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Expression of genes *ESR1* and *ESR2* encoding estrogen receptors and cognitive functioning in patients with depression

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Abstract

Introduction: *Researchers are increasingly interested in the role of sex hormones and their impact on other than reproductive systems, including mental functioning. Estrogens acting through the receptors ESR1 and ESR2 may participate in the regulation of mood and cognitive functions. The aim of the study was to evaluate the expression of genes* ESR1 *and* ESR2 *encoding estrogen receptors in depressive disorders and search for relationship between gene expression, cognitive functioning and socio-demographic data.*

Material and methods: *A total of 236 people (131 depressed patients, 105 healthy controls) participated in the study. Socio-demographic data were collected. Neuropsychological test have been carried out to evaluate cognitive functions. Venous blood was collected from all participants. RT-PCR was performed to evaluate the expression of genes* ESR1 *and* ESR2 *at mRNA level and enzyme-linked immunosorbent assay (ELISA) test to evaluate gene expression at protein level.*

Results: *Participants suffering from depression were characterized by a higher level of ESR1 expression both at the mRNA and protein level. Expression of* ESR1 *and* ESR2 *genes might be related to functioning in the examined cognitive areas. Expression of* ESR1 *and* ESR2 *genes does not differ statistically significantly in terms of gender.*

Conclusions: ESR1 *and* ESR2 *genes might play an important role in depression and cognitive functioning.*

Key words: depression; estrogen receptor; cognitive functions; ESR1; ESR2

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Introduction

Depressive disorders are a growing social problem worldwide. The latest WHO data indicate that depression affects approximately 3.8% of the population. Currently, about 280 million people in the world struggle with depression, which is considered the fourth most serious disease in the world [1]. Depression is a significant health and social problem. It is one of the main causes of disability. There are many theories about the reasons of depressive disorders, it is a multifactorial disease that has a biopsychosocial background. Searching for new genes related to the development of depressive symptoms, possible risk factors and new ways of treatment seems to be crucial. Researchers are increasingly interested in the role of sex hormones and their impact on other than reproductive systems, including mental functioning.

Estrogens act on target cells through two different types of receptors, ESR1 (alpha) and ESR2 (beta), which are nuclear receptors. In addition to the traditional route of hormonal signaling related to the regulation of gene transcription, mechanisms of rapid action of steroids, called non-genomic, have been described, in which the regulation of cell activity is carried out by interactions with regulatory proteins in the cell membrane and cytoplasm [2].

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These two receptors differ in terms of structure, show different tissue specificity and different affinity for ligands, are encoding for different genes (*ESR1* and *ESR2*). The *ESR1* gene is located in the q25.1-q25.2 portion of chromosome 6, while the *ESR2* gene is located in the q23.2-q23.3 portion of chromosome 14.

The ESR1 is most expressed in the endometrium, prostate, liver, ovaries, adipose tissue, and thyroid, while the ESR2 in the testes, adrenal glands, ovaries, adipose tissue, lymph nodes, spleen. Both receptors are also located in blood cells and areas of the brain related to cognition and emotion. The ESR1 shows greatest expression in the hypothalamus, thalamus, amygdala, cerebellum and cortex, while ESR2 in the cerebellum, thalamus, amygdala, and hypothalamus. Both receptors are also expressed in the hippocampus [3, 4]. These brain areas are involved in pathogenesis of depression.

According to monoamine theory of depression, the study in rats showed that ESR2 regulates tryptophan hydroxylase mRNA expression in serotonergic neurons of the dorsal nuclei [5].

It is likely that ESR1, working together with another transcription factor, NF-kappa B, synergistically activates the serotonin 1A receptor promoter through non-classical estrogen response elements, that is through a mechanism that does not involve direct action of the receptor with DNA [6].

Not only serotonin, but also dopamine have association with depression and estrogen receptors.

In mice lacking the *ESR2* gene in the cells of the amygdala, disorders of both dopaminergic and cholinergic transmission were observed [7]. Also, ESR1 in the hippocampus attribute to presynaptic transmission in GABA-ergic and cholinergic neurons [8].

