Single nucleotide polymorphism of the KIBRA gene in recurrent depressive disorders

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Abstract

OBJECTIVE: Depression is a disease of multifactor background. Episodic memory dysfunction is one of depression characterising disturbances, which may lead to its onset and development. Memory processes are controlled by a number of extra- and intracellular mechanisms. KIBRA, a newly discovered protein, belonging to signal transduction proteins, participates in the control of episodic memory. The presented study was designed to assess correlation between single nucleotide polymorphism (SNP) T/C (rs17070145) of the KIBRA gene and the risk of recurrent depressive disorder (DD).

METHODS: The study was carried out in a group of 181 patients with recurrent DD and 149 healthy control subjects. Genotyping was conducted by polymerase chain reaction (PCR)/restriction fragment length polymorphism (RLFP) method.

RESULTS: TThe obtained results have revealed no significant correlation between the studied polymorphism and recurrent DD. Obtained value of the disease odds ratio (ORdis) suggests that presence of T/T homozygote decreases risk of development of recurrent DD, but the result was not statistically significant.

CONCLUSIONS: Following the results, it may be concluded that the studied polymorphism does not influence either the onset mechanism or the course of recurrent DD. Even if T/C polymorphism of the KIBRA gene induces memory disturbances, they may be unspecific and unselective for recurrent DD. Further studies on the genes, which control characteristic processes of DD and influence their course, are demanded and mostly justified.

ORIGINAL ARTICLE

INTRODUCTION

Numerous researchers have been paying much attention to episodic memory, the effectiveness of which depends on proper functioning of various brain structures, including the hippocampus (Vargha-Khadem *et al.* 1997; Papassotiropoulos *et al.* 2006). The occurrence of memory dysfunction is associated with predisposition to mental diseases (Frantom *et al.* 2008). Recurrent depressive disorders (DD) is one of the psychiatric diseases which are characterised by episodic memory disturbances (Airaksinen *et al.* 2004). Additionally, a certain memory deterioration has been demonstrated for the age of patients between 35 and 40 years (Swanson 1999), at which age, the incidence of recurrent DD reveals an overtly growing trend (Chapman & Perry 2008).

Proper memory functionality is controlled by a rather complex process, involving those neurotransmitters, which take part in synaptic transmission, and molecules, which are important for intracellular signal transmission (Gundelfinger & tom Dieck 2000: Sheng & Hoogenraad 2007). Scaffolding proteins are of key importance for the integration of activities of various biological molecules which are important for memory processes. They are part of the postsynaptic density of neurons, which plays various roles in memory formation (Yamauchi 2002; Boeckers 2006).

One of scaffolding molecules, which plays an important role in the proper control of episodic memory in the hippocampus, is KIBRA, a recently identified protein of 125 kDa in weight, belonging to the family of proteins which participate in signal transmission processes (Papassotiropoulos *et al.* 2006; Schaper *et al.* 2007). KIBRA is a protein, characterised by high expression in cytosol in brain cells. This protein contains two WW domains in its structure, located on the amine end, which react with proline-rich sequences of proteins, a C2 domain, which mediates the sensitivity to calcium ions (Rizo & Südhof 1998; Kremerskothen *et al.* 2003; Bai & Chapman 2004). Additionally, KIBRA interacts with dendrin and synaptopodin, which are elements of the postsynaptic cytoskeleton, being related to the synaptic plasticity of neurons (Mundel *et al.* 1997; Kremerskothen *et al.* 2006; Duning *et al.* 2008). KIBRA is a substrate for protein kinase Cς (PKCς) (Büther *et al.* 2004), which controls such processes as growth and differentiation (Moscat & Diaz-Meco 2000; Hirai & Chida 2003). A very important function of KIBRA is its ability to bind kinase domain of protein kinase Mς (PKMς), which is a neuron specific variant of PKCς (Osten *et al.* 1996; Büther *et al.* 2004). PKCς and PKMς are important for correct synaptic transmission, long-term potentiation (LTP), synaptic plasticity, memory formation and the process of memorisation (Sacktor *et al.* 1993; Drier *et al.* 2002; Serano *et al.* 2005; Pastalkova *et al.* 2006; Shema *et al.* 2007).

Single nucleotide polymorphism (SNP) has been described for the KIBRA encoding locus, characterised by T to C substitution (rs17070145). This polymorphism occurs in the 9th intron of the gene, localised on chromosome 5 (5q34–q35.2) (Papassotiropoulos *et al.* 2006). The presence of this polymorphism influences the episodic memory and modulates the activation of the hippocampus during memory retrieval process. The presence of the T allele contributes to the occurrence of better verbal and visual episodic memory. Additionally, this allele predisposes to the occurrence of reduced activity of the memory-related brain regions (Papassotiropoulos *et al.* 2006; Schaper *et al.* 2007). The above-mentioned results confirm the participation of the KIBRA encoding locus in the neurobiological mechanisms of memory.

Taking into account the fact of disturbed episodic memory in depression, what is one of the features characteristic for depressive disorders, as well as the role of genetic factors in the development of depression and of depression-accompanying symptoms, an attempt was undertaken to evaluate the correlation between KIBRA T/C SNP (rs17070145) and recurrent DD.

