Acta Neuropsychiatrica 2016

DOI: 10.1017/neu.2015.72

ACTA NEUROPSYCHIATRICA

The role of MMP genes in recurrent depressive disorders and cognitive functions

Bobińska K, Szemraj J, Gałecki P, Talarowska M. The role of MMP genes in recurrent depressive disorders and cognitive functions.

Kinga Bobińska[1], Janusz Szemraj[2], Piotr Gałecki[1], Monika Talarowska[1]

[1]Department of Adult Psychiatry, Medical University of Lodz, Lodz, Poland; and
[2]Department of Medical Biochemistry, Medical University of Lodz, Lodz, Poland

Objective: Among the 28 metalloproteinases described so far, 23 can be found in the human organism, but only few are expressed in the human brain. The main objective of this study was to analyse the relationship between *MMP-2*, *MMP-9* and *TIMP-2* gene expression and cognitive performance.
Methods: The study comprised 234 subjects: patients suffering from recurrent depressive disorder (rDD, $n = 139$) and healthy subjects (HS, $n = 95$). The cognitive function assessment was carried out with the help of the following tests: Trail Making Test, The Stroop Test, Verbal Fluency Test and Auditory Verbal Learning Test. Gene expression on the mRNA and protein level was evaluated for *MMP-2*, *MMP-9* and *TIMP-2* in both groups using RNA extraction, reverse transcription and enzyme-linked immunosorbent assay.
Results: Both mRNA and protein expression levels of all the genes were significantly lower in rDD subjects as compared with HS. Having analysed the entire experimental group ($N = 234$), significant interrelations were found between the expression of the analysed genes and the results of the tests used to measure cognitive functions. Increased expression on both the mRNA and the protein level was associated in each case with better performance of all the tests conducted. After carrying out a separate analysis on the people from the rDD group and the HS group, similar dependencies were still observed.
Conclusions: The results of our study show decreased expression of *MMP-2*, *MMP-9* and *TIMP-2* genes on both mRNA and protein levels in depression. Elevated expression of *MMP-2*, *MMP-9*, *TIMP-2* positively affects cognitive efficiency: working memory, executive functions, attention functions, direct and delayed auditory–verbal memory, the effectiveness of learning processes and verbal fluency. The study highlights the important role of peripheral matrix metalloproteinases genes in depression and cognitive functions.

Keywords: cognition; depression; MMPs

Monika Talarowska, Department of Adult Psychiatry, Medical University of Lodz, Lodz, Poland, Aleksandrowska 159, 91-229 Lodz, Poland.

Tel: +4 842 775 1986;
Fax: +4 842 640 5058;
E-mail: talarowskamonika@wp.pl

Accepted for publication December 13, 2015

First published online February 9, 2016

Significant outcomes

- The study highlights the important role of peripheral MMP genes in depression and cognitive functions.

Limitations

- The number of patients and healthy subjects may result in the lack of statistical significance in some cases, which is the limitation affecting the conclusions of our study.

Introduction

Matrix metalloproteinases (MMPs) belong to the group of enzymes that play a particular role in the human body. They are involved in cell migration during development and in the construction of supporting tissue for all internal organs; moreover, they stimulate the growth of neurites. They are active in the production of new blood vessels and are indispensable for the correct development of the skeleton and connective tissue regeneration (1,2). Their pathologic

activity was, however, observed in many diseases such as autoimmune disorders, Alzheimer's disease (AD) (3), multiple sclerosis (SM) (4), amyotrophic lateral sclerosis (5), cancers (e.g. multiple myeloma, malignant melanoma, mammary tumour), hepatic cirrhosis or myocardial infarction (6–8).

Among the 28 metalloproteinases described so far, 23 can be found in the human organism (9,10), but only few are expressed in the human brain. These include both matrix metalloproteinase 2 (*MMP-2*) and matrix metalloproteinase 9 (*MMP-9*), the expression of which was found in the frontal cortex, the CA1–CA3 regions of the hippocampus and in the cerebellum (11). It is possible to find the tissue inhibitor of MMP 2 (*TIMP-2*) mRNA in all parts of the brain, though with lower levels in the cerebellum (11,12).

As an object of our analysis we have selected the MMPs which – due to the profile of their activity – may be significant in the aetiology and course of recurrent depressive disorders (rDD). It seems that MMPs have the ability to stimulate numerous proinflammatory mediators such as chemokine CXCL-8, interleukine 1β (IL-1β) or tumour necrosis factor α (TNF-α) (1). *MMP-2* and *MMP-9* are active in pathologic processes in the peripheral and central nervous system (CNS) (13,14). They probably have an impact on increased blood–brain barrier permeability (15,16). The presence of active forms of *MMP-2* and *MMP-9* was observed in the nuclei of neuronal and glial cells (17). The two metalloproteinases are assigned a special role in the process of neurogenesis and neuroplasticity (17). *MMP-2* and *MMP-9* have been critically implicated in synaptic circuit remodelling – including structural changes in axon/dendrite structures and dendritic spines – in both healthy subjects (HS) and the affected (18). Moreover, the level of *MMP-9* in the cerebrospinal fluid of the patients with SM is considered a potential indicator of disease activity, which may serve as a confirmation of an ongoing inflammatory process (19). On the other hand, *TIMP-2* demonstrates antiapoptotic properties (10).

The main aim of this study was to analyse *MMP-2* (gelatinase A), *MMP-9* (gelatinase B) and *TIMP-2* genes on both mRNA and protein levels in patients suffering from rDD as well as to investigate the relationship between *MMP-2*, *MMP-9* and *TIMP-2* gene expression and cognitive performance. We provided a hypothesis that *MMP-2*, *MMP-9* and *TIMP-2* gene expression levels are higher in the rDD group, which might have an influence on cognitive functions.

Material and methods

Subjects

The study was carried out on a group of 234 subjects aged 20–67 ($M = 40.61$ years, SD = 13.76), comprising both patients with rDD ($n = 139$) and control HS ($n = 95$).