Except of neurotransmitters theory of depression and its connections with estrogen receptors, the occurrence of depressive disorders is influenced by the action of the hypothalamic-pituitary-adrenal axis. A study on an animal model [9] revealed that estradiol, can dysregulate the HPA axis, acting on ESR1, another study [10] pointed both receptors as potential HPA axis modulator by estrogen during restraint stress.

The study suggests that ESR2 activation may regulate hippocampal plasticity and improve hippocampal-dependent cognition. More dendritic branches were observed in vivo. The ESR2 agonist improved performance on hippocampus-dependent memory tasks [11]. Other publications have indicated that ESR2 promotes neurogenesis, has a neuroprotective effect, reduces the inflammatory response and behaviors similar to anxiety and depression [12].

The study confirms that cognitive deficits occur in a significant proportion of patients with depression and should not be underestimated because they are a predictor of response to treatment. It happens that, despite pharmacotherapy, cognitive deficits persist as the so-called residual symptoms of depressive disorders, significantly hindering the patient's functioning [13]. The aim of our study was to evaluate the expression of genes *ESR1* and *ESR2* encoding estrogen receptors and in depressive disorders and search for relationship between gene expression, cognitive functioning and socio-demographic data.

Material and methods

Characteristics of study participants

Each study participant signed informed written consent to participate in the study, in accordance with the resolutions of the Bioethics Committee of the City of Lodz: RNN/384/11/KB, KE/1401/18. The study involved 236 participants of the Polish population aged 19–64 (39.8 ± 14.02), including 145 women and 91 men. The experimental group consisted of 131 patients of Psychiatry Department diagnosed with depressive disorder (diagnosis F32 and F33 according to ICD-10), including 76 women and 55 men, aged 19–64 (48.53 ± 11.05). The control group consisted of 105 participants, including 69 women and 35 men aged 19–64 (28.89 ± deviation 8.69). Groups were statistically significantly different in terms of age ($p < 0.001$). There was no statistically significant difference in terms of gender between groups ($p = 0.31$). The exclusion criterion for both groups was the coexistence of neurological, inflammatory and neoplastic diseases. Socio-demographic data (age, gender) were collected from all study participants and tests were performed to assess cognitive functions using: Stroop Color — Word Interference Test, Trail Making Test A & B, Verbal Fluency Test and Luria Memory Words Test. Venous blood in the amount of 5 mL was collected from all participants to perform genetic tests.

Molecular analysis

The material for the study (RNA isolation) were mononuclear cells of venous blood collected on citrate.

Protein expression assessment

The concentration of ESR1 and ESR2 proteins in the plasma of all participants was determined using ELISA immunoenzymatic tests: ESR1– Human ER alpha ELISA Kit (LifeSpan BioSciences Seattle WA USA), ESR2– Human ER beta ELISA Kit (Abbexa Ltd., Cambridge, United Kingdom) according to the protocols provided by the manufacturer.

mRNA gene expression assessment

Isolation of total RNA from the blood of all participants was performed with the InviTrap Spin Universal RNA Kit (Stratec molecular, Berlin, Germany) according to the manufacturer's instructions.

Quality analysis of isolated RNA

The quality of the isolated RNA was checked with the 2100 Bioanalyzer (Agilent Technologies). The degree of total RNA degradation was determined by electropherogram and the obtained RIN values. Only samples with a RIN > 7 were analyzed further.

Reverse transcription RT-PCR

The RT reaction was performed with the TaqMan® RNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's recommendations, using specific primers and probes Hs 00607062_gh, Hs 01584024_m1 for the *ESR1* and *ESR2* respectively, provided by Applied Biosystems. The comparative method Ct [14] was used to calculate relative gene expression at the mRNA level using the ΔΔCt calculation of standard 2-ΔΔct. Gene Expression Levels in individual samples were normalized to the reference gene *RPL13A*.

Neuropsychological tests

Stroop Color — Word Interference Test

The Stroop test was used primarily to assess verbal working memory and the effectiveness of attention processes. The test consists of two parts: RCNb (reading color name in black) and NCWd (naming color of word-different). The first part is for the test person to read as quickly as possible the words denoting the names of colors written in black on a white sheet. In the second part, subject should name the colors of the printing of individual words as soon as possible. Importantly, the color of the word print does not match the color of the designation. The result of the test is the time in seconds obtained in each of the parts and the number of errors made in each of these phases. This test assesses the ability to learn one way of doing things and the ability to switch to another model while the first one is still being recalled [15].

