were not significantly different among patient subtypes or between patients and controls. Besides, we have demonstrated that CGC, ATA, and AGA (−123, −88, +20) haplotypes were significantly associated with IFNβ response in MS patients. As SNPs on *MxA* promoter region might participate in MS patients' response to IFNβ, prior patients genotyping may increase the rate of responsiveness and help in individualized selection of treatment options.

Keywords Multiple sclerosis · MxA · Polymorphism

Introduction

Multiple sclerosis (MS) is a heterogeneous immune-related demyelinating disorder of the central nervous system [1]. The interactions between genetic and environmental risk factors are implicated in this chronic demyelinating disorder [2]. According to disease activity and progression, MS patients are phenotypically divided to distinct categories with relapsing–remitting (RR) being the most prevalent one [3]. Interferon beta (IFNβ) was the first specific disease-modifying treatment (DMT) approved for relapsing-remitting multiple sclerosis (RRMS) and is still one of the most frequent recommended drugs [4]. IFNβ as a first-line injectable DMT [5] has been shown to decrease the relapse rate and the lesion load assessed by magnetic resonance imaging (MRI), brain atrophy, and disability progression [6]. Similar to all DMTs, not all MS patients respond to IFNβ treatment. The development of neutralizing antibodies (NAbs) in patients has been shown to partially contribute in non-responsiveness [7]. High-titer NAbs, which are detected in about 15% of patients, stop IFNβ biological activity and the therapeutic effect of IFNβ. Prediction of the possibility of NAb induction in a patient would help in switch to alternative treatments and improvement of percentage of responders [8]. Recent expert consensuses have supported the measurement of IFNβ biological activity and of NAbs in the management of IFNβ-treated patients [8]. Myxovirus resistance protein A (*MxA*) is among the

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genes which are induced by IFNβ and consequently show the biological activity of IFNβ [9]. It is a member of the dynamin superfamily of large GTPases which prevent the replication of single-strand RNA viruses [10]. The quantification of MxA mRNA in the peripheral blood mononuclear cells has been shown to detect all the patients in whom IFNβ does not activate the corresponding receptor [10]. Consequently, low MxA levels are considered as biological non-responsiveness [11]. The detection of MxA protein in post-mortem brains of MS patients not treated with IFN [12] implies its role in the pathogenesis of MS as well. In addition, single nucleotide polymorphisms (SNPs) in or near the IFN-stimulated response elements (ISREs) on the *MxA* promoter region have been shown to correlate with the anti-viral activity of MxA induced by IFNβ as well as pathophysiology of MS [10]. Considering the putative role of MxA in the MS pathogenesis and patients' response to IFNβ, in order to find the association between *MxA* genotypes and the clinical effectiveness of long-term IFN-β therapy in MS, in the present study, we genotyped three functional polymorphisms in this gene in a population of Iranian MS patients including both responders and non-responders as well as healthy controls.

Material and methods

Patients and control groups

The present case-control study included 231 unrelated patients with sporadic RRMS from Tehran Hospitals and MS society of Iran and 180 healthy matched controls. The diagnosis of RRMS has been performed by specialized neurologists according to the revised McDonald criteria [13]. Patients had been treated with IFNβ-1a (intramuscular injection of 20 μg of CinnoVex [CinnaGen Co, Tehran, Iran] three-times a week) for at least 2 years and were categorized as IFNβ responders (n = 146) when there was no continued progression in the Expanded Disability Status Scale (EDSS) and no relapse during follow-up period or as non-responders (n = 85) when at least one relapse happened during follow-up in addition to an increase of at least one point in the EDSS that lasted for at least two successive visits, which were separated by a 6-month intermission. At the time of sampling, they were clinically stable (not in relapse phase).

Blood sampling

Three milliliters of peripheral blood was taken in an EDTA tube. Informed consent was obtained from all individual participants enrolled in the study. Complete personal and familial history was obtained in a questionnaire. The study was approved by the local Ethical Committee and has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Genotype determination

