Mediators of Inflammation

Matrix Metalloproteinases in Human Pathology and Physiology

Lead Guest Editor: Ewa Matuszczak
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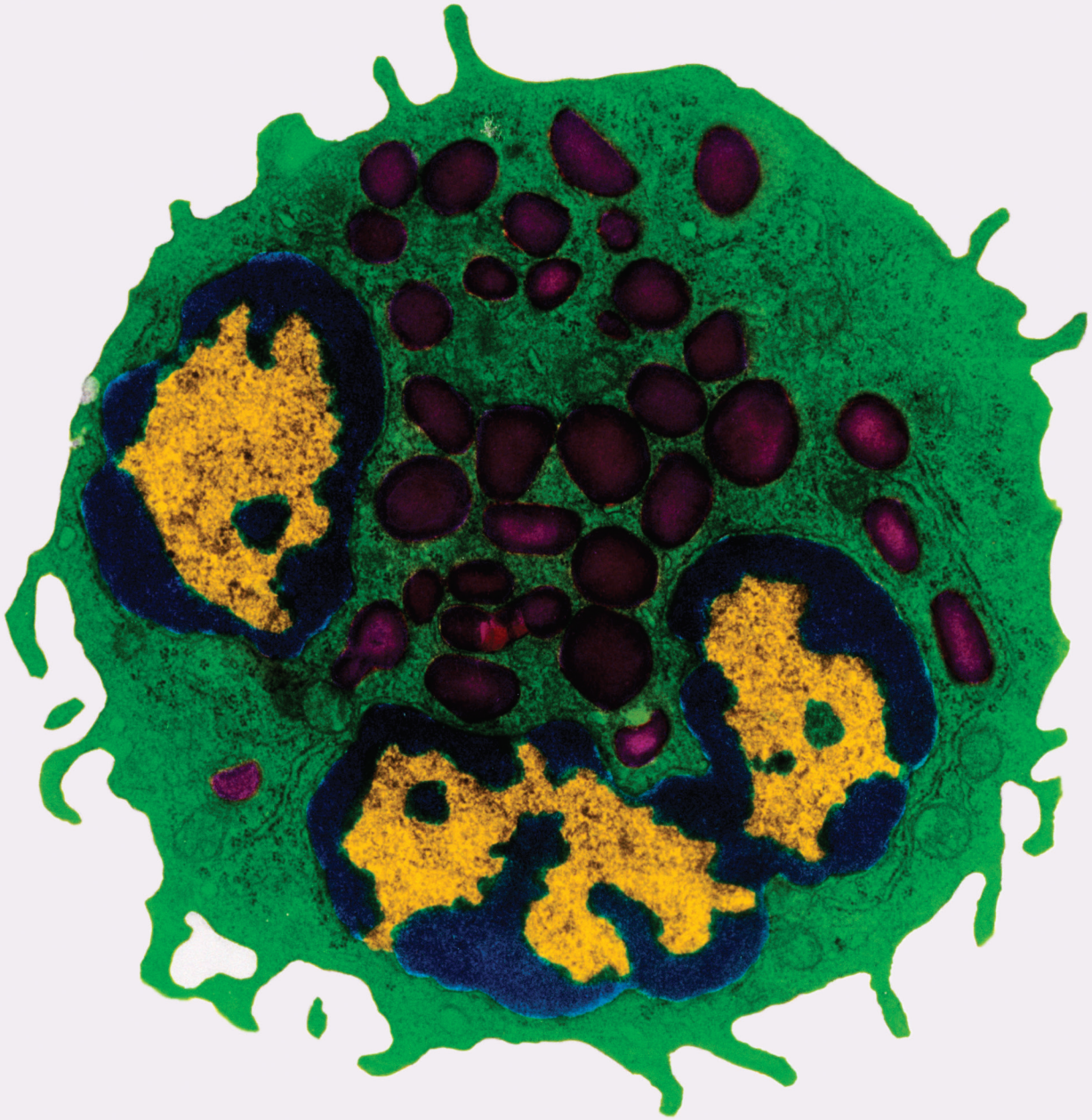

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Research Article

Assessment of Selected Matrix Metalloproteinases (MMPs) and Correlation with Cytokines in Psoriatic Patients

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Metalloproteinases (MMPs) and cytokines have a great impact on the pathogenesis of psoriasis. Cytokines, as key mediators of inflammation and autoimmune processes, play a crucial role in the regulation of MMP expression in different cell types. Parallel, MMPs have an influence on cytokine production. This interaction was not well recognized in psoriatic patients. Our study is aimed at assessing the selected serum MMP levels and their correlations with cytokine levels in the serum of psoriatic patients. We observed a significantly elevated level of pro-MMP-1 and MMP-9 in psoriatic patients' serum in comparison to the control group. We did not observe any statistically significant differences of MMP-3 and pro-MMP-10 between the psoriatic patients and the control group. We did not observe any statistically significant differences in all the studied MMP levels between the patients with and without psoriatic arthritis (PsA). MMP-3 level correlated positively with proinflammatory cytokines, i.e., IL-12p/70, IL-17A, and TNF-α as well as MMP-3 and pro MMP-1 correlated positively with IL-4 in the psoriatic patients. In the control group, a positive correlation between pro-MMP-1 and TNF-α was found. These results confirm MMPs and Th1 and Th17 cytokine interaction in the inflammatory regulation in psoriasis.

1. Introduction

Psoriasis is a frequent skin disease whose pathogenesis is still not fully understood. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes involved in many physiological processes like tissue remodeling, cell migration, angiogenesis, and epithelial apoptosis [1]. According to MMP structure, substrate specificity, and function, MMPs can be divided into 9 subgroups: collagenases (MMP-1, MMP-8, and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3 and MMP-10), stromelysin-like MMPs (MMP-11 and MMP-12), matrilysins (MMP-7 and MMP-26), transmembrane MMPs (MMP-14, MMP-15, MMP-16, and MMP-24), glycosylphosphatidylinositol- (GPI-) type MMPs (MMP-17 and MMP-25), MMP-19-like MMPs (MMP-19 and MMP-28), and other MMPs (MMP-18, MMP-20, and MMP-23) [2, 3]. Many metalloproteinases are involved in psoriasis pathogenesis. MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-12, and MMP-19 influence the migration of epidermal keratinocytes in the psoriatic epidermis [1, 4]. MMP-1 induction increases also the mobility of dermal fibroblasts [1, 5]. High MMP-9 levels can inhibit the mobility of dermal fibroblasts and epidermal keratinocytes [6, 7] and can slow down skin healing [8]. The correct balance between the different MMPs is also important for microcapillary permeability. The changes in MMP-2, MMP-3, MMP-9, and MMP-12 expression in psoriatic skin may indicate its predisposition to the growth of new capillaries [1, 9, 10]. Many MMPs are involved in the regulation of inflammatory response in psoriasis. The infiltration

of lymphocytes follows the presence of monocytes, macrophages, and neutrophils in the epidermis. Monocytes express MMP-1, MMP-7, MMP-8, and MMP-9; macrophages express MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-10, MMP-12, and MMP-13; and neutrophils secrete MMP-8, MMP-9, and MMP-25 [1, 11]. The activation of distinct T cell subsets drives the maintenance phase of psoriatic inflammation. Especially, the Th1 cytokines, interferon- (IFN-) γ, tumor necrosis factor- (TNF-) α, and interleukin- (IL-) 12, and Th17 cytokines, IL-17, IL-21, IL-22, IL-25, IL-26, and TNF-α, are responsible for keratinocyte proliferation and the maintenance of inflammation. Keratinocytes participate actively in the inflammatory cascade through cytokine (IL-1, IL-6, IFN-γ, and TNF-α), chemokine, and antimicrobial peptide (AMP) secretion [12]. All these processes lead to tissue remodeling in psoriasis.

