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The investigation of relevancy between *PIAS1* and *PIAS2* gene expression and disease severity of multiple sclerosis

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ABSTRACT

Introduction: *PIAS1* and *PIAS2* (protein inhibitor of activated STAT 1,2) play key roles in the pathogenesis of autoimmune and inflammatory diseases. This study aims to evaluate the gene expression of these factors in multiple sclerosis (MS) patients compared to healthy individuals and correlate them with the severity of MS.

Materials and methods: Sixty participants, including 30 patients with MS and 30 healthy controls were studied. The expression of *PIAS1* and *PIAS2* genes in peripheral blood samples of all participants was measured by real-time PCR. The severity of MS was evaluated using the Expanded Disability Status Scale (EDSS). Finally, we evaluated the correlation between the expression of *PIAS1* and *PIAS2* genes with disease severity.

Results: The expression of *PIAS1* gene was increased in patients with MS compared to healthy subjects (P value < .001). Also, there was a significant correlation between the expression of *PIAS1* and *PIAS2* genes with disease severity according to EDSS.

Conclusion: Our study suggests the expression of *PIAS1* and *PIAS2* genes as a prognostic and diagnostic marker in MS disease.

KEYWORDS

Multiple sclerosis; *PIAS1*; *PIAS2*; EDSS

Introduction

Multiple sclerosis (MS), a chronic autoimmune demyelinating disease of central nervous system,^[1] is mediated by autoreactive T cells^[2] and leads to progressive neurological disability.^[3] It is estimated that about 2.5 million people worldwide are diagnosed with MS, mainly young adults at the age between 20 and 40.^[4] The etiology of MS is yet unknown; however, a combination of immunological as well as environmental factors such as vitamin D deficiency, smoking,^[5] increase in

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cytomegalovirus, positivity of anti-CMV IgG and IgM antibodies,^[6] decreased *Helicobacter pylori*,^[7] imbalance of inflammatory and anti-inflammatory factors,^[8] and genetic factors^[9] are thought to be cause or contribute to MS. The classification of MS based on the severity of clinical symptoms includes: relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), progressive relapsing MS (PRMS), and primary progressive MS (PPMS).^[8] Depending on the brain regions attacked by the immune system, the patients suffer from a variety of symptoms including muscle weakness, paresthesia, ataxia, and visual disturbances.^[4] Successful therapies target the immune system indicating such system plays an important role in the disease activity.

Different combinations of the Janus kinas/Signal Transducer and Activator of Transcription (JAK/STAT) factors, utilized by over 70 cytokines as well as growth factors,^[10] initiate and orchestrate innate and adaptive immune responses.^[9] The regulation of such pathway seems to be critical; and in this regard, the Protein Inhibitor of Activated STAT (PIAS) proteins contribute in endogenous negative regulation of JAK/STAT pathway.^[11] Actually, dysregulation of JAK/STAT pathway has pathologically been implicated in some autoimmune diseases such as MS.^[3]

PIAS family consists of *PIAS1*, *PIAS2* (also known as PIASx), *PIAS3*, and *PIAS4* (also known as PIASy).^[12,13] *PIAS3* and *PIAS2* interact with STAT3 and STAT4, respectively; while *PIAS1* and *PIASy* interact with STAT1.^[13] It has been proposed that PIASs regulate the function of over 60 proteins through various molecular mechanisms^[14–16] including blocking the DNA binding activity of transcription factors, recruiting transcriptional co-receptors or co-activators, promoting protein somuylation, and sequestering transcription factors to certain subnuclear structures where co-receptor complexes are enriched.^[14,15] This family also acts as potential regulators of cell proliferation, inflammatory responses,^[17,18] and tumor development.^[19] The PIAS family expression is known to be related to many inflammatory and autoimmune diseases such as CNS inflammation,^[20–22] several oncogene conditions,^[23] type 2 diabetes mellitus,^[24] and viral infections.^[25]

Considering the effects of PIAS protein family in inflammatory conditions, we proposed the probable relation of gene expression of *PIAS1* and *PIAS2* to the autoimmune disease of MS and its severity. Such investigation may reveal a prognostic role of *PIAS1* and *PIAS2* in MS and may also suggest new therapeutic clues.

Methods and materials

Study subjects

This case–control study was performed on 30 patients with MS and 30 healthy volunteers in the Neurology Clinic of Shahid Beheshti Hospital in

Kashan city, Iran. The inclusion criteria were MS patients with ages between 20 and 60, course of disease (RRMS, PPMS, SPMS, and PRMS) identified according to McDonald criteria.^[26] All patients were treated with IFN- β . Any other chronic inflammatory/autoimmune diseases, clinical relapse, and glucocorticoid therapy during the past 1 month were considered as exclusion criteria. Healthy volunteers had no any history of chronic inflammatory and autoimmune diseases. In addition, healthy participants were matched to the patients for age and gender. The protocol was approved by the ethics committee of Kashan University of Medical Science. Written informed consent was obtained from all participants.