The patients were chosen for the experiment based on the ICD-10 inclusion criteria (F 32.0–F 32.2, F 33.0–F 33.8) (20). All the subjects were examined during hospitalisation. Axis I and II disorders (however not depressive episodes) as well as somatic diseases diagnosed and injuries of the CNS, which could have had an impact on cognitive performance, were considered exclusion criteria. Before carrying out the main procedure, case history was obtained for each single case using the standardised Composite International Diagnostic Interview (CIDI) (21).

The HS was composed of HS without any cases of psychiatric disorders in family members. The healthy controls included community volunteers who were accepted to take part in the study on the basis of the psychiatric CIDI interview criteria (21). Subjects with other psychiatric diseases, axis I and II disorders, neurological disorders, or suffering from substance abuse or dependence, were excluded from the experiment.

Based on the diagnostics, interviews and medical data, none of the participants had been diagnosed with any relevant intellectual deficit or a mental disability. At the onset of the experiment, all the subjects were not taking any medications. Neither, did they suffer from any medical illnesses, including *inter alia* infections and inflammatory or allergic reactions. The control subjects or depressed patients were not treated with the use of the drugs known to influence lipid metabolism, immune responses or endocrine functions. The control subjects had not been taking any medications for at least 2 months before blood sampling. None of the participants was overusing alcohol or smoked excessive amounts of cigarettes; neither had any of them ever taken psychotropic drugs.

Methods

Neuropsychological assessment and severity of depression

The neuropsychological assessment was conducted by trained and qualified neuropsychologists. A comprehensive neuropsychological test battery – that is the Trail Making Test (TMT), the Stroop Test, the Verbal Fluency Test (VFT) and the Auditory Verbal Learning Test (Polish version, AVLT) – was used to evaluate the full scope of cognitive functions. An assessment of depression severity was conducted using the 17-item Hamilton Depression Rating Scale (HDRS). Part A of TMT was conducted to evaluate psychomotor speed, while part B was used to assess visuospatial performance, working memory and executive functions. The Stroop Test measured the

https://doi.org/10.1017/neu.2015.72 Published online by Cambridge University Press

effectiveness of verbal working memory, executive functions and attention processes. The VFT evaluated the ability to form and fluently utter words compatible with the given criteria. In the case of evaluating auditory–verbal memory – both direct and delayed – and the effectiveness of learning processes, the Polish equivalent of the Rey AVLT, the so-called AVLT, was applied. A thorough description of the tests can be found in (22) and (23).

The HDRS, Stroop Test, TMT, AVLT and VFT were performed at the onset of the therapy in the case of the patients with rDD. All of them were examined on admission during the symptomatic phase. At that time, the patients were not taking any drugs that would have had an influence on their cognitive functions. The neuropsychological tests were performed during a single examination in the HS group. The patients were examined by the same practitioner and the same psychologist was in charge of the neuropsychological tests, including results evaluation. The HDRS test was carried out by the same psychiatrist.

Gene expression on the mRNA and protein level was evaluated for *MMP-2*, *MMP-9* and *TIMP-2* in both groups.

RNA extraction and reverse transcription. A reagent used in RNA extraction, referred to as TRIZOL (Invitrogen Life Technologies, Life Technologies Corp., Grand Island, NY, USA), was used for the extraction of total RNA from the blood samples according to the standard acid-guanidinium-phenol-chloroform method. After extraction, RNA was analysed by means of agarose gel electrophoresis. The study was carried out only in the cases with preserved 28 S, 18 S and 5 S ribosomal RNA bands, with good RNA quality. DnaseI (GIBCO, Life Technologies Corp., Grand Island, NY, USA) was utilised to digest total RNA at room temperature for a period of 15 min. A quantity of 5 µg of digested RNA underwent the process of reverse transcription at the temperature of 42°C for 60 min (total reaction volume of 20 ml) using the ImProm-II™ Reverse Transcription System kit (Promega Corp., Madison, WI, USA). The resulting cDNA was applied in a real-time PCR reaction.

Detecting gene expression based on the method of real-time RT-PCR. TaqMan™ technology-based real-time PCR was performed using a master mix, prepared in compliance with the FastStart Universal Probe Master (ROX) from Roche Applied Science (Basel, Switzerland). The online Universal ProbeLibrary (www.universalprobelibrary.com) was used to design primers and probes. The following primer sequences and probe numbers were applied: MMP-2 (forward, 5'-ACTGTTGGTGGGAACTCAGAAG-3', reverse, 5'-CAAGGTCAAT GTCAGGAGAGG-3', probe: #1), MMP-7 (forward, 5'-CGGATGGTAAGC AGTCTAGGG-3', reverse, 5'-AGGTTGGATACATC ACTGCA3TTAG-3', probe: #49), MMP-9 (forward, 5'-TGGGTGTACGACGGTGAAAA-3' , reverse, 5'-CATGGGTCTCTAGCCTGATA-3' probe: #31), TIMP-2 (forward 5'-TCTGGAAACGACATTTA TGG-3', reverse 5'-GTTGGAGGCCTGCTTATGGG-3' and 18sRNA (forward 5'-CCGATAACGAACGA GACTCTGG-3', reverse 5'-TAGGGTAGGCACACG CTGAGCC-3' probe: #29); they facilitated internal control for real-time PCR.

A final volume of 50 µl was used for real-time PCR, with 0.05 µg of cDNA, 25 µl of FastStart Universal Probe Master 2×, 250 nM of probe and 1 µM of each primer. The amplification process lasted 10 min at the temperature of 95°C and the purpose was to activate FastStart Taq DNA polymerase. The amplification and analysis of the signals constituted the objective of 40 15-s-long rounds at 95°C and 1-min rounds at 60°C. The ABI Prism 7000 Sequence Detection System (Applied Biosystems, Life Technologies Corp., Grand Island, NY, USA) was used to calculate amplification values. Every sample was assayed in triplicate in the course of independent reactions. Real-time PCR data were calculated automatically based on the data analysis module. The recorded results were subject to an analysis in accordance with the $2^{-\Delta\Delta c_t}$ method. PCR efficiency validation was performed with a standard curve. Standard curves were prepared for each gene by serial dilution.

