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Serum KIBRA mRNA and Protein Expression and Cognitive Functions in Depression

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Background: Genes participating in synaptic signalling or plasticity in brain regions such as the prefrontal cortex (PFC) and the hippocampus have been implicated in cognition. Recently, a new gene (KIBRA, *WWC1*) has been added to this group due to its impact on memory performance. Recurrent depressive disorder (rDD) is a multifactorial disease, that one of the typical features is cognitive impairment. The main objective of this study was to perform an analysis of the KIBRA gene on both mRNA and protein levels in patients suffering from rDD and to investigate the relationship between KIBRA expression and cognitive performance.

Material/Methods: The study comprised 236 subjects: patients with rDD (n=131) and healthy subjects (n=105, HS). Cognitive function assessment was based on: Trail Making Test, The Stroop Test, Verbal Fluency Test and Auditory Verbal Learning Test.

Results: Both mRNA and protein expression levels of KIBRA gene were significantly higher in healthy subjects when compared to rDD. The presented relationship is clear even after taking age, education and sex of the examined subjects into consideration. No statistically significant relationship was found in the experiments between any of the conducted tests and KIBRA gene expression on mRNA level for both the rDD and HS groups. The presented study has limitations related to the fact that patients were being treated with antidepressant. This is relevant due to the fact that some antidepressants may affect mRNA expression. Number of patients and healthy subjects may result in the lack of statistical significance in some cases.

Conclusions: 1. The results of our study show decreased expression of the KIBRA gene on both mRNA and protein levels in depression. 2. We did not find any significant relationship between KIBRA gene expression and cognitive functions in case of both the healthy subjects and the patients affected by rDD.

MeSH Keywords: **Cognition • Depressive Disorder • Genotype**

Abbreviations: **rDD** – recurrent depressive disorder; ***WWC1*** – WW-and-C2-domain-containing-protein-1; **KIBRA** – cytoplasmic protein highly expressed in the kidney and brain; **PKM**ζ – protein kinase M ζ; **SNP** – single-nucleotide polymorphism; **AD** – Alzheimer's disease; **CIDI** – Composite International Diagnostic Interview; **HDRS** – Hamilton Depression Rating Scale; **TMT** – Trail Making Test; **VFT** – Verbal Fluency Test; **AVLT** – Auditory Verbal Learning Test; **WAIS-R** – Wechsler Adult Intelligence Scale-Revised

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Background

Human cognition is a polygenic trait. Genes which participate in synaptic signalling or plasticity in brain regions like the prefrontal cortex (PFC) and the hippocampus have been implicated in cognition. A new gene (KIBRA) has been added to this group in recent times owing to its influence on memory performance [1,2].

KIBRA (also referred to as *WWC1* for WW-and-C2-domain-containing-protein-1) is a cytoplasmic protein, which is highly expressed in the kidney and brain (KIBRA), and has been recently associated with higher brain functions. *WWC1* is genetically linked with human episodic memory performance and it has been shown that its product protein (KIBRA) interacts with an atypical protein kinase, i.e. protein kinase M ζ (PKMζ). PKMζ is a candidate postsynaptic regulator of memory maintenance [3].

KIBRA was identified in the human brain and kidney for the first time in 2003. It has been associated with memory performance and synaptic plasticity recently. A single nucleotide polymorphism (SNP, *rs17070145*) was detected through genome-wide screening in the ninth intron of the KIBRA gene (T→C substitution); it was also implicated in human memory and the underlying neuronal circuitry [4]. Moreover, the KIBRA protein has critical importance for long-term potentiation and memory consolidation [5,6]. Additionally, studies conducted in recent times have indicated that KIBRA takes part in other physiological processes such as cell polarity, membrane/vesicular trafficking, mitosis and cell migration. The KIBRA protein is highly phosphorylated by numerous types of kinases in epithelial cells at the biochemical level [1].

