

## Is there a link between TNF gene expression and cognitive deficits in depression?

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Neuroinflammation is a known factor in the pathogenesis of recurrent depressive disorders. Depression is accompanied by activated immune-inflammatory pathways including increased levels of TNF $\alpha$ , sTNFR1 and sTNFR2. The purpose of this study was to analyse the *TNF- $\alpha$* , *TNFRSF1A* and *TNFRSF1B* genes on both mRNA and protein levels in patients with rDD, and to investigate the relationship between *TNF- $\alpha$* , *TNFRSF1A* and *TNFRSF1B* gene expression and cognitive performance. The study comprised 158 subjects: patients with recurrent depressive disorder (n=89) and healthy subjects (n=69). Cognitive function assessment was based on: Trail Making Test, The Stroop Test, Verbal Fluency Test and Auditory Verbal Learning Test. Both mRNA and protein expression levels of all genes were significantly higher in rDD subjects when compared to healthy controls. No statistically significant correlations were observed between the analysed variables in both the rDD group and the HS test group. The only exception was noticed in the HS test group, where increased expression of *TNFRSF1A* and *TNFRSF1B* gene negatively affected the performance of the AVLT test. However, statistically significant correlations between *TNF*, *TNFRSF1A*, *TNFRSF1B* mRNA gene expression levels and all the neuropsychological tests used in the survey for the entire group were observed. **Conclusions:** 1. The results of our study show increased expression of the *TNF*, *TNFRSF1A* and *TNFRSF1B* genes on both mRNA and protein levels in depression. 2. Elevated expression of *TNF- $\alpha$* , *TNFRSF1A* and *TNFRSF1B* negatively correlates with cognitive efficiency: working memory, executive functions, attention, auditory-verbal memory, effectiveness of learning processes and verbal fluency.

**Key words:** depressive disorder, TNF- $\alpha$ , TNFRSF1A, TNFRSF1B, cognitive impairment

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**Abbreviations:** AD, Alzheimer's disease; AVLT, the Auditory Verbal Learning Test; CFS, chronic fatigue syndrome; CNS, Central Nervous System; HDRS, Hamilton Depression Rating Scale; HS, healthy subjects; IDO, indolamine-2,3-dioxygenase; IL-6, interleukin 6; L-1 $\beta$ , interleukin 1 beta; M, mean; MMSE, Mini-Mental State Examination; OCD, obsessive-compulsive disorder; rDD, recurrent depressive disorder; SD, standard deviation; TMT, the Trail Making Test (TMT); TNFR1, tumour necrosis factor receptor 1; TNFRSF1A, tumour necrosis factor receptor superfamily member 1A; TNFRSF1B, tumour necrosis factor receptor superfamily member 1B; TNF- $\alpha$ , tumour necrosis factor alpha; TNRF2, tumour necrosis factor receptor 2; VFT, the Verbal Fluency Test

### INTRODUCTION

Neuroinflammation is a known factor in the pathogenesis of recurrent depressive disorder (rDD) (Singhal *et al.*, 2014). Depression is an immune-inflammatory illness characterized by systemic and neuroinflammatory responses and by oxidative and nitrosative stress and neuroprogression (Maes *et al.*, 1990-1991; Moylan *et al.*, 2013, Moylan *et al.*, 2014). A number of studies have shown increased expression of pro-inflammatory cytokines, such as TNF- $\alpha$  (tumour necrosis factor alpha), IL-1 $\beta$  (interleukin 1 beta), and IL-6 (interleukin 6), in the brain of patients with depression leading to neuroinflammation (Dowlati *et al.*, 2010; Hannestad *et al.*, 2011). On the other hand, there is accumulating evidence of alterations in the peripheral immune system with increased circulating levels of pro-inflammatory cytokines in depressive disorders (Anisman, 2009). The first studies indicating an increased level of TNF- $\alpha$  in patients with depression were published as early as in 2000 (Lanquillon *et al.*, 2000; Milikova *et al.*, 2001). Moreover, increased levels of TNF- $\alpha$  in plasma are linked with the fact that treatment is resistant to the use of conventional antidepressants (O'Brien *et al.*, 2007). It has also been demonstrated that TNF- $\alpha$  antagonism improves treatment of resistant depression in a subgroup of patients with high baseline inflammatory biomarkers (Rison *et al.*, 2013).