Trail Making Test A & B (TMT A&B)

The test consists of two parts (A and B). In part A, the subject connects successive numbers (from 1 to 25) with a continuous line. In part B, the subject connects consecutive numbers and letters of the alphabet alternately with a continuous line according to the formula: 1-A-2-B-3-C. Both parts should be completed as soon as possible. The test allows you to assess the visuospatial aspects of working memory and the efficiency of attention. The time, measured in seconds, is the result of each part of the test. TMT-A tests psychomotor speed and visuospatial functions. TMT-B additionally tests the ability to switch to a new task performance criterion and the ability to inhibit a previously learned rule [16].

Verbal Fluency Test

This test was used to assess the ability to create and pronounce words fluently according to a given criterion. It consists of three parts. The first two contain semantic categories and examine semantic fluency. The subject's task is to name as many words as possible from the semantic category: animals and sharp objects within 60 seconds. In the third part, the so-called letter category, examining phonemic fluency, the subject is asked to name as many words beginning with the letter "k" as possible within 60 seconds. The score is the number of words spoken correctly in each test. Phonemic fluency is mainly based on executive functions such as motor planning, concept selection, inhibition of semantic strategies and performance control, it also engages the phonological loop of working memory. Semantic fluency requires the involvement of mainly semantic memory [17].

Luria Memory Words Test

This test is used to assess immediate auditory memory, delayed memory and the effectiveness of learning processes. In the 10-word learning curve test, the subject repeats all memorized words immediately after each of the ten trials, which are read by the examiner in the same order and at the same pace. Then, after a 30-minute delay, the subject repeats the memorized words again, but without the researcher reading them again. The test result is the number of words repeated by the tested person in subsequent trials and the number of words reproduced after a thirty-minute break [18].

Results

Statistical analyses were carried out using the SPSS version 23 program. The selection of parametric/ /non-parametric tests depended on the distribution of the tested variables. The level of significance was considered to be $p < 0.05$. Trends towards statistical significance was considered to be $0.05 < p < 0.1$.

Comparison of gene expression

To examine whether healthy and depressed group differed in terms of the level of expression of genes encoding estrogen receptors, t-Student tests were

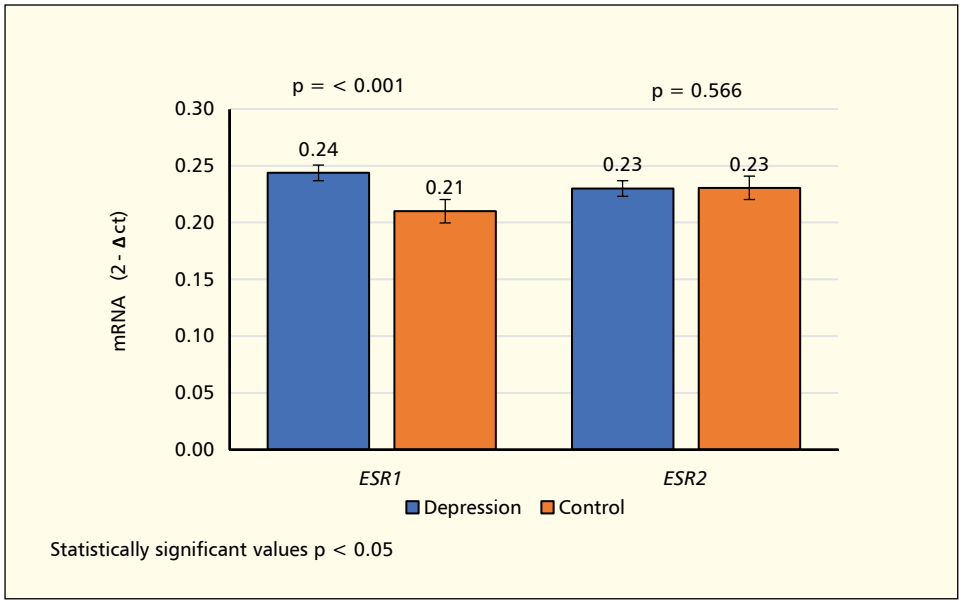

Figure 1. Comparison of *ESR1* and *ESR2* gene expression at the mRNA level in both groups