Determination of serum MMP-2,-9, TIMP-2 levels with the use of enzyme-linked immunosorbent assay (ELISA). The RayBio® Human ELISA from RayBiotech (Norcross, GA, USA) made it possible to perform a quantitative analysis of circulating human MMP-2,-9 and TIMP-2 in serum. Each serum sample was assessed three times. The recommendations worked out by the manufacturer enabled to define instructions and calculate results. The standards and samples were first pipetted into wells with immobilised antibodies specific for human MMP-2,-9, TIMP-2 and incubated afterwards. Then, after the incubation phase and washing, biotinylated antihuman MMP-2,-9, TIMP-2 antibody was added. Having washed away all unbound substances, biotinylated antibody, the horseradish peroxidase-conjugated streptavidin was pipetted into the wells, which were washed once again. Tetramethylbenzidine substrate solution was added to the wells. Colour developed proportionally to the amount of MMP-2,-9, TIMP-2 bound. Colour development stopped (Stop Solution) and its intensity was measured using the Thermo Labsystems Multiskan Ascent 354 (Lab Recyclers, Frederick, MD, USA) at 450 nm (24,25).

https://doi.org/10.1017/neu.2015.72 Published online by Cambridge University Press

Statistical analysis

The statistical analysis of the collected material involved the calculation of both descriptive and inferential data. A two-tailed critical region was employed in the testing of the statistical hypothesis.

The qualitative features of the study and control groups are expressed as frequencies (shown as percentage values). The arithmetical mean (M) was calculated to determine the average values for quantitative characteristics. Statistical dispersion measures included the range of values between the minimum and the maximum, and standard deviation.

The following tests were used in the comparison of the nonparametric variables in the examined groups: the Pearson χ^2 test for qualitative variables and the Mann–Whitney U-test for two independent groups to determine the coincidence of distributions. Spearman's R rank order correlation coefficients were estimated in order to evaluate the relationships between the variables. For all the analyses, statistical significance was defined as $p < 0.05$ (26). All data analyses were conducted in STATISTICA PL, version 10.

Ethics

The patients were all native Poles, inhabitants of central Poland. They were unrelated to one another. The individuals were selected to the test group at random; replacement sampling was excluded. The experimental group was randomly chosen among patients of the Babinski Memorial Hospital in Lodz, Poland. The HS group included selected staff members working in this hospital.

Before agreeing to take part in the experiment, the subjects were informed that participation was voluntary and the purpose of the study was explained. In addition, the participants were assured that any and all personal data and results of the tests would be kept confidential. Written informed consent was obtained from each subject according to the study protocol, which had been approved by the Bioethical Committee of the Medical University of Lodz (No NN/469/11/KB).

Results

The characteristics of the study group in terms of sex, age, education (number of years of education) and the course of the disease (rDD group) are presented in Table 1. No statistically significant differences in terms of sex were discovered between the examined groups ($\chi^2 = 1.16$, $p = 0.281$); the only differences considered the age ($Z = 9.91$, $p = 0.001$).

Average values of expression on the mRNA and the protein level for *MMP-2*, *MMP-9* and *TIMP-2*

Table 1. Demographic characteristics of the group with rDD in comparison to the HS group, and data concerning the course of the disease

Characteristics	Whole group ($N = 234$)			rDD ($n = 139$)			HS ($n = 95$)		
	N	%	(± SD)	N	%	(± SD)	n	%	(± SD)
Sex									
Female	143	61.11	–	81	58.27	–	62	65.26	–
Male	91	38.89	–	58	41.73	–	33	34.74	–
Age (years)	–	–	40.61 (±13.76)	–	–	48.19 (±11.15)	–	–	30.53 (±7.82)
Education level									
Primary	41	17.52	–	41	29.51	–	–	–	–
Secondary	111	47.44	–	69	49.63	–	42	44.21	–
College/University	82	35.04	–	29	20.86	–	53	55.79	–
rDD									
Disease duration in years	–	–	–	–	–	6.79 (±5.65)	–	–	–
Number of depression episodes	–	–	–	–	–	4.96 (±3.72)	–	–	–

%, percentage; ± SD, standard deviation; HS, healthy subjects; n, number of samples; rDD, recurrent depressive disorders.

https://doi.org/10.1017/neu.2015.72 Published online by Cambridge University Press

Table 2. Expression on the protein level and on the level of mRNA for *MMP-2*, *MMP-9* and *TIMP-2* genes in the examined group

	Whole group ($N = 234$)	rDD ($n = 139$)	HS ($n = 95$)	Mann–Whitney *U*-test	
Variable	*M* (± SD)	*M* (± SD)	*M* (± SD)	*Z*	*p*
MMP-2 protein (pg/ml)	394.24 (40.48)	373.47 (27.33)	424.63 (37.34)	−9.178	<0.0001*
MMP-2 mRNA ($2^{-\Delta\Delta c_t}$)	310.42 (33.55)	285.78 (10.18)	346.47 (20.68)	−12.983	<0.01*
MMP-9 (pg/ml)	399.21 (48.15)	371.97 (28.76)	439.07 (42.77)	−9.891	<0.0001*
MMP-9 mRNA ($2^{-\Delta c_t}$)	308.91 (27.71)	294.51 (12.07)	362.33 (24.63)	−12.983	<0.01*
TIMP-2 (pg/ml)	399.77 (44.46)	374.17 (27.89)	437.22 (37.03)	−10.316	<0.0001*
TIMP-2 mRNA ($2^{-\Delta\Delta c_t}$)	322.04 (38.01)	289.41 (9.09)	337.46 (19.89)	−12.924	<0.01*

HS, healthy subjects; *M*, mean; MMP-2, matrix metalloproteinase 2; MMP-9, matrix metalloproteinase 9; rDD, recurrent depressive disorders; SD, standard deviation; TIMP-2, tissue inhibitor of MMP 2.
*p statistically significant.