Cytokines are small proteins, which are produced by a variety of immune cells (lymphocytes, macrophages, natural killer cells etc.). They are important for cell signalling when the immune system is mounting inflammatory responses to infection and cell damage (Sudheimer *et al.*, 2014). The circulating proinflammatory cytokines such as TNF- $\alpha$  are thought to promote the depressive disorder by affecting the functions of the Central Nervous System (CNS) in multiple ways (Mikova *et al.*, 2001; Dantzer *et al.*, 2008). TNF- $\alpha$  seems to contribute to the pathophysiology of depression by activating monoamine reuptake, stimulating the hypothalamic-pituitary-adrenocortical axis (HPA) and decreasing production of serotonin due to the increased activity of indolamine-2,3-dioxygenase (IDO) (Lichtblau *et al.*, 2013). Moreover, cytokines like TNF- $\alpha$  link the non-specific immune system to the hypothalamo-pituitary-adrenocortical (HPA) axis. TNF- $\alpha$  and its soluble receptors p55 and p75, which are released during infection and inflammation, activate the HPA system at the hypothalamic, pituitary and adrenal level resulting in the release of cortisol, being the most significant negative feedback signal preventing an overshoot of the ongoing host defense (Silverman *et al.*, 2005). A chronically activated HPA axis – as occurring during chronic stress – leads to defective responses of the immune system to inflammatory challenges (Bese-

dovsky & del Reay, 2000). Exogenous TNF- $\alpha$  administration causes the release of CRH (corticotropin-releasing hormone), ACTH (adrenocorticotrophic hormone) and cortisol (Michie *et al.*, 1988).

The action of TNF- $\alpha$  depends on the stimulation of specific receptors: TNFR1 (tumour necrosis factor receptor 1/tumour necrosis factor receptor superfamily member 1A, TNFRSF1A) and TNFR2 (tumour necrosis factor receptor 2/tumour necrosis factor receptor superfamily member 1B, TNFRSF1B) (Lichtblau *et al.*, 2013).

Besides the well-established mood changes, it has been recently recognised that a generalised cognitive deficit is at the core of depression (Talarowska *et al.*, 2014). Many studies also report that elevated pro-inflammatory cytokines negatively affect hippocampal structure and cognitive functioning (Sudheimer *et al.*, 2014). Elevated levels of TNF- $\alpha$  in particular have been shown to cause a reduction in hippocampal volumes through the neurodegenerative TNFRSF1A pathway and can lead to the development of depressive-like behaviour (Eyre *et al.*, 2013). The role of TNFRSF1B has not been thoroughly examined until now.

The purpose of this study was to analyse the TNF- $\alpha$ , TNFRSF1A and TNFRSF1B genes on both mRNA and protein levels in patients with rDD, and to investigate the relationship between *TNF- $\alpha$* , *TNFRSF1A* and *TNFRSF1B* gene expression and cognitive performance. We hypothesised that levels of *TNF- $\alpha$* , *TNFRSF1A* and *TNFRSF1B* gene expression are higher in the rDD group and that this might affect cognitive functions.

## MATERIAL AND METHODS

### Subjects

The study was carried out on a group of 158 subjects aged 20-67 (M=39.86 yrs., S.D.=14.15): patients with rDD (n=89) and a control group of healthy subjects (HS, n=69). Sample recruitment and description have already been presented elsewhere (Talarowska *et al.*, 2014b).

All the patients were native inhabitants of central Poland, unrelated to one another. The individuals for the study group were selected randomly without replacement sampling. The patients were chosen to take part in the experiment based on the inclusion criteria of ICD-10 (F 32.0-7.32.2, F 33.0-F 33.8) (WHO 1993). All the subjects were examined during the course of their hospitalisation. The presence of axis I and II disorders – other than depressive episode – and the diagnosis of somatic diseases and injuries of the central nervous system (CNS), which could have affected cognitive performance, were considered exclusion criteria. In all the subjects participating in the study, case history was obtained before the main study procedure using the standardized Composite International Diagnostic Interview (CIDI) (Patten 1997). The patients from the rDD group were qualified for the study during the process of pharmacological therapy modification. They were treated with agents from the group of selective serotonin reuptake inhibitors (SSRI).

The HS comprised healthy subjects without any family history of psychiatric disorders. The healthy controls included community volunteers who were enrolled based on the criteria of the psychiatric CIDI interview (Patten 1997). The control subjects with psychiatric diagnoses, axis I and II disorders, neurological disorders, or substance abuse or dependence were excluded from the study.

Based on medical records and diagnostic interviews, none of the participants had been diagnosed with a mental disability or any relevant intellectual deficit. All the subjects were free from medical illnesses, including 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 prior to blood sampling. None of the healthy participants was a drinker or heavy smoker, and none had ever taken psychotropic drugs.