In 2006, Papassotiropoulos and colleagues [7] discovered a link between KIBRA SNP and human episodic memory among 341 healthy young adults from Switzerland, with replication in 2 additional healthy cohorts (from Switzerland and the United States). The subjects with the rs17070145 T allele – when compared to noncarriers – demonstrated better delayed recall across a selection of episodic memory tasks and reduced hippocampal activation on functional MRI during episodic memory tasks [4]. What is more, genetic variations at the KIBRA *rs17070145* polymorphism have also been associated with executive functions and Alzheimer's disease (AD) [8,9]. The experiment carried out by Wilker et al. [10] provided an opportunity to reveal a link between 2 *WWC1* SNPs (*rs10038727* and *rs4576167*) and the likelihood of PTSD development, which indicates that this memory-related gene might participate in the processes taking place as a result of traumatic stress and have an impact on the intensification of fear memories.

Although KIBRA is also found in the cerebellum and the hypothalamus, it is mainly expressed in memory-related regions of the brain, e.g. the hippocampus and the cortex. KIBRA is characterized by somatodendritic distribution and enrichment at postsynaptic density in primary hippocampal neurons [11]. During episodic remembering, differences in blood oxygenation level-dependent (BOLD) responses in the hippocampus and medial temporal lobe (MTL) cortices influenced by KIBRA have been observed [12].

Cognitive deficits present in recurrent depressive disorders (rDD), predominantly connected with episodic memory processes and the so-called frontal functions (executive functions, verbal fluency, attention, working memory), have been explored and examined very thoroughly in recent times [13–15]. Cognitive impairment, linked with the earlier onset of depressive symptoms and episode prolongation, may in return lead to an ineffective antidepressant therapy and impede full recovery, hence result in incomplete functional remission [16].

The main objective of this study was to perform an analysis of the KIBRA gene on both mRNA and protein levels in patients suffering from rDD and to investigate the relationship between KIBRA expression and cognitive performance. We formulated a hypothesis that KIBRA gene expression levels are lower in the rDD group and that it might have an impact on cognitive functions.

Material and Methods

Subjects

The study was conducted in a group of 236 subjects aged 20-67 (*M*=39.79 yrs., *SD*=14.02) – patients affected by rDD (*n*=131) and a control group of healthy subjects (HS, *n*=105). Sample recruitment and description have already been presented elsewhere [17].

Methods

Cognitive function assessment and severity of depression

Trained neuropsychologists were in charge of the neuropsychological assessment. A comprehensive neuropsychological test battery, including the Trail Making Test (TMT), the Stroop Test, the Verbal Fluency Test (VFT), the Auditory Verbal Learning Test (Polish version, AVLT) and the Digit Span from Wechsler Adult Intelligence Scale-Revised (WAIS-R), was used to examine and assess a full range of cognitive functions. The 21-item Hamilton Depression Rating Scale (HDRS) served to assess depression severity. A detailed description of the tests can be found in [18] and [19].

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For the patients with rDD, the HDRS, Stroop Test, TMT, AVLT, VFT and the Digit Span 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.

KIBRA mRNA and serum protein expression

The analyses were carried out in accordance with the methods previously described, i.e. mRNA expression [20], serum protein expression by the ELISA method (Human Kibra Elisa Kit (antibodies-online.com antibodies-online GmbH, Aachen, Germany) was used).

Blood samples from the patients were collected in 5 ml test tubes containing EDTA, which were later centrifuged at 1000 × g for 10 minutes at 4 degrees Celsius and used for the isolation of peripheral blood lymphocytes. The lymphocytes and serum were stored at –70°C until analyzed.

Determining protein concentration

150 µl of the reaction mixture was added to pits containing 150 µl of serum, diluted 10 times in 10 mM of phosphate buffered saline, pH 7.4, and incubated (2 hours, 37°C). In order to specify protein concentration, an analytical curve for serum albumin was determined. Both the examined samples and the reference samples were made parallel in 3 repetitions. Sample absorbance was measured using Multiskan Ascent Microplate Photometer (Thermo Labsystems) at λ=562 nm and total protein concentration was calculated from the standard curve equation.

