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Angiotensin-converting enzyme insertion/deletion gene polymorphism and interferon-β treatment response in multiple sclerosis patients: a preliminary report

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We investigated the effect of the functional insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme (ACE) gene on the response to interferon-β (IFN-β) therapy in Croatian and Slovenian patients with multiple sclerosis (MS). A total of 275 IFN-β treated MS patients [162 responders (Rs) and 113 nonresponders (NRs)] were genotyped by PCR. The *ACE* I/D genotype distribution and allele frequencies did not differ between female Rs and NRs. However, male NRs tended to have a greater prevalence of the DD genotype ($P = 0.073$; odds ratio: 2.64; 95% confidence interval: 0.91–7.60) and a significantly higher frequency of the D allele ($P = 0.022$; odds ratio: 2.43; 95% confidence interval: 1.13–5.20) than male Rs. Multiple forward stepwise regression analysis indicated that the negative response to IFN-β therapy was associated with the *ACE*-DD genotype in men ($\beta = 0.371$; multiple R^2 change: 0.132; $P = 0.009$) and a higher pretreatment relapse rate in both men ($\beta = -0.438$; multiple R^2 change: 0.135; $P = 0.015$) and women ($\beta = -0.208$; multiple R^2 change: 0.042; $P = 0.034$). The *ACE* I/D polymorphism and pretreatment relapse rate accounted for ~26.7% of the IFN-β response variability among the men in the sample. Further studies of a larger number of MS patients from different populations are necessary to evaluate these preliminary findings.

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Introduction

Multiple sclerosis (MS) is a multifactorial autoimmune disease that affects the central nervous system. MS is characterized by chronic inflammation, multifocal demyelination, and axon loss. Interferon-β (IFN-β), which has immunomodulatory, anti-inflammatory, and antiproliferative effects, is the first-line disease-modifying therapy (DMT) for MS patients, but a clinical response does not occur in a large proportion (30–50%) [1].

Recent pharmacogenomic observations suggest that the efficacy of IFN-β therapy depends on genetic predisposition. Despite numerous studies in the last decade identifying gene variants that possibly influence the complex response to IFN-β [1], reliable biomarkers of treatment efficacy are lacking.

In recent years, components of the renin–angiotensin system (RAS) have been shown to be involved in the pathogenesis of MS [2]. Angiotensin-converting enzyme (ACE) is the key enzyme activating the RAS by converting angiotensin I (Ang I) into angiotensin II (Ang II), the major effector molecule of the system. Ang II acts not only as a potent vasoconstrictor but also as a proinflammatory factor implicated in inflammatory/autoimmune demyelination. Ang II stimulates brain vascular endothelial target cells, influencing blood–brain barrier breakdown and allowing macrophage infiltration of the brain parenchyma, increasing microglia and astrocyte activation [3]. In addition, ACE inactivates the potent vasodilator bradykinin through the Kinin–Kallikrein system. All of the essential components of the RAS and Kinin–Kallikrein system are present in the brain. Linking both of these important systems, ACE maintains the balance critical in the control of blood–brain barrier permeability [3].

Recently, active plaques in the brains of MS patients were reported to have elevated levels of both ACE and Ang I receptor [4], which are also upregulated in the inflamed spinal cords and immune systems of mice with experimental autoimmune encephalomyelitis [2]. In addition, increased levels of ACE have been found in the serum [5] and cerebrospinal fluid [6,7] of MS patients. Among several single nucleotide polymorphisms in *ACE* that determine the activity of this enzyme and the level

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of Ang II, such as rs4343, rs4291, and the insertion/deletion (I/D) polymorphism (rs1799752), the *ACE* I/D polymorphism is the most studied RAS-related polymorphic variant and the only one investigated in the etiology of MS until recently. This functional polymorphism in intron 16 of *ACE* (17q23) is characterized by the presence or absence of a 287-bp Alu repetitive sequence and accounts for ~50% of the total phenotypic variance of circulating and tissue ACE [8].

Animal studies of treatment with ACE inhibitors have suggested that RAS blockade decreases central nervous system demyelination and inflammation, reduces the severity of experimental autoimmune encephalomyelitis symptoms, and reverses the symptoms of established disease [2,4,9,10]. In humans, ACE inhibitors are used widely in the treatment of cardiovascular disease. Recently, Doerner *et al.* [11] reported the first study of the effects of RAS inhibitors and β-blockers on disease activity in MS patients. This clinical analysis failed to show a benefit of concomitant administration of ACE inhibitors and IFN-β-1b in MS patients compared with IFN-β-1b therapy only. However, *ACE* I/D genotypes were not determined in the patients analyzed in this study.

To date, several studies have reported an association between the *ACE* I/D polymorphism and susceptibility to MS [12,13], although a recent meta-analysis of four studies carried out in Europeans of Slavic origin [12–15] reported negative results [16]. However, no studies have examined the effect of the *ACE* I/D polymorphism on the response to DMT in MS patients. Therefore, we hypothesized that the genetic variability of the ACE gene may influence the response to IFN-β therapy in Croatian and Slovenian MS patients.