## Methods

**Cognitive function assessment and severity of depression.** The neuropsychological assessment was conducted by trained neuropsychologists. A comprehensive neuropsychological test battery was administered to assess the full range of cognitive functions: the Trail Making Test (TMT), the Stroop Test, the Verbal Fluency Test (VFT) and the Auditory Verbal Learning Test (Polish version, AVLTL). Depression severity was assessed with the 17-item Hamilton Depression Rating Scale (HDRS). An examination based on neuropsychological tests was conducted once. The assessment of depression symptom intensification with the use of the HDRS scale was performed twice, i.e. at the beginning of the therapy and after 8 weeks. A detailed description of the tests is found in (Lezak 2004) and (Talarowska *et al.*, 2012).

For the patients with rDD, the HDRS, Stroop Test, TMT, AVLTL and VFT were performed at the onset of therapy. All patients were examined on admission during the symptomatic phase. At the time of examination, the patients were not taking any medications that would have had an influence on their cognitive function. In the HS group, neuropsychological tests were conducted during a single examination. The same psychologist examined the patients using neuropsychological tests, including an evaluation of the obtained results. The HDRS test was carried out by the same psychiatrist.

**Determination of serum *TNFRSF1A*, *TNFRSF1B*, *TNF $\alpha$*  level using Enzyme-Linked Immunosorbent Assay (ELISA).** For the quantitative detection of circulating serum *TNFRSF1A*, *TNFRSF1B*, *TNF $\alpha$*  actin as control Human TNFRSF1A ELISA Kit (R&D Systems, Inc Minneapolis MN, USA), Human TNFRSF1B ELISA Kit (Antibodies Atlanta GA, USA), Human TNF $\alpha$  ELISA Kit (R&D Systems, Inc Minneapolis MN, USA), Human Actin Beta (ACTb) ELISA Kit (Antibodies Atlanta GA, USA) were used. Each serum sample was determined three times. Reactions and calculation of results were performed according to the manufacturer's recommendations. The colour development was stopped (Stop Solution) and the intensity of the colour was measured by Thermo Labsystems Multiskan Ascent 354 from Lab Recyclers at 450 nm with the correction wavelength set at 540 nm.

**Real-time quantitative PCR.** Total RNA (1  $\mu$ g) was extracted from blood cells using Trizol reagent (Applied Biosystems), and was processed directly to cDNA synthesis using the TaqMan Reverse Transcription Reagents kit (Applied Biosystem) according to the manufacturer's protocol. The human *TNFRSF1A*, *TNFRSF1B*, *TNF $\alpha$*  and 18S rRNA expression were quantified by real-time quantitative PCR using ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA USA) according to the manufacturer's protocol. Briefly, 2.5, 2.0; 1.5, 1.0; 0.5 and

0.25 µl of synthesised cDNA were amplified in triplicate for 18S rRNA and each of the target genes to create a standard curve. Likewise, 2 µl of cDNA was amplified in triplicate in all isolated samples for each primer/probe combination and 18S rRNA. Each sample was supplemented with fluorescent probe Hs01042313\_m1, Hs 00961740\_m1, Hs 01113624\_g1, Hs 04194366-g1, specific for *TNFRSF1A*, *TNFRSF1B*, *TNFA*, 18S rRNA, respectively, and made up to 50 µl using TaqMan Universal PCR Master Mix (Applied Biosystems). 18S rRNA cDNA was used as an active and endogenous reference to correct for differences in the amount of total RNA added to reaction and to compensate for different levels of inhibition during reverse transcription of RNA and during PCR. Each target probe was amplified in a separate 96-well plate. All samples were incubated at 50°C for 2 minutes and at 95°C for 10 minutes, and then cycled at 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute, for 40 cycles. Fluorescence emission data were captured and mRNA levels were quantified using the critical threshold (Ct) value. Analyses were performed with ABI Prism 7000 (SDS Software). Controls without RT and with no template cDNA were performed with each assay. Relative gene expression levels were obtained using the  $\Delta\Delta C_t$  standard  $2^{-\Delta\Delta C_t}$  calculations and expressed as a fold change of a control sample (Winer *et al.*, 1999; Livak & Schmittgen, 2001). Amplification specific transcripts were further confirmed by obtaining melting curve profiles.

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

The qualitative characteristics of the experimental and control groups are expressed as frequencies shown as percentages. To characterise the average values for quantitative features, the arithmetical mean ( $M$ ) was calculated. The measures of statistical dispersion included the range of values between the minimum and the maximum, and the standard deviation (S.D.).

To compare nonparametric variables in the test groups, the following tests were used: the Pearson  $\chi^2$  for qualitative variables and the Mann-Whitney U test for two independent groups to determine the coincidence of distributions. To evaluate the relationships between the analysed variables, Spearman's R rank order correlation coefficients were estimated. For all analyses, statistical significance was defined as  $P < 0.05$  (Kirkwood and

Sterne 2003). All data analyses were performed using STATISTICA PL, version 10.