Genomic DNA was extracted from peripheral whole blood by using the salting out method. PCR amplification was carried out using Taq 2× red master mix (Ampliqon, Denmark) in a 117 FlexCycler (Analytik Jena, Germany). A promoter region including a part of exon 1 of *MxA* gene (nt −352 to +128) was amplified using the primer pair and the PCR conditions previously described [10]. The products amplified by PCR were electrophoresed on a 2% agarose gel and purified with QIAquick Gel Extraction Kit (QIAGEN, Korea). The purified PCR products were subsequently sequenced using ABI Prism3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Chi-square test was used for determination of fitness to the Hardy–Weinberg equilibrium in patient and control groups. The frequencies of alleles and genotypes in four different inheritance models including recessive, dominant, co-dominant, and over-dominant and their associations with the disease were analyzed using Chi-square test. The allelic odds ratio and its confidence interval (CI) were used to evaluate the association between disease and alleles at each SNP. Haplotype frequencies for *MxA* were calculated using SNPStats online program (http://bioinfo.iconcologia.net/SNPstats) and Haploview 4.2 (http://www.broad.mit.edu/mpg/haploview/). The estimation of pairwise linkage disequilibrium (LD) between mentioned SNPs was provided by describing D′ and r^2 value in Haploview software. For all statistical tests, the level of significance was defined as $P < 0.05$.

Results

In the present study, 231 RRMS patients including 146 responders and 85 non-responders have participated. The clinical and demographic characteristics of patients are summarized in Table 1. The non-responder group had higher EDSS ($P < 0.0001$ in a Mann–Whitney test) compared to the responder group, which is in line with the difference in responsiveness to IFNβ therapy. The allele and genotype frequencies of three SNPs at nt −123 (A or C, rs17000900), nt −88 (G or T, rs2071430), and nt +20 (A or C, rs464138) located near an ISRE have been investigated in patients and healthy subjects. Of particular note, the frequency of rs464138 AA genotype was significantly higher in responders compared with both

Table 1 Clinical and demographic characteristics of MS patients

Variables	Responders $N = 146$ (%)	Non-responders $N = 85$ (%)	p values
Gender			
Female	112 (76.7)	52 (61.2)	0.01
Male	34 (23.3)	33 (38.8)	
Age in year[a]	35.0 ± 9.3	36.5 ± 9.7	0.25
Age at diagnosis (year)[a]	30.4 ± 8.6	28.0 ± 8.6	0.04
Duration of disease (year)[a]	4.5 ± 3.9	8.6 ± 6.0	<0.001
Duration of interferon β treatment (months)	26.4 ± 2	27.1 ± 1.2	–
Relapse rate	0	3.2 ± 1.1	–
Vitamin D supplement therapy			
Yes			
Daily	68 (46.6)	32 (37.6)	
Weekly	16 (10.9)	11 (12.9)	
Irregularly	22 (15.1)	17 (20.0)	0.38
No	40 (27.4)	25 (29.5)	0.86
EDSS prior to treatment[a]	3.44 ± 0.95	3.09 ± 1.15	0.01
EDSS after 2 years of treatment[a]	2.78 ± 0.95	4.3 ± 1.32	<0.0001

[a] Items with significant difference among responders and non-responders

non-responders and healthy controls (Table 2). The rs2071430 *MxA* SNP genotype frequencies in the responder (G/G genotype frequencies = 67 of 146 or 45.9%) and non-responder (G/G genotype frequencies = 38 of 85 or 44.7%) groups were similar ($P = 0.98$ in Fisher exact test). Similarly, for rs17000900, the C/C genotype frequencies in the responder (95 of 146 or 65.1%) and non-responder (57 of 85 or 67.1%) groups were similar ($P = 0.87$ in Fisher exact test). These results show that these SNP *MxA* genotypes are not indicators of MS patients' response to IFNβ. In addition, the allele and genotype frequencies of none of SNPs were significantly different among patients and controls (Table 3). Haplotype analysis showed that CGA (−123, −88, +20) haplotype was the most frequent haplotype in both cases and controls. The AGA (−123, −88, +20) haplotype was more frequent in controls compared with MS cases ($P = 0.038$, OR (95% CI) = 1.77 (1.03–3.02)). Besides, we have demonstrated that CGC, ATA, and AGA (−123, −88, +20) haplotypes were significantly associated with IFNβ response in MS patients. AGA and ATA haplotypes were more commonly detected in responders ($P = 0.036$ and 0.07, respectively), while the CGC haplotype was more frequent among non-responders ($P = 0.003$) (Table 4). Linkage disequilibrium analysis was performed by estimating D′ and r^2 values as follows: for rs17000900/rs2071430, D′ = 0.8147 and $r^2 = 0.6059$; for rs17000900/rs464138, D′ = 0.7892 and $r^2 = 0.4762$; and for rs2071430/rs464138, D′ = 0.792 and $r^2 = 0.6426$. As anticipated from the