Our study is aimed at assessing the selected serum MMP levels and their correlations with cytokine levels in the serum of psoriatic patients.

2. Materials and Methods

2.1. Studied Group. The study was conducted in patients hospitalized in the Department of Dermatology, Venereology and Pediatric Dermatology, Medical University of Lublin, Poland, because of psoriasis exacerbation. The study comprised 58 male psoriatic patients and 29 male healthy controls. 36% of patients with coexisting joint problems met the Classification of Psoriatic Arthritis (CASPAR) criteria for psoriatic arthritis (PsA). All PsA patients had a polyarticular, asymmetrical subset of the disease (more than 5 joints were affected). Demographic data, medical history, and serum for assessment of the selected MMPs and cytokines were collected from all participants.

2.2. Assessment of Psoriasis Severity. The skin lesion severity was assessed with the use of PASI (Psoriasis Area and Severity Index), BSA (Body Surface Area), and PGA (Physician Global Assessment) scores.

2.3. Assessment of MMPs' Serum Concentrations in Psoriatic Patients and Controls. Blood samples were collected from psoriatic patients and controls and were centrifuged for 15 minutes at 1000 x g. Then, serum samples were subdivided into small aliquots to be stored at -80°C until tested for MMP levels. In the studied psoriatic patients as well as the control group, the concentrations of pro-MMP-1, MMP-3, MMP-9, and pro-MMP-10 were determined with the use of R&D Systems kits (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions.

2.4. Assessment of Cytokines' Serum Concentrations in Psoriatic Patients and Controls. The concentrations of IL-12p70, IFN-γ, IL-17A, IL-2, IL-10, IL-9, IL-22, IL-6, IL-13, IL-4, IL-5, IL-1beta, and TNF-α were detected with eBioscience Th Cell Differentiation Th1/Th2/Th9/Th17/Th22 FlowCytomix™ Multiple Analyte Detection System according to the manufacturer's protocol.

The study was approved by the Polish Local Ethics Committee.

Table 1: Clinical data of psoriatic patients.

	Study group ($N = 58$)
Age (years), min–max, $M \pm$ SD	26-73, 46.7 ± 13.9
Duration of psoriasis (years), min–max, $M \pm$ SD	1-45, 20.9 ± 12.0
Age of psoriasis onset (years), min–max, $M \pm$ SD	8-55, 25.9 ± 10.3
Duration of psoriatic arthritis, min–max, $M \pm$ SD	1-25, 10.0 ± 6.3
Positive family history, n (%)	21 (36.21)
PASI, min–max, $M \pm$ SD	8-57, 23.6 ± 12.0
BSA (%), min–max, $M \pm$ SD	4-85, 27.9 ± 20.8
PGA, n (%)	
2	8 (13.79)
3	33 (56.90)
4	12 (20.69)
5	5 (8.62)

2.5. Statistical Analyses. Statistical analysis was performed using the STATISTICA software. Mean values (M) and standard deviation (SD) were calculated for continuous variables or absolute number (n) and relative number (%) of occurrences of items for categorical variables. The following statistical tests were applied: Student's t-test to compare age, MMPs, and cytokine levels between study and control groups; Mann-Whitney's U test to compare MMPs and cytokines in patients with and without PsA; and Pearson's r correlation coefficient to correlate MMPs with cytokines as well as to correlate MMPs and cytokines with severity of psoriasis. In all statistical tests, the level of significance was set at 0.05.

3. Results and Discussion

3.1. Sociodemographic Characteristics. Sociodemographic characteristics of psoriasis patients are presented in Table 1.

The control group's age (min–max 26-72, $M \pm$ SD 47.3 ± 11.8) did not significantly differ from the studied psoriatic patients' age ($p = 0.842$).

3.2. Serum MMP and Cytokine Concentrations in Psoriatic Patients and Control Group. Comparison of metalloproteinases and cytokines between psoriatic patients and control group is presented in Table 2. We observed a significantly elevated level of pro-MMP-1 and MMP-9 in psoriatic patients' serum in comparison to the control group ($p = 0.006$ and $p = 0.017$, respectively). In a healthy skin, MMP-9 is expressed in keratinocytes and immune cells, like lymphocytes, macrophages, eosinophils, and mast cells [13]. Neutrophils synthesize MMP-9 during their maturation in the bone marrow and store it in specific neutrophil granules. MMP-9 secretion is stimulated by a variety of external stimuli and is associated with the activation of cells. Loss of MMP-9 prolongs inflammation in contact hypersensitivity [2, 14]. In previous studies, MMP-1 and MMP-9 expression was observed to be significantly elevated in a psoriatic skin

Table 2: Comparison of metalloproteinases and cytokines between psoriatic patients and control group. ∗ indicates statistically significant differences.

Metalloproteinase or cytokine	Study group ($N = 58$)	Control group ($N = 29$)	p
Pro-MMP-1 (ng/mL)	7.75 ± 4.48	5.08 ± 3.47	0.006*
Total MMP-3 (ng/mL)	17.52 ± 9.39	19.22 ± 7.08	0.391
MMP-9 (ng/mL)	1165.16 ± 472.93	903.08 ± 473.27	0.017*
Pro-MMP-10 (pg/mL)	492.98 ± 229.46	554.76 ± 253.69	0.256
IL-12p70 (pg/mL)	1.01 ± 1.65	0.92 ± 1.43	0.822
IL-17A (pg/mL)	4.83 ± 6.75	3.2 ± 5.11	0.256
IL-2 (pg/mL)	18.87 ± 20.41	13.72 ± 18.29	0.255
IL-22 (pg/mL)	169.28 ± 65.74	163.46 ± 98.60	0.744
IL-6 (pg/mL)	1.41 ± 1.55	0.79 ± 0.99	0.050*
IL-4 (pg/mL)	1.26 ± 2.69	0.93 ± 1.95	0.560
IL-5 (pg/mL)	1.72 ± 2.69	1.49 ± 2.62	0.700
IL-1beta (pg/mL)	14.35 ± 26.95	25.41 ± 39.85	0.130
TNF-α (pg/mL)	6.35 ± 6.72	7.36 ± 7.95	0.535

Table 3: Comparison of metalloproteinases and cytokines between psoriatic patients with and without arthritis.

Metalloproteinase or cytokine	Psoriasis arthritis ($N = 21$)	Psoriasis vulgaris ($N = 37$)	p
Pro-MMP-1 (ng/mL)	7.75 ± 3.61	7.75 ± 4.96	0.633
Total MMP-3 (ng/mL)	18.58 ± 11.34	16.91 ± 8.19	0.821
MMP-9 (ng/mL)	1243.84 ± 610.65	1120.51 ± 375.92	0.728
Pro-MMP-10 (pg/mL)	545.63 ± 290.93	463.10 ± 183.86	0.523
IL-12p70 (pg/mL)	1.25 ± 2.10	0.87 ± 1.34	0.619
IL-17A (pg/mL)	5.81 ± 7.07	4.28 ± 6.60	0.236
IL-2 (pg/mL)	23.31 ± 24.35	16.34 ± 17.67	0.226
IL-22 (pg/mL)	175.47 ± 64.79	165.76 ± 66.91	0.610
IL-6 (pg/mL)	1.21 ± 1.27	1.52 ± 1.70	0.484
IL-4 (pg/mL)	1.39 ± 2.99	1.18 ± 2.54	0.786
IL-5 (pg/mL)	1.57 ± 1.78	1.80 ± 3.11	0.336
IL-1beta (pg/mL)	12.33 ± 25.12	15.50 ± 28.21	0.654
TNF-α (pg/mL)	6.46 ± 5.74	6.29 ± 7.29	0.540

[15–18]. High MMP-1 and MMP-9 levels were also noticed in psoriatic patients' serum [19–23]. Choi et al. [24] demonstrated that NB-UVB irradiation upregulated MMP-1 expression at both the mRNA and protein levels. However, MMP-3 and MMP-9 were decreased after NB-UVB treatment [22]. Anti-TNF treatment decreased blood levels of MMP-1 and MMP-9 [20, 25]. MMP-9 lesional and serum levels were also reduced after therapy with anti-TNF in the study of Cordiali-Fei et al. [26].