### Ethics

All the patients were native Poles, inhabitants of central Poland and unrelated. Selection of the individuals to the test group was random, without replacement sampling. The experimental group was randomly chosen from the patients treated at the Babinski Memorial Hospital in Lodz. The HS group was selected from among the staff of this hospital.

Before deciding to participate in the study, the subjects were informed of its purpose, assured that the participation was voluntary, and guaranteed that personal data and the results of the tests would be kept confidential. Written informed consent for participation was obtained from each subject according to the study protocol that had been approved by the Bioethical Committee of the Medical University of Lodz (No. RNN/728/12/KB).

### RESULTS

Table 1 presents 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). No statistically significant differences were found between the examined groups in terms of sex ( $\chi^2=1.32$ ,  $p=0.249$ ); the only differences were the number of years of education ( $Z=-6.32$ ,  $p=0.001$ ) and age ( $Z=8.16$ ,  $p=0.001$ ).

In Table 2 and in graphs 1 and 2, average values of expression on the mRNA level and on the protein level for *TNF*, *TNFRSF1A*, *TNFRSF1B* genes in the entire examined group ( $n=158$ ) are shown and the analysed variables are compared in the rDD group ( $n=89$ ) and the group of healthy individuals ( $n=69$ ).

Statistically significant differences have been observed between the patients with recurrent depressive disorders and the individuals from the HS test group as regards all the analysed variables (Table 2). Expression of *TNF*, *TNFRSF1A*, *TNFRSF1B* genes on the mRNA level and on the protein level was significantly higher in the patients suffering from rDD than in the healthy subjects.

Tables 3–5 present the correlation between *TNF*, *TNFRSF1A* and *TNFRSF1B* mRNA and protein expression levels and the results of neuropsychological tests, separately for the rDD and HS test group. Tables 3–5 also show the results of the correlation between *TNF*, *TNFRSF1A*, *TNFRSF1B* mRNA and protein expression levels and the neuropsychological tests for the entire group.

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

Characteristics	N	rDD (n=89)			HS (n=69)		
		%	(±S.D.)	n	%	(±S.D.)	
Sex	Female	50	56.18	–	45	65.22	–
	Male	39	43.82	–	24	34.78	–
Age (yrs)		–	–	48.23 (±11.49)	–	–	29.06 (±9.01)
Education level	Primary	26	29.21	–	–	–	–
	Secondary	50	56.18	–	26	37.68	–
	College/University	13	14.61	–	43	62.32	–
rDD	Disease duration in years	–	–	7.01 (±5.54)	–	–	–
	Number of depression episodes	–	–	5.15 (±1.98)	–	–	–

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

Table 2. Expression on the protein level and on the level of mRNA for TNF, TNFRSF1A, TNFRSF1B genes in the examined group

Variable	n=158	rDD n=89	HS n=69	Mann-Whitney U test	
	M (S.D.)	M (S.D.)	M (S.D.)	Z	p
TNF protein (pg/ml)	8.22 (3.53)	11.16 (1.31)	4.47 (0.99)	10.736	<0.001*
TNF mRNA ( $2^{-\Delta\Delta ct}$ )	0.47 (0.24)	0.68 (0.09)	0.19 (0.05)	10.736	<0.001*
TNFRSF1A protein (pg/ml)	1188.92 (206.19)	1351.01 (77.66)	982.20 (111.63)	10.707	<0.001*
TNFRSF1A mRNA ( $2^{-\Delta\Delta ct}$ )	1.29 (0.11)	1.38 (0.04)	1.19 (0.06)	10.679	<0.001*
TNFRSF1B (pg/ml)	1977.09 (208.11)	2136.19 (97.04)	1774.17 (113.01)	10.499	<0.001*
TNFRSF1B mRNA ( $2^{-\Delta\Delta ct}$ )	1.68 (0.11)	1.76 (0.05)	1.58 (0.07)	10.187	<0.001*

TNF, tumour necrosis factor; TNFRSF1A, tumour necrosis factor receptor superfamily member 1A; TNFRSF1B, tumour necrosis factor receptor superfamily member 1B; rDD, recurrent depressive disorders; HS, healthy subjects; M, mean; S.D., standard deviation; \*p, statistically significant.