Patients and methods

A total of 275 (211 women, 64 men) IFN-β-treated patients who fulfilled the revised McDonald's criteria for MS [17] were recruited by collaborating clinical and genetic centers in Croatia and Slovenia. The clinical criteria for a response to IFN-β were applied after 2 years of treatment. Patients with relapse-onset MS were divided into two groups: responders [Rs; no relapses and no progression of Expanded Disability Status Scale (EDSS) score] and nonresponders (NRs; having one or more relapses and an increase in the EDSS score of at least 1 point confirmed at 6 months during the follow-up period) [18]. Informed consent was obtained from all participants. The study was carried out with the approval of the ethical committees of both centers.

Genotyping was performed by a one-step PCR [8]. Fisher's exact and χ^2-tests were used to compare the frequency of genotypes/alleles of the *ACE* I/D polymorphism between groups and deviation from Hardy–Weinberg equilibrium. The relationship between the genotypes and normally distributed variables was

Table 1 Clinical characteristics of the multiple sclerosis patients in this study

	Males ($n=64$)			Females ($n=211$)			Total ($n=275$)		
Clinical data	IFN-β R ($n=40$)	IFN-β NR ($n=24$)	*P*	IFN-β R ($n=122$)	IFN-β NR ($n=89$)	*P*	IFN-β R ($n=162$)	IFN-β NR ($n=113$)	*P*
Age at onset (mean ± SD) (years)	29.3 ± 6.8	29.1 ± 7.9	0.951	28.7 ± 8.1	27.0 ± 8.1	0.137	28.8 ± 7.8	27.4 ± 8.1	0.145
Number of relapses in previous 2 years (mean ± SD) (range)	1.5 ± 1.0 (1–5)	2.2 ± 1.6 (1–7)	0.062	1.7 ± 1.0 (1–6)	2.1 ± 1.3 (1–7)	0.031	1.7 ± 1.0 (1–6)	2.1 ± 1.3 (1–7)	0.004
EDSS score (mean + SD) (range)									
At baseline	2.9 ± 1.7 (1–6.5)	2.3 ± 2.3 (1–7.5)	0.354	2.8 ± 1.6 (1–7)	2.9 ± 1.2 (1–5.5)	0.701	2.8 ± 1.7 (1–7)	2.8 ± 1.4 (1–7.5)	0.885
At the study point	2.2 ± 1.3 (1–5.5)	3.2 ± 2.8 (1–7.5)	0.237	2.7 ± 1.7 (1–6.5)	3.8 ± 1.5 (1–7)	0.002	2.6 ± 1.6 (1–6.5)	3.8 ± 1.7 (1–7.5)	0.0003

EDSS, Expanded Disability Status Scale; IFN-β, interferon-β; NR, nonresponder; R, responder.

tested by analysis of variance. A multiple forward stepwise regression analysis including polymorphism, relapse rates, and EDSS score before IFN-β treatment was carried out to evaluate the independent effect of the *ACE* I/D polymorphism on the IFN-β treatment response. Partial coefficients of correlation were calculated to test the correlation between *ACE* I/D genotypes and the treatment response, controlling for relapse rate and EDSS score before IFN-β treatment.

Results

Of the 275 MS patients included in the study, 162 (58.9%) were classified as Rs and 113 (41.1%) as NRs to IFN-β. Both groups had a similar mean age at disease onset and mean EDSS at baseline, whereas NRs had a higher relapse rate at baseline and more severe MS than Rs at the study endpoint (Table 1).

The frequencies of *ACE* I/D genotypes and alleles in Rs and NRs are shown in Table 2. The *ACE* I/D genotype distributions did not deviate from Hardy–Weinberg equilibrium in any group. We found no difference in the *ACE* I/D genotype distribution or allele frequencies between Rs and NRs in the overall patient group or women. However, we observed a trend toward a greater prevalence of the DD genotype ($P=0.073$; odds ratio: 2.64, 95% confidence interval: 0.91–7.60) and a significantly higher frequency of the D allele ($P=0.022$; odds ratio: 2.43; 95% confidence interval: 1.13–5.20) in male NRs compared with male Rs.

The multiple forward regression analysis showed that the presence of the DD genotype significantly predicted a negative IFN-β therapy response in men ($\beta=0.371$; multiple R^2 change: 0.132; $P=0.009$), contributing toward 13.2% of the response variability. The significant impact of the DD genotype on the IFN-β therapy response in this group was confirmed by partial coefficients of correlation analysis ($r=0.434$, $P=0.009$). In women, the IFN-β therapy response did not significantly correlate with any *ACE* I/D genotype. In addition, a significant negative dependence of the response to IFN-β therapy on the pretreatment relapse rate was found in both men ($\beta=-0.438$; multiple R^2 change: 0.135; $P=0.015$) and women ($\beta=-0.208$; multiple R^2 change: 0.042; $P=0.034$). The pretreatment relapse rate explained 13.5% of the response variability in men with MS and 4.2% of the response variability in women with MS.