Table 2 Genotype frequency of three SNPs of *MxA* gene in association with responsiveness to IFN-β-1a therapy

SNP	Genotypes	Responder N (%) Group A	Non-responder N (%) Group B	Control N (%) Group C	p value		
					A vs. C	B vs. C	A vs. B
rs17000900	C/C	95 (65.1%)	57 (67.1%)	116 (64.4%)	0.16	0.44	0.88
	A/C	42 (28.8%)	24 (28.2%)	60 (33.3%)			
	A/A	9 (6.2%)	4 (4.7%)	4 (2.2%)			
rs2071430	G/G	67 (45.9%)	38 (44.7%)	83 (46.1%)	0.97	0.95	0.98
	G/T	69 (47.3%)	41 (48.2%)	86 (47.8%)			
	T/T	10 (6.8%)	6 (7.1%)	11 (6.1%)			
rs464138	A/A	72 (49.3%)	17 (20%)	60 (33.3%)	0.0035[a]	0.0034[b]	<0.0001[c]
	A/C	57 (39%)	43 (50.6%)	96 (53.3%)			
	C/C	17 (11.6%)	25 (29.4%)	24 (13.3%)			

Odds ratio with 95% CI for the significant p values

[a] 2.02 (1.26–3.25)

[b] 1.59 (0.83–3.03)

[c] 6.23 (2.77–14.03)

Table 3 Allele and genotype frequencies of three SNPs in patients and controls

	Genotype	Cases $N = 231$ (%)	Controls $N = 180$ (%)	OR(95% CI)	p value
rs17000900					
Co-dominant	C/C	152 (65.8%)	116 (64.4%)	1.00	
	A/C	66 (28.6%)	60 (33.3%)	1.19 (0.78–1.82)	0.15
	A/A	13 (5.6%)	4 (2.2%)	0.40 (0.13–1.27)	
Dominant	C/C	152 (65.8%)	116 (64.4%)	1.00	0.77
	A/C-A/A	79 (34.2%)	64 (35.6%)	1.06 (0.71–1.60)	
Recessive	C/C-A/A	218 (94.4%)	176 (97.8%)	1.00	0.076
	A/A	13 (5.6%)	4 (2.2%)	0.38 (0.12–1.19)	
Over-dominant	C/C-A/A	165 (71.4%)	120 (66.7%)	1.00	0.3
	A/C	66 (28.6%)	60 (33.3%)	1.25 (0.82–1.91)	
Allele n (%)	C	370 (80.0%)	292 (81.0%)	1.00	0.71
	A	92 (20.0%)	68 (19.0%)	0.94 (0.67–1.33)	
rs2071430					
Co-dominant	G/G	105 (45.5%)	83 (46.1%)	1.00	0.95
	G/T	110 (47.6%)	86 (47.8%)	0.99 (0.66–1.48)	
	T/T	16 (6.9%)	11 (6.1%)	0.87 (0.38–1.97)	
Dominant	G/G	105 (45.5%)	83 (46.1%)	1.00	0.89
	G/T-T/T	126 (54.5%)	97 (53.9%)	0.97 (0.66–1.44)	
Recessive	G/G-G/T	215 (93.1%)	169 (93.9%)	1.00	0.74
	T/T	16 (6.9%)	1 (6.1%)	0.87 (0.40–1.93)	
Over-dominant	G/G-T/T	121 (52.4%)	94 (52.2%)	1.00	0.97
	G/T	110 (47.6%)	86 (47.8%)	1.01 (0.68–1.49)	
Allele n (%)	G	320 (59.3%)	252 (70.0%)	1.00	0.82
	T	142 (30.7%)	108 (30.0%)	0.96 (0.72–1.30)	
rs464138					
Co-dominant	A/A	89 (38.5%)	60 (33.3%)	1.00	0.11
	A/C	100 (43.3%)	96 (53.3%)	1.42 (0.93–2.19)	
	C/C	42 (18.2%)	24 (13.3%)	0.85 (0.47–1.54)	
Dominant	A/A	89 (38.5%)	60 (33.3%)	1.00	0.28
	A/C-C/C	142 (61.5%)	120 (66.7%)	1.25 (0.83–1.88)	
Recessive	A/A-A/C	189 (81.8%)	156 (86.7%)	1.00	0.18
	C/C	42 (18.2%)	24 (13.3%)	0.69 (0.40–1.19)	
Over-dominant	A/A-C/C	131 (56.7%)	84 (46.7%)	1.00	0.043
	A/C	100 (43.3%)	96 (53.3%)	1.50 (1.01–2.22)	
Allele n (%)	A	278 (60.0%)	216 (61.0%)	1.00	0.95
	C	184 (40.0%)	144 (39.0%)	1.01 (0.76–1.33)	

physical distances, these three SNPs have significant correlations with each other.