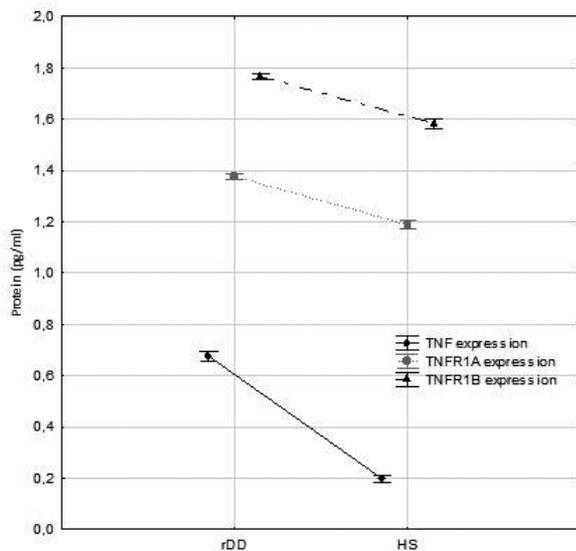


Figure 1. Average values and confidence interval (95.00%) for the analysed variables.

rDD, recurrent depressive disorders; HS, healthy subjects

No statistically significant correlations were observed between the analysed variables in both the rDD group and the HS test group. The only exception was noticed in the HS test group, where increased expression of *TNFRSF1A* and *TNFRSF1B* gene correlated negatively with the performance of the AVLT test. However, statistically significant correlations between *TNF*, *TNFRSF1A*, *TNFRSF1B* mRNA gene expression levels and all the neuropsychological tests used in the survey for the entire group (n=158) were observed.

## DISCUSSION

In recent years, an increasing number of scientific reports support the immunological hypothesis of depression. This emphasises the role of the immune system in the aetiology, course and recurrence of the disease (Maes *et al.*, 2013). Many authors who analyse this prob-

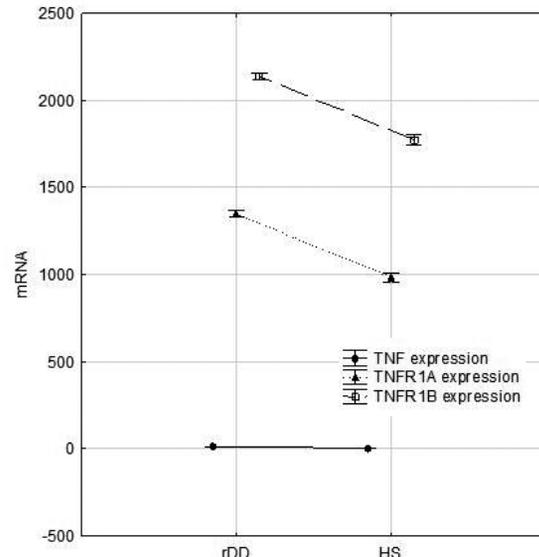


Figure 2. Average values and confidence interval (95.00%) for the analysed variables.

rDD, recurrent depressive disorders; HS, healthy subjects

lem pay attention mainly to the role of proinflammatory cytokines in behavioural changes, within the systems of neurotransmitters in CNS and in endocrine changes observed in depression. Moreover, the varying course of depressive disorders is justified by the inflammatory mechanisms and immunological disorders that lie at the foundation of the disorders (Maes *et al.*, 2013).

The hypothesis presented by the authors in the introduction was confirmed. The obtained results indicate a difference between the level of *TNF*, *TNFRSF1A* and *TNFRSF1B* gene expression on both mRNA level and protein level between the group of patients with rDD and the reference group. The people suffering from recurrent depressive disorders were characterised by higher expression of all the genes on the protein level and on the mRNA level than the group of healthy subjects. The results obtained are in agreement with the results of experiments conducted by Dannehl and cow-

Table 3. Spearman's rank correlation coefficients(R) for the variables tested – TNF

	rDD (n=89)		HS (n=69)		The whole group (n=158)	
	TNF mRNA (2- $\Delta\Delta^{\text{ct}}$ ) 0.68 (0.09)	TNF protein (pg/ml) 11.16 (1.31)	TNF mRNA (2- $\Delta\Delta^{\text{ct}}$ ) 0.19 (0.05)	TNF protein (pg/ml) 4.47 (0.99)	TNF mRNA (2- $\Delta\Delta^{\text{ct}}$ ) 0.47 (0.24)	TNF protein (pg/ml) 8.22 (3.53)
	R		R		R	
TMT A-time	-0.032	0.001	-0.004	0.013	0.482*	0.472*
TMT B-time	-0.072	-0.034	0.087	0.100	0.595*	0.585*
RCNb-time	-0.091	-0.081	0.117	0.135	0.487*	0.483*
NCWd-time	-0.198	-0.163	0.210	0.234	0.483*	0.466*
VFT-animals	-0.037	-0.016	0.079	0.079	-0.443*	-0.447*
VFT-sharp objects	0.113	0.084	-0.010	-0.022	-0.333*	-0.323*
VFT-the letter k	-0.093	-0.111	0.028	0.035	-0.386*	-0.381*
AVLT-first attempt	-0.124	-0.151	-0.203	-0.186	-0.507*	-0.503*
AVLT-number of words in 30 min	-0.018	-0.041	0.144	0.167	-0.507*	-0.505*
AVLT-average of 5 tests	-0.104	-0.111	-0.190	-0.167	-0.599*	-0.601*