Clinical assessment

The severity of MS was evaluated using the Expanded Disability Status Scale (EDSS), a clinician-administered assessment scale evaluating the functional systems of the central nervous system. Today, EDSS is used to describe disease progression in patients with MS and to assess the effectiveness of therapeutic interventions in clinical trials.^[8,27] It consists of ordinal rating system ranging from 0 (normal neurological status) to 10 (death due to MS) in 0.5 increments interval.^[28] Patients were divided into two groups of mild and severe according to their EDSS score. Patients with the score of 0.5–4.5 were placed in mild group and those with the EDSS score of 5–9.5 were placed in severe group. Patients in remission phase were enrolled in the study.

Laboratory procedures

Peripheral venous blood samples were collected from all participants. Fresh peripheral blood mononuclear cells were separated from 2 ml of anticoagulated blood by Ficoll-Hypaque (Lymphodex, Inno-Train, Germany) density gradient centrifugation. Total RNA was extracted from peripheral blood mononuclear cells using High Pure RNA Isolation Kit (Cat No: 11828665001, Roche Applied Science, Germany), and cDNA was synthesized from the extracted RNA using Transcriptor First Strand cDNA Synthesis Kit (Cat No: 04897030001, Roche Applied Science, Germany). The amounts of *PIAS1* and *PIAS2* gene expression were measured through Taqman primer probe Comparative CT method using ABI 7300 Real-Time PCR system. β -*Actin* housekeeping gene was used as endogenous control.

Statistical analysis

The statistical indices of the *PIAS1* and *PIAS2* gene expression were analyzed by independent *t* and chi-square tests. Using Pearson's correlation coefficient, we calculated the correlations between variables. All analyses were

performed using the SPSS 16 software. Data were expressed as mean \pm standard deviation (SD).

Results

The expression of *PIAS1* gene was 9.13 ± 1.96 and 6.77 ± 1.99 in MS patients and healthy controls, respectively. The expression of *PIAS1* gene was significantly higher in MS patients than in healthy subjects ($P < .001$). However, there was no significant difference in the expression of *PIAS2* gene between two groups ($P = .39$) (Table 1).

We found a significant correlation between the gene expression of *PIAS1* with EDSS score ($R = 0.65$, $P < .001$). We also found a significant correlation between the expression of *PIAS2* gene with EDSS score ($R = 0.58$, $P < .001$). The correlation between *PIAS1* and *PIAS2* gene expression was 0.32 ($P = .012$) (Table 2).

The expression of *PIAS1* gene was significantly higher in severe forms (10.3 ± 1.25) of MS than that in mild forms (8.55 ± 2.01) ($P = .018$). There was also a significant increase in the gene expression of *PIAS2* according to EDSS score. The gene expression of *PIAS2* was 7.55 ± 1.39 in mild forms of MS and 8.7 ± 1.25 in severe forms ($P = .036$) (Table 3).

Using receiver operating characteristic (ROC) curve and Area under Curve (AUC), we determined the sensitivity and specificity of both *PIAS1* and *PIAS2* gene expression as diagnostic markers of MS severity. In the best cutoff point of *PIAS1* gene expression = 7.5, sensitivity and specificity were 80% and 63.3%, respectively; and AUC was determined as 0.802. Considering

Table 1. Basic and clinical characteristic of MS patients and healthy subjects.

Variants	MS patients (n = 30)	Healthy controls (n = 30)	P value
Age (years)	38.11 ± 13.88	29.92 ± 9.52	0.019
Gender (male/female)	(5/25)	(9/21)	0.222
Disease duration (years)	6.62 ± 4.18	–	–
Type of disease			
RRMS	23 (76.7%)	–	–
PPMS	0 (0%)	–	–
SPMS	5 (16.7%)	–	–
PRMS	2 (6.7%)	–	–
Type of treatment			
No drug	2 (7.1%)	–	–
Cinovex	20 (71.4%)	–	–
Rebief	5 (17.9%)	–	–
Others	1 (3.6%)	–	–
Family history			
Positive	4 (13.3%)	–	–
Negative	26 (86.7%)	–	–
Number of relapses	4.4 ± 4.1	–	–
Treatment duration (years)	3.58 ± 3.1	–	–
EDSS			
Mild (0.5–4.5)	20 (66.6%)	–	–
Severe (5–9.5)	10 (33.3%)	–	–
PIAS1	9.13 ± 1.96	6.77 ± 1.99	< 0.001
PIAS2	7.93 ± 1.43	7.53 ± 2.09	0.392

Table 2. Pearson’s correlation coefficient between the expression of *PIAS1* and *PIAS2* genes with other variables in multiple sclerosis (MS) patients.