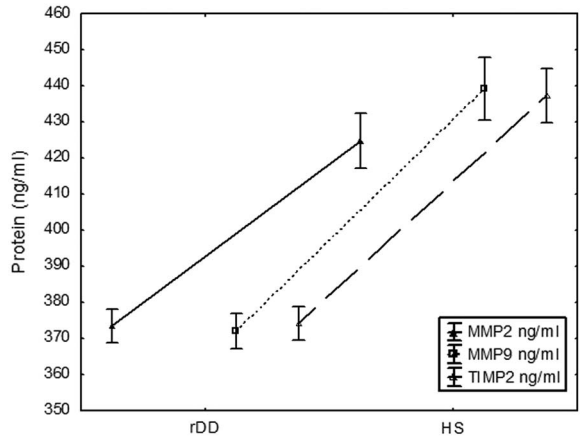

Fig. 1. Average values and confidence interval (95.00%) for the analysed variables. rDD, recurrent depressive disorders; HS, healthy subjects; MMP-2, matrix metalloproteinase 2; MMP-9, matrix metalloproteinase 9; TIMP-2, tissue inhibitor of MMP 2.

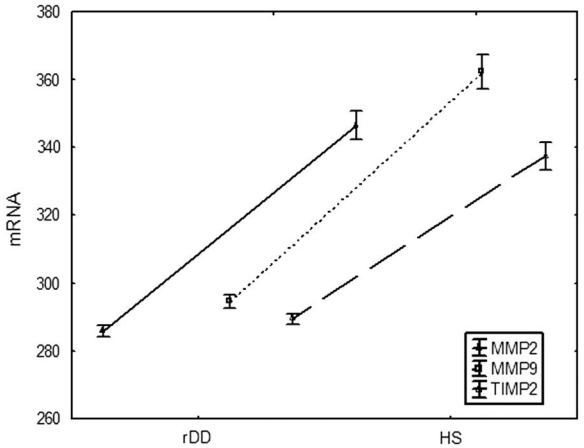

Fig. 2. Average values and confidence interval (95.00%) for the analysed variables. rDD, recurrent depressive disorders; HS, healthy subjects; MMP-2, matrix metalloproteinase 2; MMP-9, matrix metalloproteinase 9; TIMP-2, tissue inhibitor of MMP 2.

genes in the examined group ($N = 234$) are shown in Table 2 and in Figs 1 and 2, and the analysed variables are compared in the rDD group ($n = 139$) and the group of healthy individuals ($n = 95$).

Differences of statistical significance were observed between the patients with rDD and the HS in relation to all of the analysed variables (Table 2). The expression of *MMP-2*, *MMP-9*, TIMP-*2* genes on the mRNA level and the protein level was significantly greater in the HS as compared with the rDD group.

The correlation between *MMP-2*, *MMP-9*, *TIMP-2* mRNA and protein expression levels and the results of the neuropsychological tests – separately for the rDD and HS test group – are shown in Tables 3–5, which also include the results of the correlation between the *MMP-2*, *MMP-9*, *TIMP-2* mRNA and protein expression levels and the neuropsychological tests for the entire group.

Having analysed the entire experimental group ($N = 234$), significant interrelations were found between the expression of the genes and the results of the tests that measured cognitive functions. Increased expression on both the mRNA and the protein level was associated with better performance of all the tests conducted in each case.

After carrying out a separate analysis for the individuals from the rDD group and the HS group, similar dependencies were still observed; however, they were not that numerous. A summary of the results for the rDD group and HS can be found in Table 6.

Discussion

Chronic stress is considered one of the significant factors in the aetiology and course of depressive disorders (27). Its impact on the hyperactivity of the hypothalamic–pituitary–gonadal axis and on the inhibition of the immune system is emphasised (27). The research studies conducted by Lutgendorf et al. (28) revealed that *MMP-9* was found to be elevated in macrophage cells of the depressed patients, particularly in combination with high stress levels. In the paper cited, depressed patients

https://doi.org/10.1017/neu.2015.72 Published online by Cambridge University Press

Table 3. Spearman's rank correlation coefficients(*R*) for the variables tested – MMP-2

	rDD (n = 139)		HS (n = 95)		The whole group (N = 234)	
	MMP-2 mRNA ($2^{-\Delta\Delta c_t}$) M = 285.78 (±10.18)	MMP-2 protein (pg/ml) M = 373.47 (±27.33)	MMP-2 mRNA ($2^{-\Delta\Delta c_t}$) M = 346.47 (±20.68)	MMP-2 protein (pg/ml) M = 424.63 (±37.34)	MMP-2 mRNA ($2^{-\Delta\Delta c_t}$) M = 310.42 (±33.55)	MMP-2 protein (pg/ml) M = 394.24 (±40.48)
	R		*R*		*R*	
TMT A-time	−0.131	−0.244*	0.154	−0.192	−0.443*	−0.451*
TMT B-time	−0.119	−0.122	−0.136	−0.221*	−0.589*	−0.482*
RCNb-time	−0.114	−0.191*	−0.021	0.042	−0.462*	−0.349*
NCWd-time	−0.031	−0.114	−0.051	−0.209*	−0.471*	−0.404*
VFT-animals	0.044	0.102	0.179	0.141	0.451*	0.347*
VFT-sharp objects	−0.014	0.084	−0.119	0.121	0.314*	0.315*
VFT-the letter k	−0.043	0.173*	0.045	0.138	0.274*	0.323*
AVLT-first attempt	0.022	−0.012	−0.054	0.168	0.374*	0.368*
AVLT-number of words in 30 min	0.049	−0.062	−0.108	0.032	0.484*	0.382*

AVLT, Auditory–Verbal Learning Test; HS, healthy subjects; MMP-2, matrix metalloproteinase 2; NCWd, naming colour of word – different; RCNb, reading colour names in black; rDD, recurrent depressive disorder; TMT, Trail Making Test; VFT, Verbal Fluency Test.
*p statistically significant.