TNFRSF1A, tumour necrosis factor receptor superfamily member 1A; rDD, recurrent depressive disorder; HS, healthy subjects; TMT, Trail Making Test; RCBn, reading colour names in black; NCWd, naming colour of word-different; AVLT, Auditory-Verbal Learning Test; VFT, Verbal Fluency Test; \*p, statistically significant.

Table 4. Spearman's rank correlation coefficients(R) for the variables tested – TNFRSF1A

	rDD (n=89)		HS (n=69)		The whole group (n=158)	
	TNFRSF1A mRNA (2- $\Delta\Delta^{\text{ct}}$ ) 1.38 (0.04)	TNFRSF1A protein (pg/ml) 1351.01 (77.66)	TNFRSF1A mRNA (2- $\Delta\Delta^{\text{ct}}$ ) 1.19 (0.06)	TNFRSF1A protein (pg/ml) 982.20 (111.63)	TNFRSF1A mRNA (2- $\Delta\Delta^{\text{ct}}$ ) 1.29 (0.11)	TNFRSF1A protein (pg/ml) 1188.92 (206.19)
	R		R		R	
TMT A-time	0.001	0.037	0.059	0.103	0.485*	0.502*
TMT B-time	-0.031	-0.002	0.136	0.163	0.593*	0.602*
RCNb-time	-0.018	0.033	0.068	0.088	0.492*	0.514*
NCWd-time	-0.145	-0.082	-0.044	-0.012	0.438*	0.463*
VFT-animals	0.040	-0.020	0.109	0.068	-0.412*	-0.441*
VFT-sharp objects	0.018	-0.019	-0.029	-0.087	-0.355*	-0.376*
VFT-the letter k	-0.089	-0.087	-0.097	-0.120	-0.403*	-0.403*
AVLT-first attempt	-0.117	-0.154	-0.199	-0.174	-0.492*	-0.506*
AVLT-number of words in 30 min	0.016	-0.006	-0.089	-0.104	-0.527*	-0.538*
AVLT-average of 5 tests	-0.009	-0.077	-0.256*	-0.268*	-0.579*	-0.603*

TNF, tumour necrosis factor; rDD, recurrent depressive disorder; HS, healthy subjects; TMT, Trail Making Test; RCBn, reading colour names in black; NCWd, naming colour of word-different; AVLT, Auditory-Verbal Learning Test; VFT, Verbal Fluency Test; \*p statistically significant.

orkers (2014) and other authors (Khairova *et al.*, 2009; Dowlat *et al.*, 2010; Becking *et al.*, 2013; Mills *et al.*, 2013; Schmidt *et al.*, 2014; Mihailova *et al.*, 2016; Park *et al.*, 2016).

The first reports indicating the participation of pro-inflammatory cytokines in the pathogenesis of depression resulted from observations of mental changes in laboratory-tested animals and in people after administering, among others, TNF- $\alpha$ . In the tested groups the following symptoms were noticed: loss of appetite, fatigue, low mood, or reduced efficiency of cognitive processes (mainly the efficiency of memory and attention processes) (Dantzer, 2009). According to Maes and coworkers (2012), the concentration of TNF- $\alpha$  in blood serum of

the affected with symptoms of depressive disorders is, however, significantly greater than in the group of patients with chronic fatigue syndrome (CFS). In several papers it is possible to find confirmation of the relationship between increased levels of TNF- $\alpha$  and the intensified tendency towards making suicidal attempts. This correlation is observed among both adults and teens (Serafini *et al.*, 2013; O'Donovan *et al.*, 2013). What is interesting, Grosse and coworkers (2016) suggest a linear relationship between childhood sexual abuse and increased pro-inflammatory cytokine levels in depressive patients, while more recent stressful life events are not related to these inflammatory markers. The last of the conclusions seems to support the neurodevelopmental