Discussion

To the best of our knowledge, this study was the first to examine an association between the *ACE* I/D polymorphism and different IFN-β treatment responses in MS patients. The main finding is a sex-specific association of the *ACE* I/D polymorphism with the IFN-β treatment response in men. Although sex differences in the response to DMT in MS have not been studied extensively, recent findings indicate that men and women do not have the same response to IFN-β [19].

In this preliminary study, we found a significant positive association between a negative response to IFN-β treatment and the DD genotype in men with MS. The *ACE* DD genotype is associated with higher tissue and plasma levels of ACE, whereas the *ACE* II genotype is associated with lower ACE levels and the ID genotype with intermediate levels [8]. We previously reported that the *ACE* DD genotype and D allele may contribute toward an elevated risk of MS among Croatian and Slovenian men [12]; thus, the same genetic background may influence both individual IFN-β treatment responses and MS susceptibility. This finding seems to be especially interesting considering the more profound effect of the *ACE* I/D polymorphism on men compared with women in previous studies in which the *ACE* DD genotype conferred an increased risk of hypertension [20] and premature myocardial infarction in men [21]. Furthermore, one recent study reported that a number of cardiovascular genetic factors are associated with increased MS risk, suggesting that the pathobiology of these diseases overlaps [22].

As the frequency of relapse 2 years before IFN-β treatment differed significantly between Rs and NRs, we further evaluated whether the relapse rate is a reliable predictor of the treatment response, either alone or in combination with the *ACE* I/D genotype. A higher frequency of relapse 2 years before IFN-β treatment was associated with a negative response to IFN-β therapy in

Table 2 Frequency of angiotensin-converting enzyme insertion/deletion genotypes and alleles among the multiple sclerosis patients in this study

	Males ($n=64$)			Females ($n=211$)			Total ($n=275$)		
Genotype/allele	IFN-β R ($n=40$)	IFN-β NR ($n=24$)	*P*	IFN-β R ($n=122$)	IFN-β NR ($n=89$)	*P*	IFN-β R ($n=162$)	IFN-β NR ($n=113$)	*P*
ACE I/D									
DD	11 (27.5)	12 (50.0)	0.073	32 (26.3)	22 (24.7)	0.804	43 (26.5)	34 (30.1)	0.520
ID	18 (45.0)	10 (41.7)	0.795	69 (56.5)	51 (57.3)	0.914	87 (53.7)	61 (54.0)	0.964
II	11 (27.5)	2 (8.3)	0.081	21 (17.2)	16 (18.0)	0.885	32 (19.8)	18 (15.9)	0.419
D	40 (50.0)	34 (70.8)	0.022	133 (54.5)	95 (53.4)	0.817	173 (53.4)	129 (57.1)	0.393
I	40 (50.0)	14 (29.2)	–	111 (45.5)	83 (46.6)	–	151 (46.6)	97 (42.9)	–

Data are presented as *n* (%).
ACE, angiotensin-converting enzyme; I/D, insertion/deletion; IFN-β, interferon-β; NR, nonresponder; R, responder.

both men and women. The findings are consistent with those of Sorensen *et al.* [23] and Sellebjerg *et al.* [24], who identified pretreatment relapse rate as an important predictor of a negative response to IFN-β treatment in a large cohort of Danish patients.

The current study has several limitations. First, although our study had 100% power to detect a 2.0-fold increase in *ACE* D allele frequency in men and a 1.8-fold increase in women, the relatively small number of participants, especially men, limits our conclusions on the effect of the *ACE* I/D polymorphism on the IFN-β treatment response. Second, our study is based on a short period of observation (2 years) because we could not follow the patients for a longer period of time on the same drug, which certainly would have allowed for a better assessment of the IFN-β response [25].

Finally, with respect to possible comorbidities related to the ACE system, none of our patients were on ACE inhibitors or other drugs (hypolipidemic, antiplatelet) related to cardiovascular disease. However, in a small number of patients, we cannot rule out concomitant use of antispastic/anticonvulsant/anxiolytic drugs that could have potentially modulated MS severity.

Conclusion

The present study shows that the *ACE* DD genotype is associated with a negative response to IFN-β therapy in men. The *ACE* I/D polymorphism and pretreatment relapse rate accounted for ~26.7% of the IFN-β response variability in this patient group, indicating the involvement of many other genes and/or factors in the response to treatment. As the RAS is a complex system that may influences autoimmune responses, modulates T cells, and acts on macrophages through different signaling pathways, future studies should analyze additional polymorphisms in RAS genes and correlate them with the *ACE* I/D polymorphism within the scope of the response to DMT in MS. In addition, further studies of a larger number of MS patients from different populations are necessary to evaluate these preliminary findings.

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Conflicts of interest

There are no conflicts of interest.

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