We did not observe any statistically significant differences in MMP-3 and MMP-10 between the psoriatic patients and the control group ($p = 0.391$ and $p = 0.256$, respectively). In previous studies, MMP-3 was not detected in a healthy skin [27]. In a psoriatic skin, MMP-3 level was positively correlated with the level of the proinflammatory cytokine IL-22 [28]. MMP-3 appears to be essential for skin inflammation [2]. Elevated level of MMP-3 was discovered in the serum from patients with psoriasis compared to the healthy controls [22, 29]. MMP-10 was not detected in the psoriatic skin [16]. Diani et al. [23] observed elevated serum MMP-10 levels in psoriatic patients. It was noticed that the serum concentration of MMP-3 in PsA patients decreased after anti-TNF-α treatment [25, 30–33].

The mean fluorescence intensity of IFN-γ, IL-10, IL-9, and IL-13 was not intensive enough to calculate the cytokine concentration. In the psoriatic patients, IL-6 level was higher than in the control group and this difference was statistically significant ($p = 0.05$). The results were in accordance with

Table 4: Correlations between metalloproteinases and cytokines and severity of psoriasis ($N = 58$). $*$ indicates statistically significant differences.

Metalloproteinase or cytokine		PASI	BSA	sPGA
Pro-MMP-1 (ng/mL)	r	0.087	0.065	0.157
	p	0.517	0.631	0.241
Total MMP-3 (ng/mL)	r	-0.286	-0.196	-0.264
	p	0.030*	0.141	0.045*
MMP-9 (ng/mL)	r	-0.034	0.017	0.097
	p	0.800	0.898	0.468
Pro-MMP-10 (pg/mL)	r	-0.209	-0.147	-0.013
	p	0.116	0.270	0.920
IL-12p70 (pg/mL)	r	-0.154	-0.034	-0.092
	p	0.249	0.799	0.492
IL-17A (pg/mL)	r	-0.069	-0.098	-0.058
	p	0.608	0.464	0.667
IL-2 (pg/mL)	r	-0.076	-0.102	-0.094
	p	0.571	0.445	0.484
IL-22 (pg/mL)	r	-0.035	-0.113	-0.175
	p	0.794	0.398	0.189
IL-6 (pg/mL)	r	-0.090	-0.021	-0.101
	p	0.500	0.879	0.452
IL-4 (pg/mL)	r	-0.140	-0.083	-0.154
	p	0.295	0.537	0.248
IL-5 (pg/mL)	r	0.240	0.248	0.240
	p	0.069	0.060	0.070
IL-1beta (pg/mL)	r	0.115	0.092	0.167
	p	0.390	0.493	0.212
TNF-α (pg/mL)	r	-0.157	-0.120	-0.157
	p	0.239	0.370	0.238

previous studies which demonstrated the increased IL-6 serum level in psoriatic patients compared to healthy controls [34–37]. The decrease of serum IL-6 level was observed after MTX treatment [36], UVB radiation, topical steroids, infliximab, and adalimumab [38, 39]. On the other hand, ustekinumab did not affect serum IL-6 levels [39]. We did not observe any statistically significant differences in other studied cytokine levels between the psoriatic patients and control group.

3.3. Serum MMP and Cytokine Concentrations in Psoriatic Patients with and without Arthritis. Comparison of metalloproteinases and cytokines between psoriatic patients with and without arthritis is presented in Table 3. We did not observe any statistically significant differences in the analyzed MMP and cytokine levels between the psoriatic patients with and without arthritis. However, some authors recognized MMP-1 and MMP-3 as the biomarkers of PsA [25, 29, 40–42]. Diani et al. [23] observed elevation of serum MMP-1, MMP-9, and MMP-10 both in psoriatic patients and in patients with PsA.

3.4. Correlations of MMPs and Cytokines with Psoriasis Severity. Correlations of MMP and cytokine levels with severity of psoriasis are presented in Table 4. In our study, only MMP-3 levels correlated negatively with the severity of psoriasis measured by PASI and sPGA. There are not many studies to compare these results. Diani et al. [23] observed no statistically significant correlations between MMP-1, MMP-3, and MMP-9 levels and duration of psoriasis or PsA as well as the severity of the disease calculated with PASI. Flisiak et al. [19] reported that the elevated levels

Table 5: Correlations between metalloproteinases and cytokines in psoriatic patients and in control group. ∗ indicates statistically significant differences.

Cytokine		Study group ($N = 58$)				Control group ($N = 29$)			
		Pro-MMP-1 (ng/mL)	Total MMP-3 (ng/mL)	MMP-9 (ng/mL)	Pro-MMP-10 (pg/mL)	Pro-MMP-1 (ng/mL)	Total MMP-3 (ng/mL)	MMP-9 (ng/mL)	Pro-MMP-10 (pg/mL)
IL-12p70 (pg/mL)	r	0.091	0.450	0.038	0.114	-0.177	-0.177	-0.251	-0.074
	p	0.500	0.001*	0.776	0.393	0.357	0.358	0.189	0.702
IL-17A (pg/mL)	r	0.229	0.312	0.029	-0.034	-0.070	0.126	0.031	0.171
	p	0.084	0.017*	0.828	0.800	0.717	0.514	0.873	0.375
IL-2 (pg/mL)	r	-0.108	0.119	-0.194	0.190	0.133	0.092	-0.127	-0.023
	p	0.419	0.374	0.144	0.152	0.491	0.635	0.511	0.907
IL-22 (pg/mL)	r	-0.032	0.177	-0.174	-0.124	0.069	-0.029	0.143	0.172
	p	0.812	0.185	0.193	0.356	0.722	0.883	0.460	0.372
IL-6 (pg/mL)	r	0.153	0.152	-0.003	0.136	0.186	-0.042	0.211	-0.066
	p	0.253	0.254	0.980	0.309	0.334	0.828	0.271	0.733
IL-4 (pg/mL)	r	0.274	0.414	0.068	-0.011	-0.067	0.016	-0.098	0.228
	p	0.037*	0.001*	0.614	0.936	0.728	0.933	0.614	0.234
IL-5 (pg/mL)	r	0.133	-0.101	0.008	-0.166	-0.066	0.277	-0.014	-0.128
	p	0.320	0.450	0.951	0.212	0.734	0.146	0.941	0.507
IL-1beta (pg/mL)	r	0.012	0.113	0.068	0.054	0.205	0.169	-0.031	-0.185
	p	0.931	0.401	0.611	0.688	0.286	0.381	0.875	0.337
TNF-α (pg/mL)	r	0.071	0.267	-0.059	0.045	0.645	0.023	-0.122	-0.169
	p	0.599	0.043*	0.663	0.738	<0.001*	0.905	0.530	0.382

of MMP-1 in psoriatic plasma and the levels of MMP-1 were inversely correlated to disease severity. The correlation between the levels of MMP-9 and the severity of disease was noticed by Buommino et al. [20]. Serum concentration of MMP-10 presented a negative correlation with PsA duration and a positive correlation with PASI in psoriatic patients [23].