Enzyme-linked immunosorbent assay (ELISA)

The concentration of proteins KIBRA in the serum of the patients was determined using Human Kibra Elisa Kit (antibodies-online.com antibodies-online GmbH, Aachen, Germany) according to the protocols provided by the manufacturer. β-actin was used for endogenous control of protein concentration in the samples and determined with the help of Human Actin Beta (ACTb) ELISA Kit (BMASSAY) based on the manufacturer's recommendations. 100 µl of serum (ρprotein=0.5 mg/ml) was added to pits coated with antibodies specific for the analyzed proteins and incubated (1.5 hours, 37°C). The content was removed and the pits were rinsed 3 times in 10 mM of phosphate buffered saline and incubated (1 hour, 37°C) with 100 µl of biotinylated antibodies specific for the analyzed proteins. Then, the content was removed and the pits were rinsed 3 times in 10 mM of phosphate buffered saline and incubated (30 minutes, 37°C) with 100 µl of ABC Working Solution. The content was removed and the pits were rinsed 5 times in 10 mM of phosphate buffered saline and incubated (10 minutes, 37°C) with 90 µl of TMB substrate. After adding 100 µl of TMB Stop Solution, the absorbance of the samples was measured using Multiskan Ascent Microplate Photometer (Thermo Labsystems) at λ=450 nm. In order to determine protein concentration, analytical curves for the analyzed proteins were made.

Total RNA isolation

Total RNA isolation from the patients' blood was performed using InviTrap Spin Universal RNA Kit (Stratec molecular, Berlin, Germany) based on the manufacturer's recommendations. 300 µl of blood in a test tube was incubated with 300 µl of Lysis/Binding Buffer. 300 µl of Acid Phenol: Chloroform mixture was added to the cellular lysate and, after mixing, the sample was centrifuged (5 minutes, 10000 × g) to separate the aqueous phase from the organic phase. The upper fraction (aqueous) was moved to a fresh test tube, which contained 375 µl of 96% ethanol and, after mixing, the entire content was poured into a test tube with a column and a filter. After centrifugation (15 seconds, 10000 × g), the column with the filter was moved to fresh test tubes and rinsed in 700 µl of RNA Wash Solution 1, and then subjected to centrifugation (10 seconds, 10000 × g). The column with the filter was rinsed twice in 500 µl of Wash Solution 2/3 and centrifuged (1 minute, 10000 × g). The column with the filter was placed in a fresh test tube and isolated RNA was subject to elution in 30 µl of water free from nucleases (temperature of 95°C) by means of centrifugation (30 seconds, 10000 × g). Absorbance was measured using a spectrophotometer (Picodrop) at λ=260 nm in order to determine total RNA concentration. Isolated RNA was stored in temperature of –70°C.

Quality analysis of isolated RNA

The quality of total RNA was checked with Agilent RNA 6000 Nano Kit (Agilent Technologies) in accordance with the manufacturer's recommendations. 1 µl of RNA 6000 Nano dye was added to a test tube containing 65 µl of Agilent RNA 6000 Nano gel matrix and then centrifuged (10 minutes, 13000 × g). The gel-fluorescent dye mixture was applied on the surface of a Nano chip placed in a workstation. Then, 5 µl of RNA Nano marker was added to selected pits. Isolated samples of RNA and RNA size marker were subject to denaturation (2 minutes, 70°C), and then 1 µl of the sample was pipetted to selected pits of the Nano chip and mixed (1 minute, 2400 rpm). The quality of isolated RNA was checked using 2100 Bioanalyzer (Agilent Technologies). The level of degradation of total RNA was determined with the use of an electrophoretogram and RIN values recorded. Only the samples with RIN value >7 were subject to further analysis.