Table 4. Spearman's rank correlation coefficients(*R*) for the variables tested – MMP-9

	rDD (n = 139)		HS (n = 95)		The whole group (N = 234)	
	MMP-9 mRNA ($2^{-\Delta\Delta c_t}$) M = 294.51 (±12.07)	MMP-9 protein (pg/ml) M = 371.97 (±28.76)	MMP-9 mRNA ($2^{-\Delta\Delta c_t}$) M = 362.33 (±24.63)	MMP-9 protein (pg/ml) M = 439.07 (±42.77)	MMP-9 mRNA ($2^{-\Delta\Delta c_t}$) M = 308.91 (±27.71)	MMP-9 protein (pg/ml) M = 399.21 (±48.15)
	R		*R*		*R*	
TMT A-time	−0.096	−0.191*	−0.001	0.134	−0.453*	−0.346*
TMT B-time	−0.073	−0.068	−0.227*	0.033	−0.585*	−0.439*
RCNb-time	−0.021	−0.207*	0.008	−0.076	−0.423*	−0.403*
NCWd-time	−0.071	−0.137	−0.162	0.091	−0.494*	−0.359*
VFT-animals	0.021	0.183*	0.351*	−0.023	0.469*	0.348*
VFT-sharp objects	0.007	0.169*	0.054	−0.174	0.343*	0.246*
VFT-the letter k	0.052	0.147	0.022	−0.115	0.301*	0.248*
AVLT-first attempt	0.029	0.018	0.077	−0.037	0.401*	0.286*
AVLT-number of words in 30 min	0.111	0.016	−0.036	−0.124	0.511*	0.386*

AVLT, Auditory–Verbal Learning Test; HS, healthy subjects; MMP-9, matrix metalloproteinase 9; NCWd, naming colour of word – different; RCNb, reading colour names in black; rDD, recurrent depressive disorder; TMT, Trail Making Test; VFT, Verbal Fluency Test.
*p statistically significant.

demonstrated significant elevations of *MMP-9* in CD68(+) cells, adjusted for stage. Substantially greater levels of *MMP-9* in CD68(+) cells were also observed in the patients with higher levels of current stress, life stress over the last 6 months and general negative affect. On the other side, higher social support was linked with lower levels of *MMP-9* and the vascular endothelial growth factor in tumour cells (28). The expression of *MMP-2* by macrophages or tumour cells was not associated with any of the bio-behavioural factors examined to a significant extent (28). The mechanisms underlying these associations most probably involve stress hormones such as norepinephrine (NE) and cortisol (hydrocortisone). *In vitro* stimulation of isolated human macrophages with NE and hydrocortisone improved the production of *MMP-2* and *MMP-9* (29).

https://doi.org/10.1017/neu.2015.72 Published online by Cambridge University Press

Table 5 . Spearman's rank correlation coefficients(R) for the variables tested – TIMP-2

	rDD (n = 139)		HS (n = 95)		The whole group (N = 234)	
	TIMP-2 mRNA ($2^{-\Delta\Delta c_t}$) M = 289.41 (±9.09)	TIMP-2 protein (pg/ml) M = 374.17 (±27.89)	TIMP-2 mRNA ($2^{-\Delta\Delta c_t}$) M = 337.46 (±19.89)	TIMP-2 protein (pg/ml) M = 437.22 (±37.03)	TIMP-2 mRNA ($2^{-\Delta\Delta c_t}$) M = 322.04 (±38.01)	TIMP-2 protein (pg/ml) M = 399.77 (±44.46)
	R		R		R	
TMT A-time	−0.001	−0.185*	−0.069	−0.067	−0.426*	−0.435*
TMT B-time	−0.029	−0.033	−0.239*	−0.096	−0.571*	−0.443*
RCNb-time	−0.047	−0.097	0.023	0.046	−0.428*	−0.364*
NCWd-time	−0.011	−0.105	−0.119	0.039	−0.469*	−0.404*
VFT-animals	0.131	0.056	0.359*	−0.026	0.497*	0.324*
VFT-sharp objects	0.108	0.143	−0.108	−0.222*	0.343*	0.264*
VFT-the letter k	0.206*	0.104	−0.001	−0.046	0.349*	0.252*
AVLT-first attempt	0.042	0.164	0.048	−0.001	0.394*	0.362*
AVLT-number of words in 30 min	−0.001	0.081	0.096	0.061	0.503*	0.462*

AVLT, Auditory–Verbal Learning Test; HS, healthy subjects; NCWd, naming colour of word – different; RCNb, reading colour names in black; rDD, recurrent depressive disorder; TIMP-2, tissue inhibitor of MMP 2; TMT, Trail Making Test; VFT, Verbal Fluency Test.
*p statistically significant.

Table 6. Summary of the results for the rDD group and HS

	TMT A-time	TMT B-time	RCNb-time	NCWd-time	VFT-animals	VFT-sharp objects	VFT-the letter k	AVLT-first attempt	AVLT-number of words in 30 min
					rDD (n = 139)				
MMP-2 (pg/ml)	↑		↑				↑		
MMP-2 ($2^{-\Delta\Delta c_t}$)									
MMP-9 (pg/ml)	↑		↑		↑	↑			
MMP-9 ($2^{-\Delta\Delta c_t}$)									
TIMP-2 (pg/ml)	↑								
TIMP-2 ($2^{-\Delta\Delta c_t}$)							↑		
					HS (n = 95)				
MMP-2 (pg/ml)		↑		↑					
MMP-2 ($2^{-\Delta\Delta c_t}$)									
MMP-9 (pg/ml)									
MMP-9 ($2^{-\Delta\Delta c_t}$)		↑			↑				
TIMP-2 (pg/ml)						↓			
TIMP-2 ($2^{-\Delta\Delta c_t}$)		↑			↑				

AVLT, Auditory–Verbal Learning Test; HS, healthy subjects; MMP-2, matrix metalloproteinase 2; MMP-9, matrix metalloproteinase 9; NCWd, naming colour of word – different; RCNb, reading colour names in black; rDD, recurrent depressive disorders; TIMP-2, tissue inhibitor of MMP 2; TMT, Trail Making Test; VFT, Verbal Fluency Test.

The obtained results do not confirm the revelations quoted above and the hypothesis made in the introduction was confirmed only partially. We demonstrated a significant influence of MMPs on the efficiency of cognitive processes in both patients with rDD and the group of HS. It turned out that the increased expression of each of the analysed genes enhanced the efficiency of many cognitive functions: attention, immediate and delayed memory, working memory and executive functions. Increased expression of *MMP-2*, *MMP-9* and *TIMP-2* was, however, confirmed in the HS.