3.5. Correlations between MMPs and Cytokines. Correlations between MMPs and cytokines in psoriatic patients and in the control group are presented in Table 5. Positive correlations between MMP-3 level and proinflammatory cytokines IL-12p/70, IL-17A, and TNF-α were observed in the psoriatic patients ($p = 0.001$, $p = 0.017$, and $p = 0.043$, respectively, Figure 1). MMP-3 and pro-MMP-1 correlated positively with IL-4 in the psoriatic patients ($p = 0.001$ and $p = 0.037$, respectively). In the control group, a positive correlation between pro-MMP-1 and TNF-α was found ($p < 0.001$).

It is already recognized that both MMPs and cytokines have the great impact on the pathogenesis of psoriasis [1, 2, 12]. Cytokines, as key mediators of inflammation and autoimmune processes, play a crucial role in the regulation of MMP expression in different cell types [13, 43, 44]. It was noticed that MMP-1 is upregulated by IL-1beta and TNF-α. IL-4 has an inhibitory effect, whereas IFN-γ and IL-6 can present both stimulating and downregulating actions [44]. MMP-3 and MMP-10 production is elevated by IL-1beta and TNF-α. TGF-beta and IFN-γ presented the inhibitory effect. The upregulation of MMP-9 was observed by TGF-beta, TNF-α, IFN-γ, and IL-1beta [18, 43, 45, 46]. Some studies have also shown induction of MMP-9 protein expression by IL-18 [47, 48]; others did not observe the IL-18 influence on MMP-9 expression [43]. Anti-inflammatory Th2 cytokines, such as IL-4 and IL-10, are reported to downregulate MMP-9 [43, 47, 49]. IL-12 was shown to downregulate MMP-9 in stromal cells [50] and has no effect on MMP-9 induction in peripheral blood monocytes or T cells [43, 47]. Elevated IL-18, IL-12, and TNF-α levels observed in autoimmune disorders may influence the increased MMP activity associated with these conditions. On the other hand, MMPs have also an impact on the inflammatory process by activation of TGF-beta or degradation of IL-1beta, as well as by releasing TNF-α or by influence on cytokine receptors such as IL-6 and TGF-alpha [43].

We decided to study the correlations between Th1/Th2/Th9/Th17/Th22 cytokines and selected MMPs, because the understanding of the cytokine and MMP interactions is essential for better diagnosis and treatment of psoriasis, which is nowadays recognized as autoimmune and autoinflammatory disease. The results of Amezcua-Guerra et al. [51] suggest that sera from patients with psoriasis may influence the response of monocytes to stimulation with IFN-γ observed as the increased production of MMP-9.

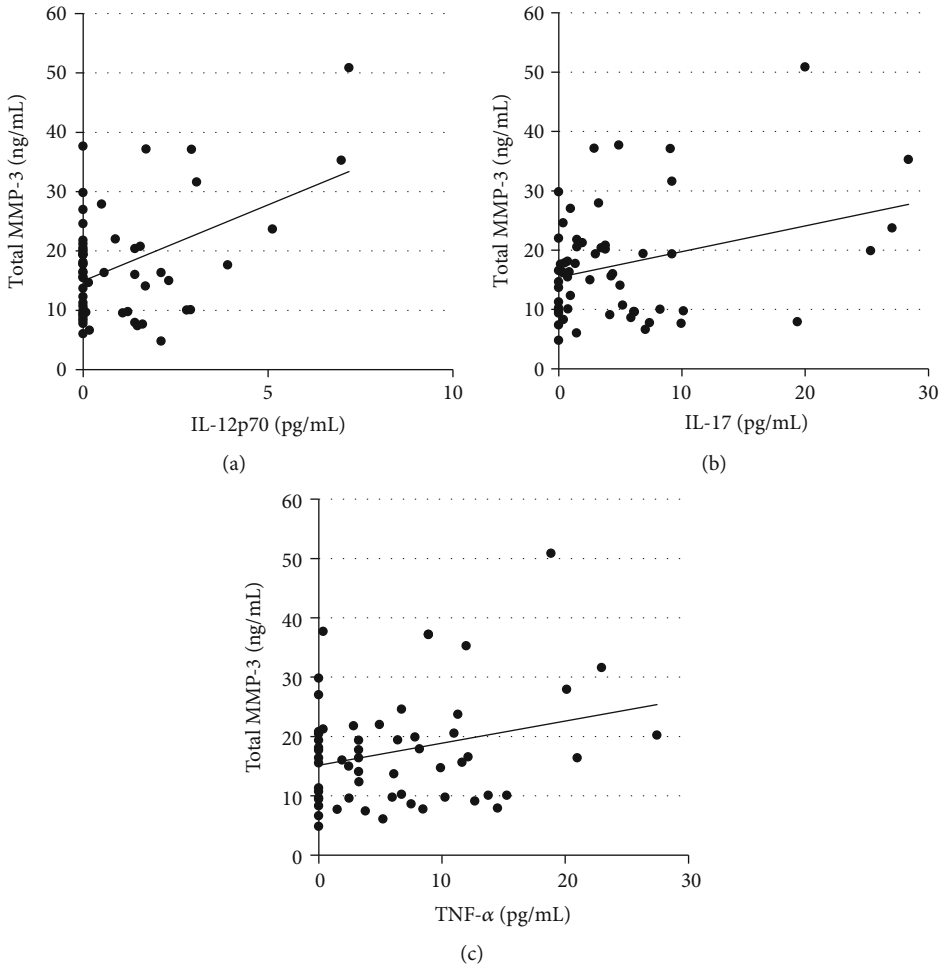

Figure 1: Correlations of total MMP-3 with (a) IL-12p70, (b) IL-17A, and (c) TNF-α.

These results suspect the existence of a primed state in inflammatory cells of psoriatic patients [51]. However, according to the PubMed search, there are no studies comparing the levels of MMPs and cytokines in psoriatic patients. There are only a few studies comparing the levels of MMP-1, MMP-3, or MMP-9 before and after anti-TNF treatment [20, 25, 26, 30–33]. The decrease of MMPs after anti-TNF treatment can suggest the important influence of TNF-α on MMPs' production.

4. Conclusions

Metalloproteinases and cytokines have the great impact on the pathogenesis of psoriasis. However, interactions between them in psoriasis are not as well recognized as in other autoimmune disorders. Positive correlations between MMP-3 level and proinflammatory cytokines IL-12p/70, IL-17A, and TNF-α recognized in our study confirm MMPs and Th1 and Th17 cytokines' cross talk in the inflammatory regulation in psoriasis.

Data Availability

The data used in this study are available from the corresponding author upon request.

Ethical Approval

All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflicts of Interest

The authors declare no competing interests.