	PIAS1		PIAS2	
	Correlation coefficient	P value	Correlation coefficient	P value
PIAS1	1	–	0.322	0.012
PIAS2	0.322	0.012	1	–
EDSS	0.656	< 0.001	0.588	0.001
Number of relapses	0.374	0.042	0.314	0.091
Treatment duration	0.294	0.121	0.225	0.241
Duration of disease	0.308	0.098	0.112	0.557
Age	0.346	0.012	–0.048	0.733

Table 3. *PIAS1* and *PIAS2* gene expression according to MS severity.

Variant	EDSS group	Number	Mean ± SD	P value
PIAS1	Mild (0.5–4.5)	20	8.55 ± 2.012	0.018
	Severe (5–9.5)	10	10.30 ± 1.252	
PIAS2	Mild (0.5–4.5)	20	7.55 ± 1.395	0.036
	Severe (5–9.5)	10	8.7 ± 1.252	

Table 4. Sensitivity and specificity of *PIAS1* and *PIAS2* gene expression in ROC curve.

Inflammatory markers	Optimal cutoff point	Sensitivity	Specificity	LR+	LR–	AUC
PIAS1	7.5	80%	63.3%	2.17	3.16	0.802
PIAS2	6.5	80%	33.3%	1.19	1.665	0.554

the optimal cutoff point of *PIAS2* gene expression = 6.5, sensitivity was 80% and specificity was 33.3%, and AUC was determined as 0.554 (Table 4) (Figure1).

Discussion

The present study aimed to evaluate the gene expression of *PIAS1* and *PIAS2* in MS patients. Rare studies evaluated PIASs in patients with MS. In a study by Catherine O’Doherty et al.,^[20] a significant relevancy between the gene expression of *PIAS1* and MS as well as responding to IFN-B treatment has been shown. Multi-allelic combinations analysis determined a JAK2-IL10RB-GBP1-PIAS1 combination was most significant. Evaluating the potential underlying biallelic patterns showed JAK2–IL10RB as the core element. However, the triplet including quartet with an additional *PIAS1* allele or *GBP1* allele demonstrated to be more influential.^[20] By evaluating RRMS peripheral blood mononuclear cells in the active and stable phases and its differences, a specific genomic signature for RRMS was determined, which *PIAS-1* were determined is related to the active phase of RRMS.^[29] However, in contrast to the results of these studies, there was no significant change in gene expression of *PIAS* genes between autism spectrum disorder patients and healthy subjects.^[30] The

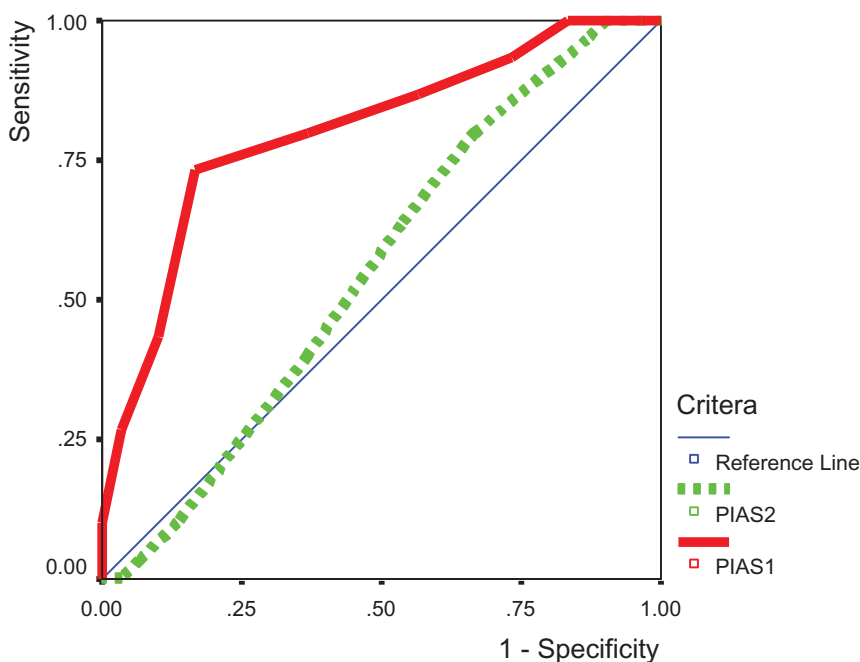


Figure 1. Sensitivity and specificity of *PIAS1* and *PIAS2* gene expression.

differences in diseases and its pathophysiology might explain the discrepancies among the results of studies.