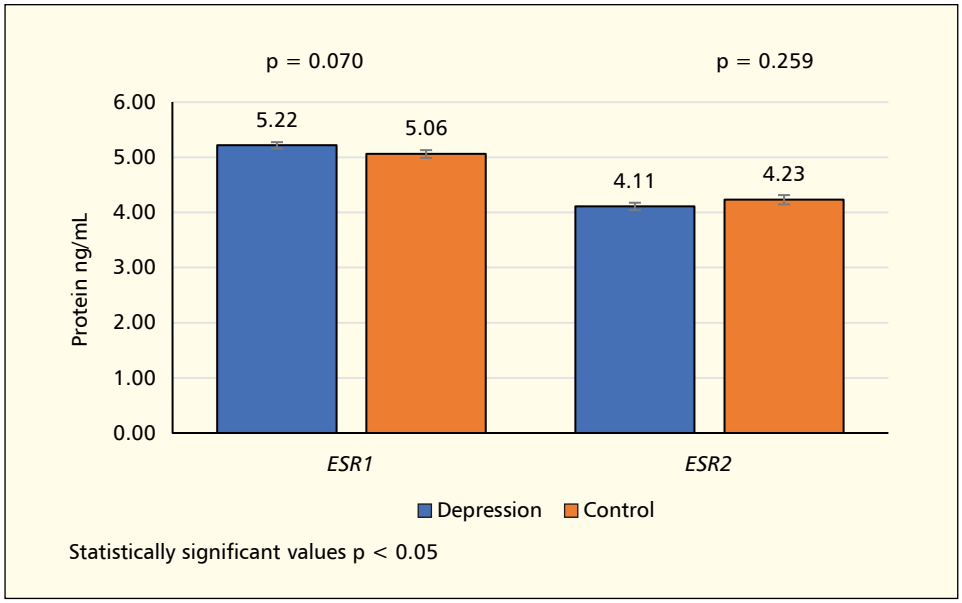

Figure 2. Comparison of *ESR1* and *ESR2* gene expression at the protein level in both groups

performed. The results of the analyses are presented in Figures 1 and 2. Patients suffering from depression had a significantly higher level of expression of the *ESR1* gene at the level of mRNA ($p < 0.001$) and protein (tendency level $p < 0.070$).

Correlation between cognitive functioning, age and gene expression

Multiple Pearson correlation analyses were performed to investigate the potential relationships between the levels of expression of the studied genes and the cognitive functioning and age in the group of all study participants (Tab. 1), control (Fig. 3) and experimental group (Fig. 4). The Pearson's correlation coefficient values were interpreted as: 0–0.3 — weak correlation, 0.31–0.50 — moderate correlation, 0.51–0.70 — strong correlation, 0.71–1.0 — very strong correlation.

According to all participants, except the scores of the Trail Making Tests, the other scales consistently suggest that the higher the level of *ESR1* (mRNA), the worse the scores on the scales assessing cognitive functions. In the experimental group a negative correlation between *ESR1* (mRNA) expression and results in the Luria test after 30 minutes and positive correlation with the results in the Stroop A test (time) and Stroop B (mistakes) and the Trail Making Test A&B results

Table 1. Matrix of r-Pearson correlations of variables in a study for all study participants

	ESR1 mRNA		ESR2 mRNA		ESR1 protein		ESR2 protein	
Variables	r	p	r	p	r	p	r	p
Age	0.292	< 0.001	0.056	0.400	0.036	0.584	–0.045	0.500
LT 1	–0.158	0.017	0.09	0.166	–0.01	0.885	0.06	0.335
LT 30	–0.217	< 0.001	0.08	0.234	–0.06	0.370	0.04	0.561
Stroop A time	0.260	< 0.001	–0.11	0.099	0.02	0.729	–0.04	0.566
Stroop A mistakes	–0.04	0.504	–0.05	0.410	–0.02	0.790	–0.02	0.794
Stroop B time	0.252	< 0.001	–0.294	< 0.001	0.09	0.163	0.00	0.977
Stroop B mistakes	0.250	< 0.001	–0.137	0.039	0.02	0.738	–0.03	0.649
Fluency "K"	–0.279	< 0.001	0.04	0.546	0.00	0.997	0.02	0.774
Fluency "animals"	–0.148	0.026	0.177	0.007	–0.01	0.873	0.06	0.377
Fluency "sharp"	–0.191	0.004	0.199	0.003	0.03	0.705	0.04	0.504
TMT A	0.286	< 0.001	–0.259	< 0.001	0.05	0.414	–0.03	0.686
TMT B	0.331	< 0.001	–0.13	0.053	0.07	0.282	–0.04	0.585