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Research Article

Impact of *MMP2* rs243865 and *MMP3* rs3025058 Polymorphisms on Clinical Findings in Alzheimer's Disease Patients

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Alzheimer's disease (AD) is a chronic neurodegenerative disease of the central nervous system with higher prevalence in elderly people. Despite numerous research studies, the etiopathogenesis of AD remains unclear. Matrix metalloproteinases (MMPs) are endopeptidases involved in the cleavage of extracellular matrix proteins and basement membrane compounds. In the brain, the pathological role of MMPs includes the disruption of the blood-brain barrier leading to the induction of neuroinflammation. Among various MMPs, MMP-2 and MMP-3 belong to candidate molecules related to AD pathology. In our study, we aimed to evaluate the association of *MMP2* rs243865 and *MMP3* rs3025058 polymorphisms with AD susceptibility and their influence on age at onset and MoCA score in patients from Slovakia. Both MMP gene promoter polymorphisms were genotyped in 171 AD patients and 308 controls by the PCR-RFLP method. No statistically significant differences in the distribution of *MMP2* rs243865 (-1306 C>T) and *MMP3* rs3025058 (-1171 5A>6A) alleles/genotypes were found between AD patients and the control group. However, correlation with clinical findings revealed later age at disease onset in *MMP2* rs243865 CC carriers in the dominant model as compared to T allele carriers (CC vs. CT+TT: 78.44 ± 6.28 vs. 76.36 ± 6.39, $p = 0.036$). The results of *MMP3* rs3025058 analysis revealed that 5A/6A carriers in the overdominant model tended to have earlier age at disease onset as compared to other *MMP3* genotype carriers (5A/6A vs. 5A/5A+6A/6A: 76.61 ± 5.88 vs. 78.57 ± 6.79, $p = 0.045$). In conclusion, our results suggest that *MMP2* rs243865 and *MMP3* rs3025058 promoter polymorphisms may have influence on age at onset in AD patients.

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disease of the central nervous system characterized by progressive memory loss, confusion, and cognitive dysfunction. It is the cause of 60 to 70% of dementia cases. The AD prevalence is estimated at 4.4% in people aged 65 years to 22% in people aged 90 years and older [1]. There are two types of AD: early-onset AD that manifests in people under the age of 65 and the much more common late-onset AD that affects people over 65. The major risk factors for AD are advanced age, genetic predisposition, chronic diseases, head injuries, and other factors [2]. The histopathological characteristics in the AD brain include senile plaques composed of the extracellular accumulation of the amyloid β peptide and intraneuronal fibrillar aggregates of hyperphosphorylated tau proteins [3, 4].

The etiopathogenesis of AD remains unclear. One of the possible mechanisms of AD progression is related to

neuroinflammation caused by matrix metalloproteinases (MMPs). MMPs are calcium-dependent zinc-containing endopeptidases that are involved in many physiological processes via cleavage of extracellular matrix components and basement membrane compounds. In the brain, MMPs play various roles involving neurogenesis, axonal growth, angiogenesis, tissue remodeling after injury, and inflammation [5]. To date, there are more than 25 MMPs described in humans. They are classified according to their abilities to cleave substrates or domain organization in collagenases (MMP-1, MMP-8, and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10, and MMP-11), matrilysins (MMP-7 and MMP-26), membrane-type (MT) MMPs (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24, and MMP-25), and others (MMP-12, MMP-19, MMP-20, MMP-21, MMP-23, MMP-27, and MMP-28) [5].

The role of MMPs was studied in different neurodegenerative diseases such as multiple sclerosis (MS), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and Alzheimer's disease [6]. In PD pathogenesis, MMP-3 has been involved in dopaminergic neurodegeneration, neuroinflammation, and barrier leakage [7]. Regarding MS, it was found that MMP-9 digests myelin basic protein, which causes demyelination and drives MS progression [8]. Furthermore, the MMP-9 degrade the endothelial basement membrane, which facilitates leukocyte extravasation and their migration into the brain [9]. The pathological role of MMP-9 was also reported in relation to amyotrophic lateral sclerosis. In the $SOD1^{G93A}$ transgenic mouse model for ALS, genetic deletion of MMP-9 as well as its pharmacological inhibition has delayed muscle denervation [10]. In addition, some studies reported increased levels of MMP-9 in plasma and CSF of ALS patients considering MMP-9 as an early biomarker of the disease [11, 12]. Finally, MMP-3 and MMP-9 levels were increased in CSF from Huntington's disease patients and correlated with disease severity [13]. It was also found that MMP-10 cleaves huntingtin in the neurons to small N-terminal fragments thought to be toxic [14].

In our study, we have focused on MMP-2 and MMP-3 due to their involvement in the processes related to neurodegeneration. MMP-2 is a 72 kDa protein also known as gelatinase A. It is produced by most connective tissue cells including endothelial cells, osteoblasts, fibroblasts, and myoblasts cells. It is capable of hydrolyzing type IV collagen, which is the main component of the basement membrane followed by elastin, endothelin, fibroblast growth factor, plasminogen, TGF-β, and MMP-9 and MMP-13. The synthesis and secretion of MMP-2 can be stimulated by a variety of stimuli during various pathological processes, such as tumor invasion, atherosclerosis, and inflammation. In the brain, MMP-2 is crucial for neurite outgrowth and neuronal plasticity [15].

MMP-3, also known as stromelysin1, is a 54 kDa protein produced by various cells including the macrophages, stromal fibroblasts, endothelial cells, immune cells, and synovial cells. It cleaves an extensive range of extracellular matrix (ECM) molecules including collagen types 3, 4, 5, 9, 10, and 11, fibronectin, elastin, gelatins, laminins, and proteoglycans. In addition, it is involved in the proteolysis of various adhesion molecules like E-cadherin and L-selectin, growth factors like heparin-binding EGF-like growth factor, cytokines like TNF and IL-1β, and proforms of other MMPs like proMMP-1, proMMP-3, and proMMP-9. In the brain, MMP-3 is essential for neurite outgrowth, neuronal plasticity, and remyelination [16, 17].

Both MMP-2 and MMP-3 are assumed to be involved in AD pathogenesis. It was shown that AD patients have elevated levels of MMP-3 in the brain, especially in the microglia of white matter and senile plaques [18]. Increased MMP-3 levels in serum, plasma, and CSF of AD patients were observed as well [19–24]. In AD patients, there was an increase in MMP-2 expression in astrocytes surrounding amyloid plaque and a decrease in the MMP-2 plasma level compared to controls [25–27]. MMP-2 and MMP-3 were shown to cleave Aβ peptides to nontoxic fragments demonstrating a protective role in AD [28–30]. In addition, MMP-3 degrades tau protein preventing its aggregation [31]. On the other hand, increased MMP-2 and MMP-3 expression induced by toxic Aβ1-42 oligomers is related to the disruption of the blood-brain barrier (BBB) leading to neuroinflammation and AD progression [32–34].

It is known that the expression level may be influenced by functional polymorphic variants in the gene promoters. *MMP2* gene is located on chromosome 16q21 in the human genome, whereas *MMP3* gene is located on chromosome 11q22.3 [5]. *MMP2* rs243865 (-1306 C>T) and *MMP3* rs3025058 (-1171 5A>6A) are two common single nucleotide polymorphisms in the promoter region, which are associated with modified MMP expression levels. The role of *MMP2* and *MMP3* promoter gene polymorphisms in predisposition to AD development has been analysed in only few studies with controversial results. An association of *MMP3* -1171 5A allele and 5A/5A genotype with the risk of AD in *APOE*ε4-positive patients was reported in two studies [35, 36]. Conversely, the association of *MMP3* -1171 6A allele with risk of AD was also found [37]. In other studies, no association of *MMP3* -1171 5A>6A polymorphism with AD susceptibility and clinical findings was observed [38–40].

Therefore, the objective of our study was to evaluate the association of *MMP2* rs243865 (-1306 C>T) and *MMP3* rs3025058 (-1171 5A>6A) polymorphisms with AD susceptibility and their influence on clinical findings in patients from Slovakia.