Do the results recorded by our team confirm the role of MMPs in neurogenesis and neuroplasticity? This is a far-reaching conclusion, yet it does not seem to be random. Structures of the frontal lobes as well as the hippocampus were involved in each cognitive function analysed by us (30), while structural and functional changes in their scope were confirmed many times in the people suffering from rDD (30).

Based on the experiments conducted, Bjerke et al. (31) suggests that the *MMP-9* enzyme is engaged in the degeneration of the white matter (46 patients without any symptoms of dementia and with small vessel disease diagnosed were evaluated). Moreover, according to Mizoguchi et al. (32), an increase in *MMP-9* expression in the hippocampus affects the development of cognitive impairment in mice.

https://doi.org/10.1017/neu.2015.72 Published online by Cambridge University Press

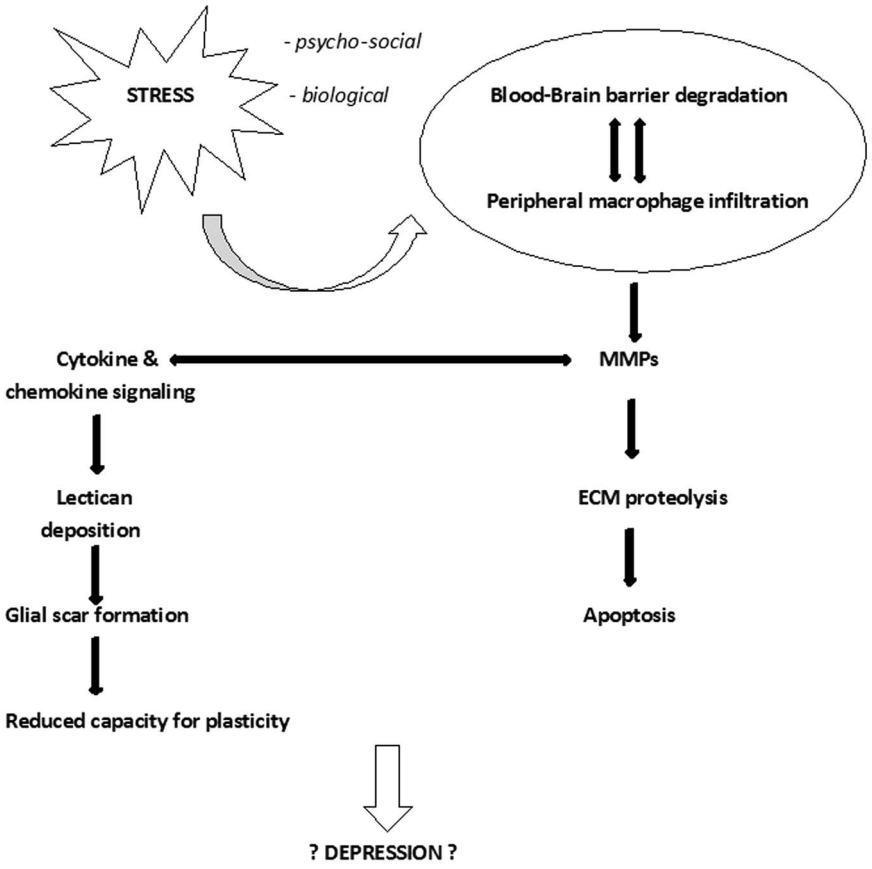

Fig. 3. MMPs and depression aetiology. MMPs, matrix metalloproteinases; ECM, extracellular matrix.

The treatment with the inhibitors of *MMP-2* or *MMP-2/9* prevented BBB breakdown in the hippocampus and the treatment with inhibitors of *MMP-2*, *MMP-9* or *MMP-2/9* prevented BBB breakdown in the cortex (33). Meanwhile, a team of scientists led by Li et al. (34) recorded results that were compatible with the ones obtained by our team. The authors examined 52 HIV carriers and 21 seronegative individuals. Plasma *MMP-2* levels were significantly reduced in an early HIV infection and correlated with the altered white matter integrity and atrophic brain changes. What is more, MMP-9 levels were higher in the treated subjects than in the naive HIV subgroup (34). In the experiments carried out with the use of an animal model, it was possible to determine that *TIMP-2* deficient mice show a defective memory function (35). In addition, Jaworski et al. (35) revealed that neither male nor female mice deficient in *TIMP-2* (knock-out) exhibited prepulse inhibition of the startle reflex, which may suggest deficits in preattentional sensorimotor gating. Knock-out mice and mice expressing a mutant truncated *TIMP-2* (knock-down) demonstrated deficits in fear-potentiated startle. Moreover, the use of *MMP-9* inhibitors reduces the efficiency of the visuospatial memory (36). In recent years, the participation of *MMP-9* in the phenomenon of long-term potentiation (LTP) has been confirmed (37). *MMP-9* plays a critical role in LTP maintenance in the Schaffer collateral-CA1 pathway and in the acquisition of the hippocampus-dependent memory. Recent studies have revealed that in the mossy fibre-CA3 (MF-CA3) projection, where LTP induction and expression are largely presynaptic, MMPs blockade disrupts LTP maintenance, and that LTP induction is associated with increased *MMP-9* expression (38). Martín-Aragón et al. (39) did not find any differences in the activity of *MMP-2* and *MMP-9* in the plasma of patients with AD and mild cognitive impairment, and in the plasma of HS selected taking age and sex into account.

From the perspective of the results recorded by us, the impact of chronic stress on the reduction of hippocampal volume is also significant (40). The hippocampus is one of the brain regions which display substantial vulnerability to chronic stress, marked structural changes in dendritic complexity and spine volume and number (41). The studies by van der Kooij et al. (42) showed that *MMP-9* activity

https://doi.org/10.1017/neu.2015.72 Published online by Cambridge University Press

is implicated in the reduction in perisynaptic nectin-3 expression specifically in the CA1 region. The authors demonstrated the involvement of *MMP-9* in chronic stress-induced alterations in social behaviours (including reduced sociability and social memory) and CA1-mediated cognition.