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RT-PCR reverse transcription

An RT reaction was carried out using TaqMan® RNA Reverse Transcription Kit (Applied Biosystems) based on the manufacturer's recommendations, using specific Hs00392086_m1, Hs04194366_g1probes, respectively for KIBRA and RPL13A genes, delivered by Applied Biosystems. The samples were incubated (30 minutes, 16°C and 30 minutes, 42°C) in a thermocycler (Biometra). Reverse transcriptase was inactivated (5 minutes, 85°C) and the obtained cDNA was stored in temperature of –20°C.

Real-Time PCR reaction – scanning miRNA panel

Real-Time PCR reaction was conducted using TaqMan® Universal PCR Master Mix, No UNG (Applied Biosystems) according to the protocol provided by the manufacturer. The reaction mixture ratio was presented in the table. To calculate relative expression of miRNA genes, the Ct comparative method was used [12]. The level of KIBRA gene expression in particular tissues was normalized in relation to RPL13A reference gene.

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, at 60°C for 30 seconds and at 72°C for 1 minute; 40 cycles were performed in total. 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 ΔΔCt standard $2^{-\Delta\Delta ct}$ calculations and expressed as a fold change of the control sample [21,22]. Amplification specific transcripts were further confirmed by obtaining melting curve profiles.

Statistical analysis

The collected material was subject to a statistical analysis, which included calculation of both descriptive and inferential statistical data. A 2-tailed critical region was employed in statistical hypothesis testing.

The qualitative characteristics of the experimental and control groups are expressed as frequencies shown as percentages. The arithmetical mean (*M*) was calculated with the aim of characterizing the average values for quantitative features. Statistical dispersion measures included the range of values between the minimum and the maximum, and the standard deviation (*SD*).

The Shapiro-Wilk test was used to analyze distributions. The Pearson χ^2 (qualitative variables) and the Mann-Whitney U test for 2 independent groups were administered to compare the nonparametric variables in the test groups.

It was possible to evaluate the relationship between the KIBRA gene on mRNA and protein levels owing to the estimation of the Spearman's R rank order correlation coefficients.

The dependencies between results of neuropsychological tests and the remaining variables were analyzed using the linear regression model. The Stroop Test (time) and TMT were subject to a logarithmic transformation. Single- and multiple-factor analyses were conducted. Statistically significant variables in the single factor analysis were taken into consideration in the multiple-factor analysis.

Statistical significance was specified as $p<0.05$ [23] for all the analyses; data analyses and calculations were performed using STATISTICA PL, version 10.

Ethics

All the patients were native Poles and inhabitants of central Poland. They were not related to one another. The individuals were selected to the test group at random, without replacement sampling. The experimental group was randomly selected from the patients treated at the Babinski Memorial Hospital in Lodz. The HS group was selected from among the staff of this hospital.

Before making a decision to participate in the study, the subjects were provided with information of its purpose, assured that the participation was voluntary, and guaranteed that all personal data and results of the tests would be kept confidential and not disclosed to any third party. Each subject provided written informed consent for participation 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 by sex, age, education and the course of the disease (rDD group). Statistically significant differences between the 2 groups have been found in terms of sex (χ^2=1.46, p=0.027), education (Z=3.18, p=0.001) and age (Z=10.44, p=0.001).

The value of the Spearman coefficient of rank correlation for the KIBRA gene on mRNA and protein levels was very high (R=0.99). We found that further analyses for evaluation of WWC1 gene expression on mRNA and protein levels with the selected variables were identical.