LT-1 — first trial in Luria Test; LT 30 — trial after 30 minutes in Luria Test; r-Pearson —correlation coefficient; p — statistical significance; TMT A — Trail Making Test A; TMT B — Trail Making Test B

Statistically significant values p < 0.05

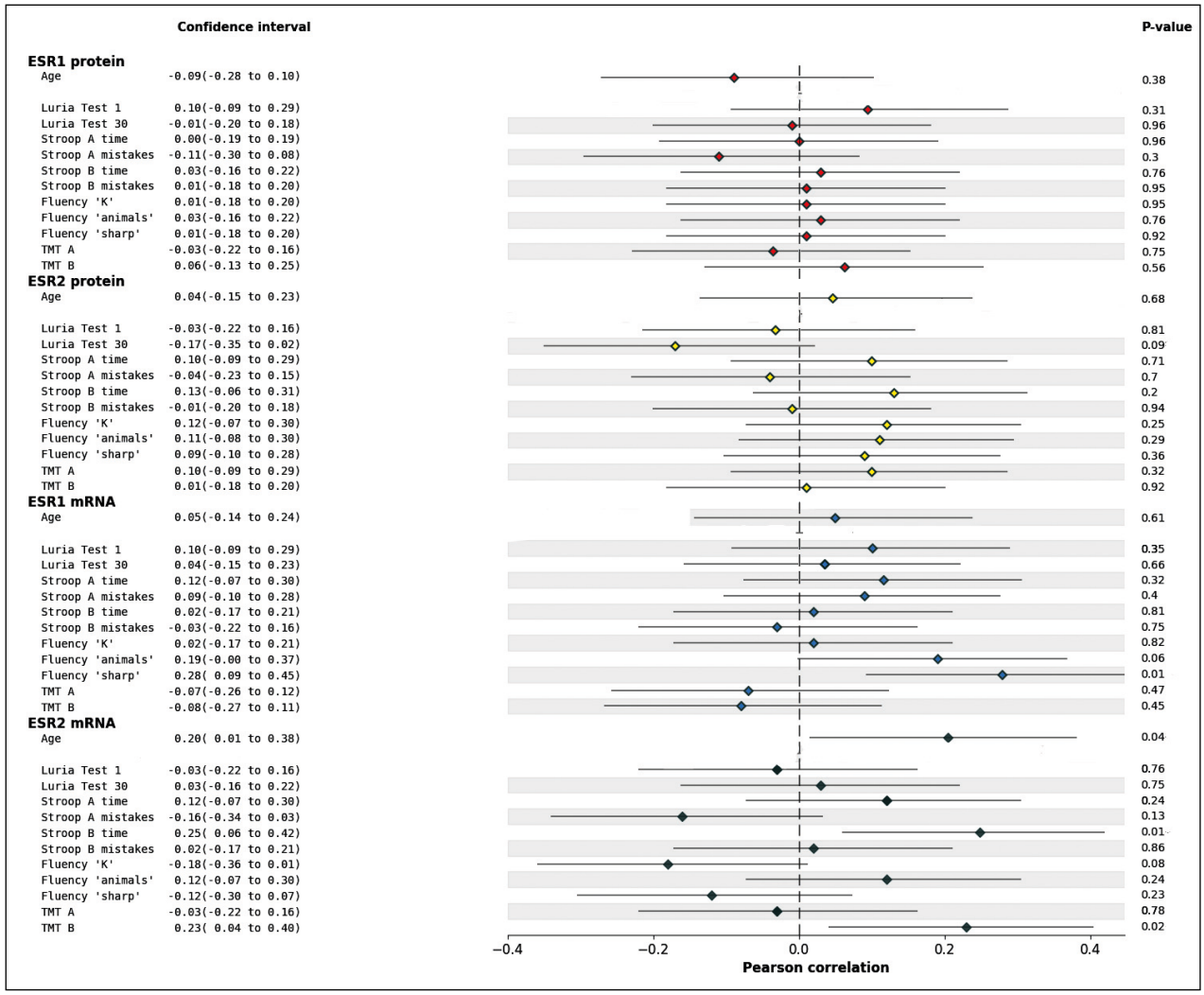