Summary

The mechanism of MMPs influence on the efficiency of cognitive functions in the subjects with rDD has not been thoroughly examined yet. The results of the studies conducted so far do not provide a clear answer to the question about the positive or negative impact of MMPs on the cognitive efficiency in both HS and the patients suffering from somatic or mental diseases. Nevertheless, we may propose a hypothesis based on the results of the experiments conducted in a group of patients treated due to schizophrenia (43,44). According to Chopra et al. (44), the overexpression of MMPs and the imbalance between MMP and tissue inhibitors of metalloproteinase are associated with various disturbances of the extracellular matrix in the schizophrenic brain. What is more, degradation of cell adhesion molecules is one of the main mechanisms, whereby MMPs are shown to affect neural plasticity (45), control synaptic activity in the hippocampus and impact learning and memory (46). Figure 3 presents possible paths of engagement of MMPs in the aetiology of depression (47).

Conclusions

1. The results of our study show decreased expression of *MMP-2*, *MMP-9* and *TIMP-2* genes on both mRNA and protein levels in depression.
2. Elevated expression of *MMP-2*, *MMP-9* and *TIMP-2* positively affects cognitive efficiency: working memory, executive functions, attention functions, direct and delayed auditory–verbal memory, the effectiveness of learning processes and verbal fluency.
3. The study highlights the important role of peripheral MMPs genes in depression and cognitive functions.

Acknowledgements

Authors' contribution: K.B. – has made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; has been involved in the drafting of the manuscript or revising it critically for important intellectual content; has given the final approval of the version to be published; agrees to be accountable for all aspects of the work and ensuring that the questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. J.S. – has made substantial contributions to the analysis and interpretation of data; has been involved in the drafting of the manuscript or revising it critically for important intellectual content. P.G. – has made substantial contributions to conception and design; has been involved in the drafting of the manuscript or revising it critically for important intellectual content; has given the final approval of the version to be published. M.T. – has made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; has been involved in the drafting of the manuscript or revising it critically for important intellectual content; has given the final approval of the version to be published.

Financial Support

This study was supported with scientific research grants from Medical University of Lodz, no. 503/5-062-02/503-51-005 and no. 503/5-062-02/503-51-006.

Conflicts of Interest

None.

References

1. Yong VW, Krekoski CA, Forsyth PA, Bell R, Edwards DR. Matrix metalloproteinases and diseases of the CNS. Trends Neurosci 1998;**21**:75–80.
2. Romi F, Helgeland G, Gilhus NE. Serum levels of matrix metalloproteinases: implications in clinical neurology. Eur Neurol 2012;**67**:121–128.
3. Stomrud E, Björkqvist M, Janciauskiene S, Minthon L, Hansson O. Alterations of matrix metalloproteinases in the healthy elderly with increased risk of prodromal Alzheimer's disease. Alzheimers Res Ther 2010;**2**:20.
4. Kurzepa J, Bartosik-Psujek H, Suchozebrska-Jesionek D, Rejdak K, Stryjecka-Zimmer M, Stelmasiak Z. Role of matrix metalloproteinases in the pathogenesis of multiple sclerosis. Neurologiaineurochirurgiapolska 2005;**39**:63–67.
5. Demestre M, Parkin-Smith G, Petzold A, Pullen AH. The pro and the active form of matrix metalloproteinase-9 is increased in serum of patients with amyotrophic lateral sclerosis. J Neuroimmunol 2005;**159**:146–154.
6. Rosenberg GA, Sullivan N, Esiri MM. White matter damage is associated with matrix metalloproteinases in vascular dementia. Stroke 2001;**32**:1162–1168.
7. Rosenberg GA, Prestopnik J, Adair JC et al. Validation of biomarkers in subcortical ischaemic vascular disease of the Binswanger type: approach to targeted treatment trials. J Neurol Neurosurg Psychiatry 2015;**86**:1324–1330.
8. Yang Y, Hill JW, Rosenberg GA. Multiple roles of metalloproteinases in neurological disorders. Prog Mol Biol Transl Sci 2011;**99**:241–263.
9. Kowalski M, Walczak A, Majsterek I. Matrix metalloproteinases (MMPs): modern molecular markers of

https://doi.org/10.1017/neu.2015.72 Published online by Cambridge University Press