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

Characteristics		rDD (n=131)			HS (n=105)		
		N	%	(±SD)	n	%	(±SD)
Sex	Female	76	58.01	–	69	65.71	–
	Male	55	41.99	–	36	34.28	–
Age (yrs)		–	–	48.53 (±11.05)	–	–	28.91 (±8.69)
Education level	Primary	10	7.63	–	–	–	–
	Secondary	95	72.52	–	48	45.71	–
	Higher	26	19.84	–	57	54.29	–
Course of the disease	Disease duration in years	–	–	6.71 (±7.53)	–	–	–
	Number of depression episodes	–	–	2.05 (±1.98)	–	–	–
KIBRA mRNA ($2^{-\Delta\Delta ct}$)		–	–	84.7 (±34.6)	–	–	134.4 (±31.7)
KIBRA protein(pg/ml)		–	–	0.4 (±0.2)	–	–	0.7 (±0.2)

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

KIBRA gene expression at mRNA was significantly lower in the rDD group when compared to HS ($p<0.01$) (Figure 1). The presented relationship is evident even after taking into account age, education and sex of the examined subjects. The average level of KIBRA gene expression measured at the mRNA level for the whole group was: $M=0.53$ ($SD=0.21$).

Based on the multi-factor analysis of the relationship between *WWC1* gene expression, education and age are associated with gene expression, however direct causes are not explained (Table 2). They may have a direct impact on the factors linked with the prevalence of the disease, which requires further studies and verification.

Having found out that age and education are confounding factors, the rDD and HS groups differed from one another in the following tests: AVLT after 30 minutes ($p=0.001$), verbal fluency test ($p=0.007$), digit symbols test ($p=0.001$), TMT part A ($p=0.017$), and TMT part B ($p<0.001$).

A statistically significant relationship was not found between any of the conducted tests and KIBRA gene expression on mRNA level for both the rDD and HS groups.

The relationship between the level of depression intensity, measured according to the HDRS scale, and KIBRA gene expression on mRNA level was evaluated (Figure 2) at a further stage of the analysis. No statistically significant relationships were confirmed in this case, either.

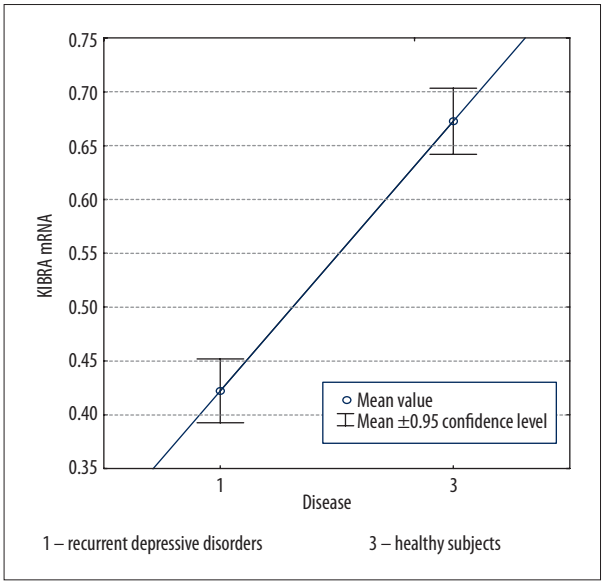

Figure 1. Corrected average values of mRNA in the examined groups.

Discussion

In the brain of a rodent and a human being, KIBRA is expressed in the structures related to memory (i.e. the hippocampus and cortex) as well as in the cerebellum and the hypothalamus [24]. When analyzing and examining the process of brain development, it has been shown that expression decreases from juvenile postnatal stages to an adult animal [24]. Statistically

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Table 2. Analysis of the relationship between KIBRA gene expression on mRNA level and selected predictors.

		Single factor			Multi factor		
		B	Se	p	b	se	p
Group	HS	ref.					
	rDD	–0.25	0.02	0.000	–0.28	0.03	0.000
Education	Higher	ref.					
	Secondary	–0.11	0.03	0.000	–0.04	0.02	0.151
	Vocational	–0.15	0.04	0.001	0.03	0.04	0.451
	Primary	–0.21	0.07	0.001	–0.04	0.06	0.478
Age	Linear trend	–0.01	0.00	0.000	0.00	0.00	0.222
Sex	Female	ref.					
	Male	–0.01	0.03	0.842		–	

B – regression coefficient; se – standard error of regression coefficient; p – level of statistical significance; rDD – recurrent depressive disorders; HS – healthy subjects.