Figure 3. Correlations between expression of genes and variables in the control group. Statistically significant values p < 0.05

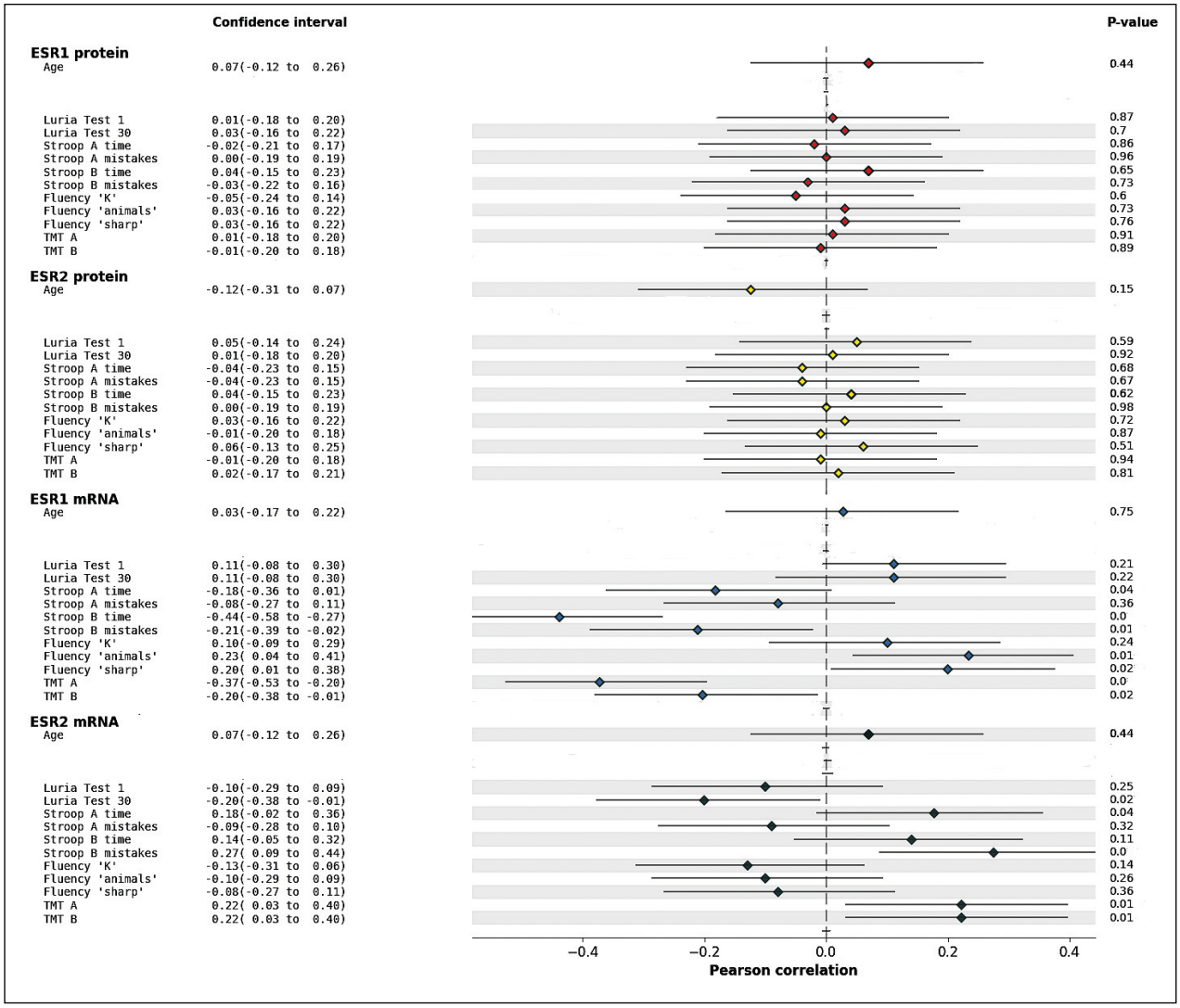

Figure 4. Correlations between expression of genes and variables in the group with depression. Statistically significant values $p < 0.05$