2. Materials and Methods

2.1. Study Groups. The investigated patient group included 171 unrelated individuals (53 men and 118 women, mean age: 79.68 ± 6.03 years) meeting criteria for late-onset Alzheimer's disease according to the ICD-10 classification [41]. AD patients were recruited at random via several psychiatric clinics throughout Slovakia. The average age at disease onset was 77.56 ± 6.39 years. The reference cohort in our case-control study comprised 308 unrelated volunteers (111 men and 197 women with a mean age of 76.23 ± 8.13 years). Montreal Cognitive Assessment (MoCA) was selected as the screening test for cognitive impairment in this study [42]. The cut-off score of 26 from 30 has been considered for normal cognition. Determination of the *APOE*ε4 allele

as a known genetic risk factor for AD was performed in both study groups and implemented as a stratification factor in further analyses. Detailed parameters of the study groups are summarized in Table 1.

All control individuals were without any personal or family history of AD, and they were randomly recruited from a larger population sample. All AD patients and controls were Caucasians of Slovak descent. Written informed consent for enrolling in the study and for personal data management was obtained from all AD patients or their legally authorized representatives as well as from the control subjects. All the investigations were carried out in accordance with the International Ethical Guidelines and the Declaration of Helsinki. The study was approved by the Independent Ethical Committee of the University Hospital Bratislava and the Faculty of Medicine, Comenius University in Bratislava.

2.2. Genotyping. Both patient and control DNAs were isolated from EDTA-treated whole blood by a modified salting out procedure [43]. Genotyping of *APOEε4* allele was performed by the determination of rs429358 (C>T) and rs7412 (T>C) polymorphisms in the fourth exon using direct sequencing as described previously [44].

The *MMP2* rs243865 (-1306 C or T allele) was investigated by PCR followed by restriction fragment length polymorphism analysis (RFLP). Primer sequences, PCR conditions, and *XspI* (Thermo Fisher Scientific, U.S.A.) enzyme cleavage were used as reported by Benesova et al. [45]. A 188 bp product was amplified by PCR reaction. After digestion, either an intact 188 bp PCR fragment (allele C) or two fragments of 162 bp and 26 bp (allele T) were produced.

The *MMP3* rs3025058 (-1171 5A or 6A) was genotyped by PCR-RFLP as described by Dragovic et al. [46]. A 120 bp PCR product flanking the polymorphic site was amplified and afterwards digested with the restrictase *PdmI* (Thermo Fisher Scientific, U.S.A.). After digestion, either an intact 120 bp PCR fragment bearing allele with 6 adenines (6A) or two fragments of 97 bp and 23 bp consisting of 5 adenine (5A) allele were produced.

2.3. Statistical Analysis. Allele and genotype frequencies were determined by direct counting. Genotypes were tested for their fit to Hardy-Weinberg equilibrium using the chi-squared goodness-of-fit test. Statistical significance of differences in allele and genotype frequencies between AD patients and control group was evaluated by the Pearson chi-squared test using the InStat statistical software (GraphPad Software, Inc., San Diego, USA). The p values, odds ratios (OR), and 95% confidence intervals (95% CI) were calculated in codominant, dominant, recessive, and overdominant inheritance models. The multivariate logistic regression analysis adjusted for sex, age, and *APOEε4* carriage status as possible modifying factors was performed by the SNPstats web software available at https://snapstat.net/snpstats/. The correlation between *MMP2* and *MMP3* gene promoter polymorphisms and clinical variables as age at onset and MoCA score was evaluated by the Student t-test with Welch correction. The p value of <0.05 was considered statistically significant.

3. Results

3.1. Characteristics of the Study Groups. The demographic and clinical characteristics of the study groups are shown in Table 1. 171 AD patients and 308 unrelated controls were included in the study. There was no statistically significant difference between the AD group and controls in relation to gender ($p = 0.31$), with females having higher prevalence in both AD patients (69.01%) and controls (63.96%). The mean age at examination was significantly higher in the AD group than in controls (79.68 versus 76.23 years; $p < 0.0001$), while an opposite trend was observed for the MoCA score having a lower value in AD patients (14.54 versus 27.52; $p < 0.0001$). The significantly higher prevalence of *APOEε4* risk allele was found in the AD group compared to controls (39.18% vs 19.16%, $p < 0.0001$). The mean age at disease onset was 77.56 ± 6.39 years.

3.2. Genotyping of MMP2 rs243865 and MMP3 rs3025058 Polymorphisms in Promoter Region. Allele and genotype frequencies of *MMP2* rs243865 (-1306 C>T) and *MMP3* rs3025058 (-1171 5A>6A) observed in AD patients and control group are shown in Tables 2 and 3. Genotype frequencies of both polymorphisms fit the Hardy-Weinberg equilibrium in AD patients ($p = 0.05$ and $\chi^2 = 4.13$ for *MMP2*; $p = 0.59$ and $\chi^2 = 0.29$ for *MMP3*) as well as in controls ($p = 0.56$ and $\chi^2 = 0.33$ for *MMP2*; $p = 0.17$ and $\chi^2 = 1.93$ for *MMP3*). Genotyping of the SNP variants at *MMP2* -1306 C>T and at *MMP3* -1171 5A>6A revealed no statistically significant differences in either allele ($p = 0.95$, OR = 1.01 for *MMP2*; $p = 0.68$, OR = 1.07 for *MMP3*) or genotype ($p > 0.05$, OR = 0.82-1.37 for *MMP2*; $p > 0.05$, OR = 0.93 -1.16 for *MMP3*) frequencies between the two studied groups. Multivariate analysis of association between the two SNPs and AD risk adjusted for age, sex, and *APOEε4* positivity as potential confounding variables revealed no changes in comparison with the univariate analysis ($p > 0.05$, OR = 0.85 -1.32 for *MMP2*, Table 2; $p > 0.05$, OR = 0.98-1.06 for *MMP3*, Table 3). Stratification of study groups according to their *APOEε4* carriage status was also performed. Analyses in *APOEε4*-positive and *APOEε4*-negative groups revealed no statistically significant differences in the distribution of *MMP2* -1306 C>T and *MMP3* -1171 5A>6A genotypes between AD patients and control group (data not shown).

3.3. Association of MMP2 rs243865 and MMP3 rs3025058 Genotypes with Clinical Features in AD Patients. The association between *MMP2* rs243865 (-1306 C>T) and *MMP3* rs3025058 (-1171 5A>6A) genotypes and clinical features as age at disease onset and MoCA score was investigated. Correlation of clinical findings with *MMP2* -1306 C>T genotypes revealed that CC carriers in the dominant model had later age at disease onset when compared to T allele carriers (CC vs. CT+TT: 78.44 ± 6.28 vs. 76.36 ± 6.39, $p = 0.036$, Table 4). This association remained significant even after adjustment for sex and *APOEε4* positivity ($p = 0.024$). Moreover, CT carriers in the overdominant model tended to have earlier disease onset as compared to other *MMP2* genotype

Table 1: Demographic and clinical characteristics of AD patients and controls.

Parameter	AD subjects ($n = 171$)	Controls ($n = 308$)	p value
Female/male ratio	118/53	197/111	0.31
Age at examination (y); mean ± SD	79.68 ± 6.03	76.23 ± 8.13	<0.0001
Age at onset (y); mean ± SD	77.56 ± 6.39	—	—
MoCA score, mean ± SD	14.54 ± 5.80	27.52 ± 1.44	<0.0001
APOEε4 positivity (yes/no)	67/104	59/249	<0.0001

Abbreviations: *n*: number; SD: standard deviation; MoCA: Montreal Cognitive Assessment; y: years. Differences in age and MoCA score between the two groups were examined by Welch's corrected *t*-test. Differences in sex were assessed using the Pearson chi-squared test. $p < 0.05$ is considered statistically significant.