Discussion

Prior identification of patients' responsiveness to DMTs has a critical role in individualized selection of treatment modalities. Genetic variants participate not only in the susceptibility to MS but also in the patients' responses to DMTs [7]. Among putative biomarkers for identification of IFNβ bioactivity and patients' responsiveness, MxA has been proven to be one of the most reliable markers [8]. In the present study, we demonstrated a significant role for rs464138 in determination of patients' response to IFNβ. Cunningham et al. have reported *MxA* among genes containing polymorphisms associated with

Table 4 *MxA* promoter and exon 1 estimated haplotype frequencies

−123	−88	+20	Total	Cases	Controls	OR (95% CI)	p value	Responders	Non-responders	OR (95% CI)	p value
C	G	A	0.239	0.248	0.229	1.00	–	0.26	0.23	1.00	–
A	T	C	0.110	0.116	0.104	0.97 (0.65–1.44)	0.862	0.12	0.12	1.16 (0.66–2.04)	0.607
C	G	C	0.171	0.171	0.170	1.08 (0.76–1.52)	0.668	0.13	0.24	2.11 (1.30–3.44)	0.003
C	T	C	0.155	0.159	0.151	1.03 (0.72–1.47)	0.887	0.15	0.18	1.40 (0.84–2.31)	0.193
C	T	A	0.111	0.110	0.112	1.10 (0.74–1.63)	0.649	0.12	0.09	0.88 (0.48–1.59)	0.663
A	G	C	0.103	0.102	0.104	1.11 (0.74–1.66)	0.630	0.09	0.12	1.44 (0.81–2.57)	0.216
A	G	A	0.052	0.040	0.065	1.77 (1.03–3.02)	0.038	0.05	0.01	0.26 (0.08–0.91)	0.036
A	T	A	0.059	0.054	0.065	1.29 (0.78–2.12)	0.321	0.08	0.01	0.19 (0.05–0.63)	0.007

response to recombinant IFNβ and presented rs17000900 as an SNP associated with responsiveness of MS patients to IFNβ therapy [14]. In addition, rs2071430 has been noticed as one of the important determinants of response to IFN in patients with chronic hepatitis C virus (HCV) infection [15]. We detected higher frequency of rs464138 AA genotype in responders compared with non-responders. A previous study has shown that rs464138 AA genotype results in the highest MxA expression in human bronchial epithelial cells compared to the AC and CC [16]. Another in vitro study demonstrated that substitution of the A by C at position +20 significantly diminishes expression rate implying a functional role for this SNP [17]. However, we could not detect any associations between two other SNPs and MS risk or response to IFNβ which is in line with Weinstock-Guttman et al.'s results which indicate no association between *MxA* genotype at these two SNPs and clinical, MRI, and *MxA* gene expression in MS patients treated with IFN-β therapy [18].

On particular note, we have demonstrated that CGC, ATA, and AGA (−123, −88, +20) haplotypes were significantly associated with IFNβ response in MS patients. AGA and ATA haplotypes were more commonly detected in responders, while CGC haplotype was more frequent among non-responders. The ATA (−123, −88, +20) haplotype is consisted of the three alleles which have been shown to result in higher expression compared with their counterpart allele [16]. Furuyama et al. have reported that MS patients with −88T haplotype express elevated levels of MxA protein in response to IFNs, while patients with −123A have higher MxA expression without IFNs [10]. Hijikata et al. [19] have demonstrated more strong responsiveness to IFNs in chronic HCV patients with −123A/−88T haplotype compared with those having −123C/−88G haplotype which is in line with our results. The crucial significance of the concurrent presence of −123A and −88T variants for maximizing MxA expression has been noted in another study as well [17]. On the other hand, the CGC (−123, −88, +20) haplotype has been shown to result in low or medium MxA expression depending on the presence of other variants at −309 and −101 positions [17]. MxA has been suggested to exert an apoptosis-promoting activity [10] which can contribute in responsiveness to IFNs as well. It is possible that induction of apoptosis in peripheral immune cells by higher level of MxA leads to amelioration of MS [10]. The results of Gniadek et al.'s [20] study which showed the increased apoptosis of peripheral immune cells by IFNβ treatment provide further evidences for this hypothesis.