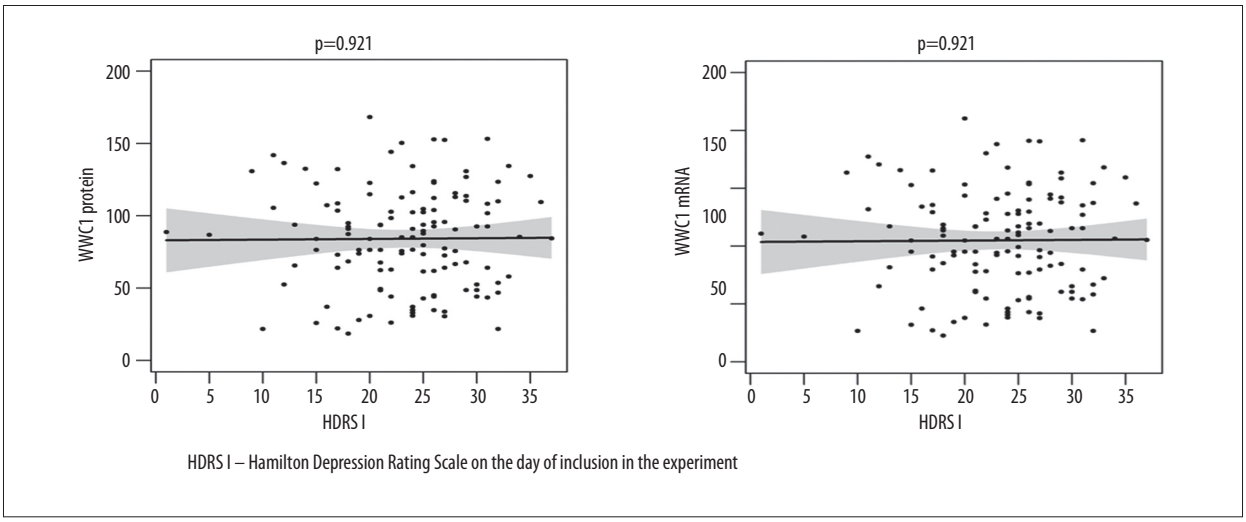

Figure 2. Average values of WWC1 gene expression on mRNA and protein levels depending on the results of the Hamilton tests during admission.

significant trends were described by Corneveaux et al. [25] who also demonstrated increased neuronal expression of KIBRA in the hippocampus, middle temporal gyrus and posterior cingulate cortex of AD brains, and reduced neuronal expression of gene coding for its binding partners. They hypothesized that a number of the same molecular processes having impact on episodic memory performance (including synaptic neurotransmission, long-term potentiation and neuronal plasticity) also contribute to the pathological and clinical features of AD [25]. Moreover, according to Papassotiropoulos et al. [7], KIBRA RNA is expressed in the entire brain with a peak in memory-related structures, i.e. the hippocampus and the temporal lobe.

The working hypothesis specified in the introduction of this study was confirmed only partially. The obtained results indicate a difference in the level of KIBRA gene expression on both mRNA level and protein level between the group of patients suffering from rDD and the healthy subjects. The people affected by recurrent depressive disorders – even after considering the confounding factors such as age, education and sex – were characterized by lower expression of the KIBRA gene on mRNA level and protein level as compared to the group of healthy subjects. However, no statistically significant relationship was identified between the reduced level of KIBRA gene expression and cognitive functions of the patients from the rDD group. Moreover, intensification of depression was not associated with a fall in KIBRA gene expression in the group of patients with rDD. Neither did KIBRA gene expression in the examined group correlate with cognitive functions of the healthy people. The presented study constitutes the first analysis of

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the relationship between KIBRA gene expression and cognitive functions of the affected subjects from the rDD group. We shall use analyses regarding healthy individuals and other groups of disorders when discussing the results.