Table 2: Allele and genotype frequencies of *MMP2* polymorphism rs243865 (-1306 C/T) in AD patients and controls.

SNP/model	Allele/genotype	AD subjects ($n = 171$)	Controls ($n = 308$)	Univariate analysis		Multivariate analysis	
				p	OR (95% CI)	p	OR (95% CI)
rs243865	C	252 (73.68%)	455 (73.86%)			—	—
	T	90 (26.32%)	161 (26.14%)	0.95	1.01 (0.75-1.36)	—	—
Codominant	CC	98 (57.31%)	170 (55.19%)		1.00		1.00
	CT	56 (32.75%)	115 (37.34%)	0.46	0.84 (0.56-1.27)	0.62	0.88 (0.57-1.34)
	TT	17 (9.94%)	23 (7.47%)		1.28 (0.65-2.52)		1.25 (0.61-2.57)
Dominant	CC	98 (57.31%)	170 (55.19%)		1.00		1.00
	CT+TT	73 (42.69%)	138 (44.81%)	0.65	0.92 (0.63-1.34)	0.76	0.94 (0.63-1.40)
Recessive	CC+CT	154 (90.06%)	285 (92.53%)		1.00		1.00
	TT	17 (9.94%)	23 (7.47%)	0.35	1.37 (0.71-2.64)	0.44	1.32 (0.65-2.65)
Overdominant	CC+TT	115 (67.25%)	193 (62.66%)				
	CT	56 (32.75%)	115 (37.34%)	0.31	0.82 (0.55-1.21)	0.44	0.85 (0.56-1.29)

Abbreviations: CI: confidence interval; *n*: number; OR: odds ratio. Allele and genotype frequencies are given as absolute numbers with percentages in parentheses. Univariate analysis is based on the Pearson chi-squared test. Multivariate analysis is adjusted for sex, age, and *APOEε4* positivity. $p < 0.05$ is considered statistically significant.

Table 3: Allele and genotype frequencies of *MMP3* polymorphism rs3025058 (-1171 5A/6A) in AD patients and controls.

SNP/model	Allele/genotype	AD subjects ($n = 171$)	Controls ($n = 308$)	Univariate analysis		Multivariate analysis	
				p	OR (95% CI)	p	OR (95% CI)
rs3025058	6A	171 (50.00%)	318 (51.62%)			—	—
	5A	171 (50.00%)	298 (48.38%)	0.68	1.07 (0.82-1.39)	—	—
Codominant	6A/6A	41 (23.98%)	76 (24.67%)		1.00		1.00
	5A/6A	89 (52.04%)	166 (53.90%)	0.82	0.99 (0.63-1.57)	0.98	1.00 (0.62-1.63)
	5A/5A	41 (23.98%)	66 (21.43%)		1.15 (0.67-1.98)		1.06 (0.59-1.88)
Dominant	6A/6A	41 (23.98%)	76 (24.67%)		1.00		1.00
	5A/6A+5A/5A	130 (76.02%)	232 (75.33%)	0.86	1.04 (0.67-1.61)	0.94	1.02 (0.64-1.62)
Recessive	6A/6A+5A/6A	130 (76.02%)	242 (78.57%)		1.00		1.00
	5A/5A	41 (23.98%)	66 (21.43%)	0.52	1.16 (0.74-1.80)	0.82	1.05 (0.66-1.68)
Overdominant	5A/5A+6A/6A	82 (47.96%)	142 (46.10%)				
	5A/6A	89 (52.04%)	166 (53.90%)	0.70	0.93 (0.64-1.35)	0.90	0.98 (0.66-1.45)

Abbreviations: CI: confidence interval; *n*: number; OR: odds ratio. Allele and genotype frequencies are given as absolute numbers with percentages in parentheses. Univariate analysis is based on the Pearson chi-squared test. Multivariate analysis is adjusted for sex, age, and *APOEε4* positivity. $p < 0.05$ is considered statistically significant.

Table 4: Analysis of association between *MMP2* rs243865 (-1306 C/T) genotypes and clinical findings.

Parameter	CC ($n = 98$)	CT ($n = 56$)	TT ($n = 17$)	p/p^* CM	p/p^* DM	p/p^* RM	p/p^* OM
Age at onset, mean ± SD (y)	78.44 ± 6.28	76.23 ± 5.81	76.81 ± 8.32	0.11/0.07	0.036/0.024	0.62/0.68	0.058/0.034
MoCA score, mean ± SD	15.20 ± 6.01	13.56 ± 5.68	14.54 ± 4.03	0.32/0.29	0.16/0.13	1.00/0.92	0.14/0.13

Abbreviations: CM: codominant model; DM: dominant model; RM: recessive model; OM: overdominant; SD: standard deviation; MoCA: Montreal Cognitive Assessment; *n*: number; y: years. *p* values were calculated using Welch's corrected *t*-test. $^*p$ values adjusted for sex and *APOEε4* positivity. $p < 0.05$ is considered statistically significant.

Table 5: Analysis of association between *MMP3* rs3025058 (-1171 5A/6A) genotypes and clinical findings.

Parameter	5A/5A ($n = 41$)	5A/6A ($n = 89$)	6A/6A ($n = 41$)	p/p^* CM	p/p^* DM	p/p^* RM	p/p^* OM
Age at onset, mean ± SD (y)	79.02 ± 5.51	76.61 ± 5.88	78.12 ± 7.91	0.11/0.11	0.09/0.09	0.52/0.53	0.045/0.048
MoCA score, mean ± SD	14.65 ± 4.55	14.25 ± 6.10	14.97 ± 6.56	0.84/0.88	0.89/0.85	0.63/0.71	0.59/0.63

Abbreviations: CM: codominant model; DM: dominant model; RM: recessive model; OM: overdominant; SD: standard deviation; MoCA: Montreal Cognitive Assessment; *n*: number; y: years. *p* values were calculated using Welch's corrected *t*-test. $^*p$ values adjusted for sex and *APOEε4* positivity. $p < 0.05$ is considered statistically significant.

carriers in adjusted models (CT vs. CC+TT: 76.23 ± 5.81 vs. 78.21 ± 6.58, $p = 0.034$, Table 4).

The correlation of investigated clinical findings with *MMP3* -1171 5A>6A genotypes is shown in Table 5. Statistical analysis revealed that 5A/6A carriers in the overdominant model seemed to have younger age at disease onset when compared to other *MMP3* genotype carriers (5A/6A vs. 5A/5A +6A/6A: 76.61 ± 5.88 vs. 78.57 ± 6.79, $p = 0.045$). After adjustment for sex and *APOEε4* positivity, the significant association of 5A/6A carriers in the overdominant model with an earlier disease onset was also found ($p = 0.048$). On the other hand, the correlation of the MoCA score with both *MMP2* rs243865 and *MMP3* rs3025058 genotypes did not reveal any significant differences ($p > 0.05$, Tables 4 and 5).

4. Discussion

MMPs are zinc-containing endopeptidases that are suggested to be associated with the pathogenesis of many neurodegenerative diseases due to their involvement in microglial activation, T-leukocyte infiltration, and blood-brain barrier dysfunction [6]. In AD patients, both beneficial and detrimental effects of MMPs have been suggested. It has been reported that MMPs could degrade amyloid β and play important roles in the extracellular Aβ catabolism and clearance [28–30, 47]. On the other hand, MMPs could contribute to AD pathogenesis by disruption of the blood-brain barrier, cell apoptosis, and initiation of inflammation [33, 34].