were observed. These results also suggest that higher expression of *ESR1* (mRNA) may be associated with cognitive deficits.

With regard to the increased level of *ESR2* (mRNA), a moderate negative correlation with the results in the Stroop B test and Trail Making Test A and weak correlation with the results of the verbal fluency test in the semantic parts "animals" and "sharp objects" were noticed.

In the experimental group the level of *ESR2* (mRNA) expression correlates negatively with the results of the Stroop B tests and the results of the Trail Making Test A&B. In addition, there is a positive correlation with verbal fluency score. This allows us to conclude that in the group of patients with depression, the higher the level of expression of the *ESR2* gene at the mRNA level, the better results in tests assessing cognitive functioning.

Weak positive correlations between age and *ESR1* (mRNA) among all study participants and control group (all participants: r-Pearson's coefficient: 0.292; p value: < 0.001; control group: r-Pearson's coefficient: 0.205; p value: 0.044) has been noticed.

Gene expression- gender differences

T-Student tests examining gender differences in the level of expression of *ESR1* and *ESR2* genes were performed. There were no statistically significant differences in the level of expression of *ESR1* and *ESR2* genes between women and men in the study groups (Tab. 2, 3).

Discussion

In our research, we decided to look at the expression of estrogen receptors among patients with depression and healthy volunteers. Patients suffering from depression were characterized by a higher level of *ESR1* expression both at the mRNA and protein levels than control group.

Table 2. Relationship between *ESR1* and *ESR2* gene expression and gender in the control group

	Women		Men				95% CI		
Gene expression	*M*	*SD*	*M*	*SD*	*t*	*p*	*LL*	*UL*	d Cohen
ESR1 mRNA	0.21	0.04	0.20	0.05	0.81	0.421	–0.01	0.02	0.17
ESR2 mRNA	0.23	0.04	0.23	0.04	1.11	0.272	–0.01	0.02	0.24
ESR1 protein	5.04	0.64	5.09	0.68	–0.33	0.740	–0.32	0.23	0.07
ESR2 protein	4.24	0.84	4.22	0.81	0.13	0.900	–0.33	0.37	0.03

CI — confidence interval; d Cohen — effect size; LL — lower limit; M — mean; p — significance level; SD — standard deviation; t — t-Student test value; t — statistically significant values $p < 0.05$; UP — upper limit

Table 3. Relationship between *ESR1* and *ESR2* gene expression and gender in the group with depression

	Women		Men				95% CI		
Gene expression	*M*	*SD*	*M*	*SD*	*t*	*p*	*LL*	*UL*	d Cohen
ESR1 mRNA	0.25	0.07	0.24	0.06	0.97	0.334	–0.01	0.03	0.17
ESR2 mRNA	0.24	0.03	0.23	0.05	1.15	0.251	–0.01	0.02	0.20
ESR1 protein	5.26	0.70	5.16	0.59	0.82	0.416	–0.13	0.32	0.14
ESR2 protein	4.15	0.78	4.04	0.83	0.79	0.433	–0.17	0.39	0.14

CI — confidence interval; d Cohen — effect size; LL — lower limit; M — mean; p — significance level; SD — standard deviation; t — t-Student test value; t — statistically significant values $p < 0.05$; UP — upper limit

There is a scant literature on the human population looking for relationships in the studied range. A study published in 2020 [19] indicated that healthy people have a higher level of *ESR1* expression, but it concerned a much smaller sample and covered a narrower age group.