The results of the studies described in literature, which refer to the links between the KIBRA gene and cognitive functions of examined subjects, are not clear or evident either. Several authors confirmed a positive correlation between human cognitive functions and KIBRA SNP (*rs17070145*). According to Muse et al. [6] (232 healthy subjects), *WWC1* T carriers were characterized by significantly better delayed recall performance than CC individuals (p=0.006). The relationship between increasing age and recall scores (immediate and delayed) was also significantly different between *WWC1* genotype groups. A significant negative association between hippocampal formation activity and increasing age was confirmed for the CC group, while no such association was observed in the T carrier group. Hayashi et al. [26] and Yasuda et al. [27], who performed studies on a group of Japanese subjects, recorded identical results to the ones presented above. In the latter work, the T allele carriers had significantly better verbal memory, attention/concentration and delayed recall performance than the C/C carriers. Furthermore, the C/T carriers and the T/T carriers had better delayed recall performance than the C/C carriers [26]. A significant association of SNP rs17070145 with both episodic and working memory was also found by Milnik et al. [28], who indicated that SNP located within KIBRA explained 0.5% variance for episodic memory tasks and 0.1% variance for working memory tasks in samples of primarily Caucasian background. Papassotiropoulos et al. [7] and Bates et al. [5] observed KIBRA's influence on delayed memory, however with no effects on immediate recall. The scientists interpreted that KIBRA was not of importance for the processes linked with early memory formation; nevertheless, its role in consolidation or delayed retention was significant [5].

The results recorded by Wersching et al. [29] differed from the ones presented above. The authors did not find any main effects of the KIBRA *rs17070145* genotype on cognitive functions (psychomotor speed, working memory, verbal memory, word fluency, executive functions). However, they did find a significant correlation between sex and the rs17070145 genotype in terms of the cognitive domain of working memory (women performed significantly worse than men in the group of T-allele carriers). Wang et al. [9] also demonstrated increased synchronization in the posterior cingulate cortex and the medial prefrontal cortex as well as in the right anterior insula, bilateral caudate nuclei, and bilateral dorsal anterior cingulate cortices (dACC) by KIBRA C-allele in comparison to the individuals with a TT genotype. Additionally, carriers of the KIBRA C-allele were characterized by a smaller volume of the grey matter in the MPFC and bilateral dACCs than TT individuals. Meanwhile, no significant genotype differences in the synchronization of the visual network or the sensorimotor network were found. This association is reversed in adults with subjective memory complaints and populations vulnerable to memory deficits (e.g. in traumatic brain injuries) [30]. According to Palombo et al. [31], young (22.2±3.7 years old) carriers of the KIBRA T-allele (*rs17070145*) had a larger hippocampal volume as opposed to noncarriers. The structural differences observed were specific to the cornu ammonis fields and dentate gyrus regions of the hippocampus. The 2 areas mentioned in the preceding sentence are linked with episodic memory processes.

Moreover, the authors cannot reach an agreement whether CC polymorphism of the KIBRA gene has an influence on increasing the risk of dementia development. According to Alemeida et al. [2], the CC polymorphism leads to the weakening of episodic memory efficiency in old age, but is not conducive to mild cognitive impairment development. What is more, Sédille-Mostafaie et al. [32], who examined a group of 75- and 76-year-old individuals, did not find any significant relationship between development of dementia and KIBRA (*rs17070145*) SNPs, while Hayashi et al. [26] hypothesized that KIBRA gene did not show any signs of association with AD in the Japanese cohort.