Table 5. Spearman's rank correlation coefficients(R) for the variables tested – TNFRSF1B

	rDD (n=89)		HS (n=69)		The whole group (n=158)	
	TNFRSF1B mRNA (2- $\Delta\Delta^{\text{ct}}$ ) 1.76 (0.05)	TNFRSF1B protein (pg/ml) 2136.19 (97.04)	TNFRSF1B mRNA (2- $\Delta\Delta^{\text{ct}}$ ) 1.58 (0.07)	TNFRSF1B protein (pg/ml) 1774.17 (113.01)	TNFRSF1B mRNA (2- $\Delta\Delta^{\text{ct}}$ ) 1.68 (0.11)	TNFRSF1B protein (pg/ml) 1977.09 (208.11)
	R		R		R	
TMT A-time	0.011	0.016	0.073	0.102	0.453*	0.485*
TMT B-time	-0.011	0.011	0.127	0.151	0.565*	0.601*
RCNb-time	0.017	0.009	0.082	0.067	0.489*	0.488*
NCWd-time	-0.061	-0.061	-0.056	-0.032	0.437*	0.456*
VFT -animals	-0.041	-0.059	0.043	0.064	-0.431*	-0.452*
VFT-sharp objects	0.045	0.041	-0.157	-0.085	-0.358*	-0.353*
VFT-the letter k	-0.075	-0.096	-0.098	-0.094	-0.383*	-0.406*
AVLT-first attempt	-0.061	-0.053	-0.289*	-0.183	-0.481*	-0.464*
AVLT-number of words in 30 min	-0.051	-0.073	-0.118	-0.096	-0.523*	-0.549*
AVLT-average of 5 tests	-0.071	-0.069	-0.301*	-0.267*	-0.578*	-0.586*

TNFRSF1B, tumour necrosis factor receptor superfamily member 1B; rDD, recurrent depressive disorder; HS, healthy subjects; TMT, Trail Making Test; RCNb, reading colour names in black; NCWd, naming colour of word-different; AVLT, Auditory-Verbal Learning Test; VFT, Verbal Fluency Test; \*p statistically significant.

theory of depression development (Galecki & Talarowska, 2016).

The direct relationship between inflammatory processes and the development of depression is also indicated in the papers which evaluate the level of TNF- $\alpha$  in blood serum of patients suffering from other diseases, including Alzheimer's disease (AD), with or without an accompanying diagnosis of depression. The serum levels of TNF- $\alpha$  were elevated in AD patients with depression compared to the control group or AD patients without depression. Furthermore, a strong inverse correlation was observed between MMSE (Mini-Mental State Examination) scores and serum levels of TNF- $\alpha$  in AD subjects with depression (Khemka *et al.*, 2014). In addition, depressive disorders have frequently been observed in association with peripheral inflammatory cytokine activation in several medical conditions, including viral infections, rheumatoid arthritis, coronary heart disease, metabolic disorders (diabetes type 1 and type 2) or cancer (El-Tantawy *et al.*, 2008; Oliveira Miranda *et al.*, 2014; Singhal *et al.*, 2014), as well as among pregnant women, in whom the presence of symptoms of depressive disorders had been noticed (Christian *et al.*, 2011). The hypothesis of an elevated level of TNF- $\alpha$  as a consequence of active stressors (e.g. experiencing domestic violence in childhood) has also been confirmed several times (Kiecolt-Glaser *et al.*, 2011; Himmerich *et al.*, 2013). Elevated concentrations of TNF- $\alpha$  in blood serum have been noticed among patients diagnosed with obsessive-compulsive disorder (OCD) (Fontenelle *et al.*, 2012). It is necessary to mention that an increased level of TNF- $\alpha$  is observed in the patients suffering from depression accompanying medical conditions characterised by a chronic inflammatory component such as breast cancer (Bower *et al.*, 2011) and cardiovascular diseases (Szczepeńska-Sadowska *et al.*, 2010). It is, however, possible to find papers in which the said relationship (depression symptoms and TNF- $\alpha$  level increase) is not confirmed (Simpson *et al.*, 2016).

The presented study also provides an evaluation of the relationship between TNF- $\alpha$  gene expression and cognitive functions.

In the case of the entire group tested (n=158), a negative influence of increased expression of TNF genes on the efficiency of all the analysed variables has been indicated: operational memory (visual and spatial, and verbal and auditory), speed of information processing, attention, short-term memory, and the ability of acquiring new information (declarative memory).

There are not many experiments that would analyse the correlation between TNF gene expression and gene expression for its receptors, and the cognitive functions of the patients with rDD. Referring to the available literature, it is worth quoting the work by Naude and coworkers (2014), in which the functional roles of TNFRSF1A and TNFRSF1B in learning and memory, motor performance and anxiety-like behaviour were analysed. Results from this study show that deletion of TNFRSF1B impairs novel object recognition, spatial memory recognition, contextual fear conditioning, and motor performance, and can increase anxiety-like behaviour in young adult mice. However, the results presented by us do not differentiate the level of performing the neuropsychological tests depending on the expression of the gene that encodes the TNFRSF1A or TNFRSF1B receptor. Increased expression of both the genes is negatively correlated with cognitive performance.