Referring to the inflammatory theory of depression, it is worth recalling the results of a study published in 2015, presenting that estrogens accelerate the resolution of inflammation in macrophages [20]. By acting on the ESR1 receptor, estradiol reduces the time spent by macrophages in inflammation. Expression of gene *ESR1* and *ESR2* was observed in the study. Increased expression of *ESR1* gene through the action of IL-4 has been noted. This information, taking into account the inflammatory theory of depression and the presence of increased concentrations of circulating pro-inflammatory cytokines in depressed patients, seem to be in line with our results.

We didn't note statistically significant differences in *ESR2* gene expression between the groups. However, a study by Gaspar et al. [21] revealed association of decreased whole-blood *ESR2* expression and depression, indicating that ER-β agonism could be possibly beneficial. Selective estrogen receptor modulator — raloxifen — can bind to both estrogen receptor, but has higher affinity to ESR2. Binding to ESR2 lead to increase in *ESR2* reporter gene expression but minimal after binding to ESR1. Studies indicate that this substance improves verbal memory reduces anxiety behavior and depression in postmenopausal women [22].

Our study revealed that the expression of the *ESR1* and *ESR2* gene at the mRNA level might be related to cognitive functioning. The higher expression of *ESR1* at the mRNA level might be correlated with worse general cognitive functioning. The study of *ESR2* (mRNA) expression in the experimental group allows us to conclude that the higher the level of expression of the *ESR2* gene at the mRNA level, the better cognitive functioning.

These results are supported by previous publications that describe the relationship of ESR2 with cognitive processes. A study published in 2008 based on a mouse model showed that ESR2 activation can regulate hippocampal synaptic plasticity and improve memory function. The ESR2 agonist improved performance on hippocampus-dependent memory tasks [23]. Knockout of the *ESR2* gene in a group of female individuals caused impairment of spatial memory responsible for recording and retrieving information needed to plan a course to a place and recall the location of an object or the occurrence of an event [24]. The hippocampus is responsible for this type of memory.

Subsequent publications have indicated that ESR2 promotes neurogenesis, modulates the neuroendocrine response to stress, has neuroprotective effects, reduces the inflammatory response and behaviors similar to anxiety and depression [12].

According to the epidemiology of depression and its relation with estrogen receptors, searching for reasons for gender discrepancy seems to be crucial. We didn't notice statistically significant differences. The study conducted on an animal model also showed no statistically significant differences in the expression of estrogen receptor genes depending on sex in most of the tissues studied, with the exception of kidneys (higher expression of *ESR1* in males) and gonads (higher expression of *ESR2* in females) [25].

We noticed weak positive correlations between age and *ESR1* expression at mRNA among all study

participants and control group. However, in our experiment, groups were statistically significantly different in terms of age ($p < 0.0010$), which is a limitation. These results do not seem to be consistent with other scientific reports, which indicate that *ESR1* expression decreases in the hippocampus with age, but the molecular mechanisms leading to the loss of *ESR1* require further investigation [26].

Our study has certain limitations. It was assumed that the expression of the examined genes in the collected material (peripheral blood) to some extent corresponds to the expression in the central nervous system [27]. Gene expression in the periphery can influence endocrine changes, which can then affect the functioning of the central nervous system.

In our study, we did not check estradiol level. Moreover, pharmacotherapy (antidepressants) taken by experimental group might affect the estrogens level, receptors and cognitive functioning [28–30]. For these reasons, the results should be treated with caution.

Conclusions

Participants suffering from depression were characterized by a higher level of *ESR1* expression both at the mRNA and protein level. Expression of *ESR1* and *ESR2* genes might be related to functioning in the examined cognitive areas. Expression of *ESR1* and *ESR2* genes does not differ statistically significantly in terms of gender.

Article information

Data availability statement

The data that support the findings of this study are available upon request from author Maria Kozłowiec.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and ap-proved by the Bioethical Committee of the Medical University of Lodz (No. RNN/384/11/KB, KE/1401/18).

Author contributions

Conceptualization — M.K., M.G., A.O. and P.G; formal analysis — M.K., M.G., A.O., P.G.; funding acquisition — P.G.; investigation — M.K.,M.G., A.O., P.G. and J.S. (biochemical analysis); methodology — M.K., M.G., A.O. P.G. and J.S. (biochemical methodology); writing — original draft — M.K.; writing —review and editing — M.K., M.G., A.O., P.G., J.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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