The study provides an evaluation of the correlation between KIBRA gene expression and cognitive functions in the subjects suffering from rDD. As mentioned before, it is the first study of this kind to provide results for patients affected by recurrent depressive disorders. We observed statistically significant differences in KIBRA gene expression between the rDD group and HS; however, no significant influence of this variable on the occurrence of depression symptoms and their intensification was found. In one of our earlier works [33], we examined a relationship between SNP T/C (*rs17070145*) of the KIBRA gene and the risk of rDD (181 patients with rDD and 149 healthy control subjects). Based on the results obtained, no significant correlation between the studied polymorphism and rDD was revealed. The value of the disease odds ratio (ORdis) we recorded suggests that the presence of the T/T homozygote reduces the risk of recurrent DD development, but the result was not statistically significant. In the available literature it is possible to find a paper dedicated to the evaluation of KIBRA's influence on cognitive functions in patients suffering from psychosis. Vassos et al. [34], based on a sample of 544 subjects (including patients with psychosis, their unaffected relatives as well as healthy individuals), demonstrated a relationship between a common T/C polymorphism of the KIBRA gene (*rs17070145*) and episodic memory performance. After an analysis of the combined sample, a significant association was found between the KIBRA T allele and improved performance in the single principle component of the memory measures, which included immediate and delayed logical and visual memory from the Wechsler Memory Scale. The authors

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observed an association of KIBRA with immediate and delayed logical memory in the unaffected individuals (patients' relatives and healthy control subjects); while a link between KIBRA and delayed visual memory was confirmed in the case of the patients with psychosis. These reports are also confirmed by Pantzar et al. [35] and Liu et al. [36].

The presented results do not make it possible to confirm a direct influence of the KIBRA gene on the occurrence of cognitive disorders and depression intensification in patients with rDD. However, they show that expression of this gene is significantly different in the group of patients suffering from rDD and in the reference group, which indicates that further tests and experiments are necessary.

A different question defining new lines of research may be effect of KIBRA expression on depression pathogenesis. We mentioned earlier that the KIBRA gene has been associated with episodic memory performance and synaptic plasticity [35]. This points to the involvement in episodic memory functioning in both the hippocampus and frontal lobes. A series of human brain structures are engaged in the processes of episodic memory as a type of declarative memory. They are mainly the medial parts of the temporal lobes, the diencephalon, and the frontal lobes (the hippocampus, the thalamus, the amygdala nucleus, and the prefrontal area) [37]. Notably, dysfunctions or neuroanatomical changes in each of those regions are evident in depression [38]. Each of the aforementioned structures is also involved in the body's response to stressful stimuli [39]. Dysregulation of the hypothalamic-pituitary-adrenal axis is one of the factors associated with the occurrence of symptoms of depression [40]. The hippocampus is particularly susceptible to stress-induced functional changes and dysregulation of the HPA axis, where a drop in the expression of the brain-derived neurotrophic factor (BDNF), deterioration of long-term potentiation (LTP), and inhibition of neurogenesis in the dentate gyrus (DG) are observed [41]. Stress affects cognitive processes. Its mild intensification facilitates remembering; however, strong stressors applied during training deteriorate the process of memorizing information [42]. Volume reduction of the grey matter in the medial prefrontal cortex and the left temporal gyrus is observed in people reporting traumatic experiences [43].

These data suggest that declarative memory (and episodic memory) might be a viable therapeutic target for cognitive remediation strategies, given the impact of cognition on diverse clinical outcomes [44]. However, there are no studies concerning the possible effect of antidepressants on KIBRA expression.

Conclusions

1. The results of our study show reduced expression of the KIBRA gene on both mRNA and protein levels in the course of depression.
2. We did not find any significant relationship between KIBRA gene expression and cognitive functions in healthy subjects or patients affected by rDD.

Limitations

The presented study has limitations related to the fact that patients were being treated with antidepressant. This is relevant due to the fact that some antidepressants may affect mRNA expression.

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.

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