The object of our study was MMP-2 and MMP-3 as candidate molecules related to AD pathology. In AD patients, an increase in MMP-2 and MMP-3 expression in the astrocytes surrounding amyloid plaques was reported [26, 48]. Moreover, increased MMP-3 levels in serum, plasma, and CSF in AD patients were also found [20–23]. Conversely, the decrease in MMP-2 plasma level in AD patients compared to controls was reported [26]. A negative correlation between MMP-3 plasma levels and the MMSE score was found [21].

As gene polymorphisms can modify gene expression and function, the aim of the study was to analyse the association of *MMP2* rs243865 and *MMP3* rs3025058 polymorphism with AD susceptibility and clinical findings in the Slovak Caucasian population. The *MMP2* rs243865 at position -1306 (C>T) and *MMP3* rs3025058 at position -1171 (5A>6A) in the promoter region have been associated with changes in MMP expression levels. The C to T transition at position -1306 prevents Sp1 binding to gene promoter leading to lower MMP-2 expression [49, 50]. Therefore, TT carriers have lower promoter activity and lower MMP-2 enzyme activity compared with CC carriers [51]. Regarding *MMP3* -1171 5A/6A polymorphism, its 5A allele has been reported to have greater transcriptional activity than the 6A allele [49].

To the best of our knowledge, genetic predisposition of *MMP2* rs243865 (-1306 C>T) and *MMP3* rs3025058 (-1171 5A>6A) to AD development has been analysed in only few studies. Our results showed no genetic association between *MMP2* rs243865 and *MMP3* rs3025058 polymorphism and AD susceptibility as reported by others [38–40]. On the other hand, studies in Finns and Italians reported a significantly higher occurrence of *MMP3* -1171 5A allele and 5A/5A genotype in *APOEε4*-positive AD patients [35, 36]. Moreover, Helbecque et al. [52] found association of *MMP3* -1171 6A/6A genotype with increased risk of dementia in *APOEε4*-negative AD patients from France. Finally, Baig et al. [37] reported association of *MMP3* -1171 6A allele with risk of AD in the UK. The discrepancies in genetic differences within the AD populations could reflect differences in various European regions or may be caused by differences in sample sizes, study design, and statistical methods.

In this study, the analysis of association of *MMP2* rs243865 (-1306 C>T) and *MMP3* rs3025058 (-1171 5A>6A) genotypes with clinical features such as age at onset and MoCA score was also performed. We found a significant association of *MMP2* -1306 CC genotype in the dominant model with later age at disease onset in crude analysis and

adjusted models. Furthermore, we observed that *MMP3* -1171 5A/6A carriers in the overdominant model tended to have lower age at disease onset when compared to other *MMP3* genotype carriers. As *MMP2* -1306 C allele is associated with higher promoter activity, it can be hypothesized that CC genotype has protective effect on AD development. Furthermore, the -1171 5A allele as a high MMP-3 producer has been associated with the pathogenesis of various diseases like acute myocardial infarction [53], breast cancer [54, 55], head and neck squamous cell carcinoma [56], and lung cancer [57].

The impact of *MMP2* rs243865 and *MMP3* rs3025058 on clinical features, including age at onset and MoCA score, has not yet been reported. Reitz et al. [39] performed an analysis of *MMP3* polymorphism with clinical findings such as cognitive MMSE performance over time, hippocampal volume, or severity of periventricular and subcortical white matter lessions. They did not find any correlation of *MMP3* genotypes or haplotypes with the above-mentioned clinical features [39]. Another study by Reitz et al. [40] investigated the association of *MMP3* -1171 5A>6A, 2092A>G, 9775T>A, and 6658T>C SNPs and their haplotypes with plasma Aβ1–40 and Aβ1–42 levels in AD patients [40]. There was no association between *MMP3* -1171 5A>6A or 6658T>C and Aβ1–40 or Aβ1–42 levels in crude or adjusted models. However, haplotype analysis showed that haplotype 2 (6A-G-T-T) was linked with significantly higher levels of plasma Aβ1–42 as compared with haplotype 1 (5A-A-T-T) ($p = 0.0002$) [40].

The role of MMP-2 and MMP-3 expression levels in AD pathology is not yet well defined. It seems that decreased MMP-2 levels in AD patients correlate with impaired degradation of Aβ peptides [58]. Conversely, Aβ-induced expression of MMP-2 and MMP-3 may contribute to the breakdown of BBB and induction of neuroinflammation [32–34]. A recent study described that Aβ-induced MMP-3 may contribute to NGF degradation leading to cholinergic atrophy and cognitive deficits in AD males [59]. Thus, it is unclear whether changes in MMP levels contribute to AD progression or might have beneficial effects on AD patients.

As MMPs seem to be involved in AD pathogenesis, their utility as therapeutic targets has been also investigated. One possibility relies on promoting MMP activities resulting in Aβ degradation. In the APP mouse model, a novel rhamnoside derivative PL402 was reported to promote Aβ cleavage via upregulation of MMP-3/MMP-9 [60]. However, this therapeutic approach should be taken with caution as it cannot be excluded that proteolytic degradation of amyloid plaques could release toxic Aβ products and other neurotoxins. On the other hand, GM6001 and another MMP inhibitor, minocycline, were reported to efficiently reduce upregulated MMP-2 and MMP-9 and prevent inflammation and oxidative stress associated with cerebral amyloid angiopathy in AD mice [61, 62].

We intend to further investigate other MMPs, such as MMP-9, which will also help to understand their role in AD etiology. Similarly to MMP-2 and MMP-3, MMP-9 could also act in Aβ degradation thus preventing deposition of Aβ [30]. MMP-9 is also involved in BBB disruption and induction of neuroinflammation [63]. *MMP9* rs3918242 at position -1562C/T is another candidate polymorphism for AD susceptibility. It was found that this C to T substitution prevents the binding of a nuclear transcription repressor protein to the *MMP9* gene promoter thus increasing its transcription [64]. A protective effect of the *MMP9* -1562 T allele having greater promoter activity in *APOE*ε4-negative AD patients was reported [65]. Thus, *MMP* gene promoter variants seem to be promising biomarkers whose role in the pathogenesis of AD warrants further investigations.

5. Conclusion

Among various MMPs, MMP-2 and MMP-3 belong to candidate molecules involved in AD pathogenesis. This is the first study investigating the impact of *MMP2* rs243865 and *MMP3* rs3025058 promoter polymorphisms on clinical features, including age at onset and MoCA score in AD patients. While no genetic association of *MMP2* rs243865 and *MMP3* rs3025058 with the risk of AD was found, our results suggest that *MMP2* rs243865 CC genotype and *MMP3* rs3025058 5A/6A genotype may have influence on the age of AD onset. In conclusion, *MMP2* rs243865 and *MMP3* rs3025058 polymorphisms may contribute to modification of certain clinical parameters in AD patients.

Abbreviations

AD: Alzheimer's disease
ALS: Amyotrophic lateral sclerosis
APOE: Apolipoprotein E
APP: Amyloid β precursor protein
Aβ: Amyloid β
BBB: Blood-brain barrier
CSF: Cerebrospinal fluid
ECM: Extracellular matrix
HD: Huntington's disease
MMP: Matrix metalloproteinase
MMSE: Minimental state examination
MoCA: Montreal Cognitive Assessment
MS: Multiple sclerosis
NGF: Nerve growth factor
PD: Parkinson's disease
RFLP: Restriction fragment length polymorphism analysis
TGF-β: Transforming growth factor-β
TNF: Tumor necrosis factor.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors report no conflict of interests.

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