Mechanistically, Members of PIAS family have been shown to interact with various STATs.^[14] PIAS3 and PIAS2 interact with STAT3 and STAT4, respectively; and PIAS1 and PIASy interact with STAT1. PIAS1 and PIAS3 exert their negative regulation by blocking the DNA binding of STAT1 and STAT3, respectively.^[13,15] On the other hand, PIAS2 and PIASy repress the transcriptional activity of STAT1 and STAT4 by recruiting co-repressor molecules such as histone deacetylases.^[13] Particularly, PIAS1 is a key regulator of the inflammation cascade of innate immunity.^[24] Such role of PIAS1 has been suggested in the control of autoimmune conditions such as insulin sensitivity in type 2 diabetes,^[24] essential thrombocytopenia,^[31] and primary hyperaldosteronism.^[32] PIAS1 overexpression has also been implicated in the regulation of several oncogenic conditions^[23] such as multiple myeloma,^[12] breast,^[33] prostate,^[34] colon,^[19] and lung cancer^[23] as well as infections of HIV^[35] and HSV1.^[36,37] In neurogenic tissues, PIAS1 expression is linked to neuronal plasticity as well as neuro-inflammation; and from this point of view, its expression may be associated with Huntington.^[22]

In line with the results of other study,^[29] our data showed an increased gene expression of PIAS1 in MS patients that was correlated to disease severity according to EDSS score. Considering the role of PIAS1 as an inhibitory factor of JAK-STAT pathway, we may conclude that the overexpression of PIAS1 in MS patients acts as a protective mechanism to decrease the disease severity.

Although, such mechanism would not be adequate to compensate the disease development, it may suggest a possible therapeutic clue in autoimmune condition of MS. Such association has also been shown in the case of other inflammatory cytokine inhibitors like SOCS1 and SOCS3.^[38,39] The relationship described above between *PIAS1* and clinical conditions has also been shown in the case of *PIAS2*. For example, *PIAS2* is overexpressed in several malignancies such as malignant melanoma^[40] as well as thyroid cancer^[41] and viral infections such as HBV and HCV.^[25,42] *PIAS2* also plays some roles in neuronal system. A study suggested the novel role of *PIAS2* somuylation in brain development and plasticity.^[43] From this point of view, *PIAS2* alterations may contribute in some brain-related diseases such as schizophrenia.^[21] In a recent study, Vavougios et al. showed a significant relation between *PIAS2* gene expression and MS disease.^[44] Unlike the latter study,^[44] we found no significant relation between gene expression of *PIAS2* and MS disease. However, the gene expression of *PIAS2* was associated to disease severity. According to EDSS score, *PIAS2* gene expression was significantly higher in severe forms of MS than in mild forms. Our study showed a significant correlation between the expression of both *PIAS1* and *PIAS2* genes with disease severity according to EDSS score. This finding suggests *PIAS1* and *PIAS2* as predictors of severe forms of MS which is independent of sex, treatment duration, and the duration of the disease. We also demonstrated that the specificity of *PIAS1* gene expression was higher than *PIAS2* gene expression in the diagnosis of MS. It may reveal the potential role of *PIAS1* as a new diagnostic factor for MS disease.

The limitation of our study was that we did not monitor the gene expression of *PIAS1* and *PIAS2* longitudinally. This limitation allowed just a cross-sectional analysis of their changes of only limited robustness. Other factors except for *PIAS1* and *PIAS2*, such as other *PIAS* genes may affect the MS severity which should be determined in future studies. Unfortunately, due to funding limitations, we did not assess the gene expression of other *PIAS* genes in the patients with MS. Moreover, further studies are needed to confirm those findings with larger sample size.

Conclusion

Our findings show the elevation of expression of *PIAS1* gene in patients with MS compared to healthy controls. We demonstrated that the *PIAS1* and *PIAS2* gene expression is correlated to disease severity according to EDSS. These findings may reveal a new diagnostic approach to MS severity and also suggests new therapeutic approaches to this disease.

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Declaration of interest

The authors declare no conflict of interest.

Authors' contributions

HN contributed in the conception or design of the work, analysis and drafting of the manuscript. EK, HA, and SA contributed in conception and manuscript drafting. The final version was confirmed by all authors for submission

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