open-angle glaucoma diagnosis and therapy. Post Hig Med Dosw 2008;**62**:584–585.
10. Agrawal SM, Lau L, Yong VW. MMPs in the central nervous system: where the good guys go bad. Semin Cell Dev Biol 2008;**19**:42–51.
11. Kaczmarek L, Lapinska-Dzwonek J, Szymczak S. Matrix metalloproteinases in the adult brain physiology: a link between c-Fos, AP-1 and remodeling of neuronal connections? EMBO J 2002;**21**:6643–6648.
12. Michaluk P, Kaczmarek L. Matrix metalloproteinase-9 in glutamate-dependent adult brain function and dysfunction. Cell Death Differ 2007;**14**:1255–1258.
13. Renaud S, Erne B, Fuhr P et al. Matrix metalloproteinases-9 and -2 in secondary vasculitic neuropathies. Acta Neuropathol 2003;**105**:37–42.
14. Mannello F, Medda V. Nuclear localization of matrix metalloproteinases. Prog Histochem Cytochem 2012;**47**:27–58.
15. Rascher G, Fischmann A, Kroger S, Duffner F, Grote EH, Wolburg H. Extracellular matrix and the blood-brain barrier in glioblastoma multiforme: spatial segregation of tenascin and agrin. Actaneuropathologica 2002;**104**:85–91.
16. Candelario-Jalil E, Thompson J, Taheri S et al. Matrix metalloproteinases are associated with increased blood-brain barrier opening in vascular cognitive impairment. Stroke 2011;**42**:1345–1350.
17. Wójcik-Stanaszek L, Sypecka J, Szymczak P et al. The potential role of metalloproteinases in neurogenesis in the gerbil hippocampus following global forebrain ischemia. PLoS One 2011;**6**:e22465.
18. Pawlak R, Rao BS, Melchor JP, Chattarji S, McEwen B, Strickland S. Tissue plasminogen activator and plasminogen mediate stress induced decline of neuronal and cognitive functions in the mouse hippocampus. Proc Natl Acad Sci USA 2005;**102**:18201–18206.
19. Fainardi E, Castellazzi M, Bellini T et al. Cerebrospinal fluid and serum levels and intrathecal production of active matrix metalloproteinase-9 (MMP-9) as markers of disease activity in patients with multiple sclerosis. Mult Scler 2006;**12**:294–301.
20. World Health Organization. ICD-10 classification of mental & behavioural disorders. Geneva: World Health Organization, 1993.
21. Patten S. Performance of the Composite International Diagnostic Interview Short Form for major depression in community and clinical samples. Chron Dis Can 1997;**3**:18–24.
22. Lezak MD. Neuropsychological assessment. New York, Oxford: Oxford University Press, 2004.
23. Talarowska M, Gałecki P, Maes M, Bobińska K, Kowalczyk E. Total antioxidant status correlates with cognitive impairment in patients with recurrent depressive disorder. Neurochem Res 2012;**37**:1761–1767.
24. Winer J, Jung CK, Shackel I, Williams PM. Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes in vitro. Anal Biochem 1999;**270**:41–49.
25. Luz Sampieri C, Pena S, Ochoa-lara M, Zeneto-Cuevas R, Leon-Cordoba K. Expression of matrix metalloproteinases 2 and 9 in human gastric cancer and superficial gastritis. World J Gastroenterol 2010;**16**:1500–1505.
26. Kirkwood B, Sterne J. Essential medical statistics, 2nd edn. Hoboken: Wiley-Bleckwell, 2003.
27. Maes M, Lambrechts J, Bosmans E et al. Evidence for a systemic immune activation during depression: results of leukocyte enumeration by flow cytometry in conjunction with monoclonal antibody staining. Psychol Med 1992;**22**: 45–53.
28. Lutgendorf SK, Lamkin DM, Jennings NB et al. Biobehavioral influences on matrix metalloproteinase expression in ovarian carcinoma. Clin Cancer Res 2008;**14**:6839–6846.
29. Lutgendorf SK, Cole S, Costanzo E et al. Stress-related mediators stimulate vascular endothelial growth factor secretion by two ovarian cancer cell lines. Clin Cancer Res 2003;**9**:4514–4521.
30. Gałecki P, Talarowska M, Anderson G, Berk M, Maes M. Mechanisms underlying neurocognitive dysfunctions in recurrent major depression. Med Sci Monit 2015;**21**: 1535–1547.
31. Bjerke M, Jonsson M, Nordlund A et al. Cerebrovascular biomarker profile is related to white matter disease and ventricular dilation in a LADIS substudy. Dement Geriatr Cogn Dis Extra 2014;**4**:385–394.
32. Mizoguchi H, Takuma K, Fukuzaki E et al. Matrix metalloprotease-9 inhibition improves amyloid beta-mediated cognitive impairment and neurotoxicity in mice. J Pharmacol Exp Ther 2009;**331**:14–22.
33. Barichello T, Generoso JS, Michelon CM et al. Inhibition of matrix metalloproteinases-2 and -9 prevents cognitive impairment induced by pneumococcal meningitis in Wistar rats. Exp Biol Med (Maywood) 2014;**239**:225–231.
34. Li S, Wu Y, Keating SM et al. Matrix metalloproteinase levels in early HIV infection and relation to in vivo brain status. J Neurovirol 2013;**19**:452–460.
35. Jaworski DM, Boone J, Caterina J et al. Prepulse inhibition and fear-potentiated startle are altered in tissue inhibitor of metalloproteinase-2 (TIMP-2) knockout mice. Brain Res 2005;**1051**:81–89.
36. Wright JW, Brown TE, Harding JW. Inhibition of hippocampal matrix metalloproteinase-3 and -9 disrupts spatial memory. Neural Plast 2007;**2007**:73813.
37. Nagy V, Bozdagi O, Matynia A et al. Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. J Neurosci 2006;**26**:1923–1934.
38. Wiera G, Wozniak G, Bajor M, Kaczmarek L, Mozrzymas JW. Maintenance of long-term potentiation in hippocampal mossy fiber-CA3 pathway requires fine-tuned MMP-9 proteolytic activity. Hippocampus 2013;**23**:529–543.
39. Martín-Aragón S, Bermejo-Bescós P, Benedí J et al. Metalloproteinase's activity and oxidative stress in mild cognitive impairment and Alzheimer's disease. Neurochem Res 2009;**34**:373–378.
40. Anacker C, Scholz J, O'Donnell KJ et al. Neuroanatomic differences associated with stress susceptibility and resilience. Biol Psychiatry 2015; doi: 10.1016/j. biopsych.2015.08.009 [Epub ahead of print].
41. Donohue HS, Gabbott PL, Davies HA et al. Chronic restraint stress induces changes in synapsę morphology in stratum lacunosum-moleculare CA1 rat hippocampus: a stereological and three-dimensional ultrastructural study. Neuroscience 2006;**140**:597–606.
42. van der Kooij MA, Fantin M, Rejmak E et al. Role for MMP-9 in stress-induced downregulation of nectin-3 in hippocampal CA1 and associated behavioural alterations. Nat Commun 2014;**5**:4995.
43. Bienkowski P, Samochowiec J, Pelka-Wysiecka J et al. Functional polymorphism of matrix metalloproteinase-9 (MMP9) gene is not associated with schizophrenia and with its deficit subtype. Pharmacol Rep 2015;**67**: 442–445.

44. Chopra K, Baveja A, Kuhad A. MMPs: a novel drug target for schizophrenia. Expert Opin Ther Targets 2015;**19**:77–85.
45. Wright JW, Harding JW. Contributions of matrix metalloproteinases to neural plasticity, habituation, associative learning and drug addiction. Neural Plast 2009;**2009**:579382.
46. Ethell IM, Ethell DW. Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. J Neurosci Res 2007;**85**:2813–2823.
47. Leonardo CC, Pennypacker KR. Neuroinflammation and MMPs: potential therapeutic targets in neonatal hypoxic-ischemic injury. J Neuroinflammation 2009;**6**:13.

https://doi.org/10.1017/neu.2015.72 Published online by Cambridge University Press