At this point it is worth mentioning the research studies by Smagula and coworkers (2016). The authors demonstrated that immunological markers are associated with brain structure in late-life depression. TNF- $\alpha$  had a significant positive relationship with white matter hyperintensity volume (WMH), but only eotaxin was associated with the executive function (set-shifting performance as measured with the Trail-making test).

Providing a response to the question concerning the cause of the mentioned relationships is not an easy task. The results of experiments carried out in recent years show that proinflammatory cytokines (including TNF)

are constitutively expressed in normal brain, although not so long ago there was an opinion that inflammatory cytokines were only expressed in the brain in response to pathological stimuli (Khairova *et al.*, 2009).

TNF is a proinflammatory cytokine, which is released during neuronal activity and plays a crucial role in regulating the strength of synaptic transmission (Marin & Kipnis, 2013). TNF has also been shown to inhibit long-term potentiation (LTP) and synaptic plasticity in the hippocampus and potentially contribute to excitotoxicity (Pickering & O'Connor, 2007). According to Butler and coworkers (2004), the pathophysiological levels of TNF- $\alpha$  have been shown to inhibit LTP in the CA1 region, as well as the dentate gyrus of the rat hippocampus.

TNF signalling through the TNFR1 receptor modulates synaptic strength by changing the expression of AMPA receptors (AMPA, synaptic  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors) in the postsynaptic compartment (Dummer *et al.*, 2002). Gliosecreted TNF was identified as an important factor in mediating synaptic scaling (Stellwagen & Malenka, 2006). TNF also seems to play a role in adult hippocampal neurogenesis (Iosif *et al.*, 2006). Signalling through TNFRSF1A seems to act as a negative regulator of neurogenesis, as its deletion results in an increase in the number of newly produced neurons, whereas TNFRSF1B signalling is a positive regulator, as indicated by the drop in neurogenesis following its deletion (Jurgens *et al.*, 2012).

Moreover, Sudheimer and coworkers (2014) have demonstrated that elevated levels of waking cortisol in conjunction with higher concentrations of TNF- $\alpha$  are associated with smaller hippocampal volumes among older adults. In addition, independent of cortisol, higher levels of TNF- $\alpha$  are also associated with smaller hippocampal volumes. Authors (Sudheimer *et al.*, 2014) suggest that the dynamic balance between the hypothalamic-pituitary-adrenal axis and inflammation processes may explain hippocampal volume reductions. Similar results, indicating the negative relationship between the level of proinflammatory cytokines (including TNF- $\alpha$ ) and the cognitive function, have been obtained by Jurgens and coworkers (2012) (experiments based on an animal model). Additionally, Pandey and coworkers (2012) reported increased expression of the TNF- $\alpha$  encoding gene in the area of the prefrontal cortex (field 10 according to Brodmann) among 24 victims of suicide attempts. This correlation was statistically greater among the victims of suicide attempts than 24 individuals from the reference group (similar results were also obtained by Janelidze and coworkers, 2011). It has been known for a long time that the prefrontal cortex is particularly active in a series of mental processes connected with planning and executing actions, and the so-called executive functions, and their weakening has been demonstrated many times among patients suffering from rDD (Talarowska *et al.*, 2013).

The significant relationships observed in the current study between TNF gene expression and cognitive efficiency suggest that immune disturbance may be involved in cognitive deficits. The presented results also indicate that immuno-inflammatory processes may play a central role in cognitive deficits, not only in depressive disorders. Future studies with larger arrays of cytokine profiles may provide more sensitive and specific modes of diagnostics in determining rDD aetiology and provide guidance in individual therapies (Young *et al.*, 2014; Dahl *et al.*, 2016; Park *et al.*, 2016).

## CONCLUSIONS

1. The results of our study show increased expression of the *TNF*, *TNFRSF1A* and *TNFRSF1B* genes on both mRNA and protein levels in depression. 2. Elevated expression of *TNF- $\alpha$* , *TNFRSF1A* and *TNFRSF1B* correlates negatively with cognitive efficiency: working memory, executive functions, attention functions, direct and delayed auditory-verbal memory, effectiveness of learning processes and verbal fluency.

## 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.

Additionally, the difference in the level of education and the differences in mean age of the examined subjects may be significant for the analysis of the obtained results.

## Competing interests

None to declare.

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