We found that the AGA (−123, −88, +20) haplotype was more frequent in controls compared with MS cases. Furuyama et al. have detected a significantly higher frequency of the haplotype with −88T and −123A in MS patients which was associated with over-expression of MxA. However, they did not detect AGA haplotype in their samples [10]. MxA has different and sometime contradictory roles in the pathogenesis of MS. Its involvement in proapoptotic pathways is a double-edge sword itself as it can lead to apoptosis in neural cells or immune cells with different clinical consequences. In addition, it has a role in the disturbance of the blood-brain barrier and exposure of glial cells to cytokines on one hand and a protective role against viral infections on the other hand [10]. Taken together, its involvement in MS pathogenesis seems to be more complicated than its role in the determination of therapeutic response.

Using the HaploReg v4 software which is a tool for discovery of annotations of the non-coding genomic variants [21], we found that rs464138 modifies the affinity for binding of NRSF_disc1, PAX5_known3, Sin3Ak-20_disc6, UF1H3BETA, Zfx, and p300_known1. Although the role of these transcription factors has not been assessed in MS, at least some of them might be associated with the pathophysiology of MS. For instance, the repressor element-1 silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) is a master regulator of neuronal gene expression which participates as a modular scaffold for dynamic recruitment of epigenetic regulatory factors to genomic loci that contain the repressor element-1 (RE1) binding motif. It represses RE1 containing neuronal genes in neural stem cells and non-neuronal cells and participates in the regulation of neuronal lineage maturation and plasticity [22]. *PAX5* is among the genes that have been shown to be highly methylated in cell-free plasma DNA obtained from MS patients compared with normal subjects [23]. *ZFX* is among differentially expressed genes in normal-appearing white matter from primary or secondary progressive MS patients [24]. Finally, the ubiquitous nuclear phosphoprotein p300 has been shown to be up-regulated in systemic sclerosis and neurodegenerative disease [25] which implies a putative role for it in the pathogenesis of MS. However, future researches are needed for evaluation of the functional network between these transcription factors and their aberrant regulation in MS pathogenesis.

In addition, haplotype analysis showed CGA (−123, −88, +20) haplotype as the most frequent haplotype in both cases and controls. Furuyama et al. have showed that CGC (−123, −88, +20) haplotype was the most common haplotype in Japanese MS patients and healthy subjects [10]. In addition, they reported that −123A allele constantly coincided with −88T and never coexisted with −88G [10]. However, we detected AGA and AGC (−123, −88, +20) haplotypes in both patients and healthy subjects which contradicts Furuyama et al. [10] observation and put their explanation regarding higher frequency of −123A in responders in Cunningham et al.'s study [14] under question. However, such distinct haplotype pattern is consistent with the supposition that these variants segregate at different frequencies in different populations. On the other hand, the detected allele frequencies in our study is in accordance with the results of a previous study which demonstrated low prevalence of the high-MxA-

producer genotypes AA (−123) and TT (−88) in Asian and Western European populations [17].

The genomic location of *MxA* near the Down's syndrome critical region (DSCR) [26] as well as over-expression of MxA in Down's syndrome as the result of gene dosage effect [27] might be involved in the supposed protective effect of Down's syndrome against MS [28]. However, although we detected higher frequency of haplotypes associated with higher MxA expression in responders compared with non-responders, there is no data regarding the effect of AGA (−123, −88, +20) haplotype (as the protective haplotype detected in the present study) on MxA expression.

In brief, in the present study, we demonstrated associations between rs464138 AA genotype in *MxA* gene and IFNβ responsiveness in MS patients. The anti-viral activity of MxA which might participate in the control of viral infections has been suggested as the underlying mechanism of associations between MxA SNPs and IFNβ treatment response in MS patients [18]. However, as the role of MxA in MS is complicated, future studies should focus on the determination of the exact mechanism of MxA participation in MS pathogenesis or treatment response. In addition, due to the relatively small sample size of the current study, the statistical power of the study is insufficient to draw firm conclusions about the results. So, further studies with larger sample sizes are needed to confirm the results.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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