METHODS

The study involved 181 patients, treated for recurrent DD (102 females – 56.35%). The diagnosis was established according to ICD-10 criteria (F33.0–F33.8) (World Health Organization 1992). In all the qualified cases, medical history was obtained, using standardized Composite International Diagnostic Interview (CIDI) (World Health Organization 1992). Additionally, the number of depression episodes, duration of the disease, the age at onset were assessed in each patient. The control group (CG) consisted of 149 healthy subjects (83 females – 55.7%) with family history negative for psychiatric disorders. The healthy controls included community volunteers, enrolled to the study on the criteria of the psychiatric CIDI interview (World Health Organization 1992). Neither DD nor CG demonstrated any statistically significant differences with respect to age and gender (p>0.05). The correlations between genotype distribution and Hardy-Weinberg equilibrium in DD patients and the CG were: DD χ^2=4.26; CG χ^2=2.265; respectively. Both patients and controls with other psychiatric diagnoses, concerning axis I and II disorders, were excluded from the study. All the patients and control subjects were native, unrelated Poles, inhabitants of the central Poland. An informed consent was obtained from all the participants of the study which had earlier been approved by the local Bioethics Committee No RNN/626/09/KB.

DNA was extracted from whole blood according to the GTC method (Sambrock & Russel 2000). The KIBRA rs17070145 polymorphism was analyzed, using 0.1 μg genomic DNA, 200 μM each dNTP, 5x GoTaq buffer solution, 1u GoTaq polymerase (Promega, Madison WI USA), 0.5 μM KIBRA primers 5'-TTTACTCCCAG-CACACACTC-3' and 5'-CACAATGAACAAGGCT-

GTGG-3'. After a 5 min denaturing step, amplification was performed according to the following cycling profile: 94°C for 30 sec, 59°C for 30 sec and 72°C for 30 sec (28 cycles). The final elongation step was 10 min at 72°C. An amplification product of 160 bp was digested with restriction enzyme Mnl I (New England BioLabs). The polymorphism was visualized by separating the digested amplification products on 6% polyacrylamide gel. The T allele created the following three products: 122 bp, 28 bp and 10 bp, C allele four products: 68 bp, 54 bp, 28 bp and 10 bp.

The results are presented as means with standard deviations (SD) or percentages (%). In order to examine the association between KIBRA polymorphism and recurrent DD, allele and genotype frequencies were compared between the study patients and the controls, using the χ^2 test. Post-hoc power analysis was performed with the use of non – central χ^2 distribution. The analysis of association was based on 95% CI for the disease odds ratios, (OR_{dis}) calculated according to Bland and Altman (2000). Departures from Hardy and Weinberg`s equilibrium were determined by comparison of the observed genotype frequencies with expected genotype frequencies. In all the analyses, $p \leq 0.05$ was accepted as the level of statistical significance.

RESULTS

The distribution of genotypes and the allele frequency of the SNP (T/C) within the 9th intron of KIBRA gene (rs17070145) in the whole group of patients are presented in Table 1. No statistically significant differences were observed, either in genotype distribution or allele frequency. Post-hoc power analysis showed that power of testing was over 80% (large samples but small effect sizes). Table 1 presents also the distribution of –C and –T alleles, as well as OR_{dis}, in relation to the presence of –C allele; -T allele or T/T homozygote in the KIBRA gene in the whole studied populations. The remaining genotypes were treated as one group (C/C + C/T) in

calculation of OR_{dis} for the presence of T/T homozygote. Obtained value of $OR_{dis} = 0.5$ suggests that presence of T/T homozygote decreases risk of development of recurrent DD, in comparison with subjects without that homozygote, but the result was not statistically significant.

No statistically significant differences were observed either in genotype distribution or allele frequency related to sociomedical characteristics, regarding CG or DD. Statistical analysis, concerning the correlations for the age at onset, duration of the disease, the number of episodes in the DD group is presented in Table 2.

DISCUSSION

Depression is a disease entity which, taking into account its prevalence, consequences, as well as its still rather unclear aetiology and mechanism, provides a broad background for investigation.

An evaluation of neurobiological changes suggests that depression is associated with structural and functional abnormalities in such regions of the brain as the hippocampus and the cortex (Monkul *et al.* 2003; Drevets *et al.* 2008), which are important for correct functionality of the episodic memory (Ekstrom & Bookheimer 2007). Various studies on depression confirm the occurrence of episodic memory disturbances in this disease entity. Airksinen *et al.* (2006; 2007) and Simons *et al.* (2009) reported that memory disturbances and poor memory functioning could be a predictive factor for the development of depressive disorders, while they may also affect the course of this disease on the other. Kessing (1998) confirmed the correlation between depression and episodic memory dysfunction, having demonstrated an increased number of depression episodes in patients with the above-mentioned disturbances.

Proper memory functioning is controlled by a multifactorial mechanism, the functionality of which also depends on genetic factors (Heuntelman *et al.* 2007;

Tab. 1. Genotype distribution and allele frequency (T/C) within the 9th intron of the KIBRA gene (rs17070145) polymorphism in DD and CG.

Genotypes	C/C n(%)	C/T n(%)	T/T n(%)	*p*-value	OR_{dis} for T/T; 95% CI
DD sample (n=181)	87 (48.07%)	85 (46.96%)	9 (4.97%)	*p*=0.241[1]	0.5; 0.2–1.3
CG sample (n=149)	61 (40.94%)	75 (50.34%)	13 (8.72%)		
Alleles	**–C** n(%)	**–T** n(%)	***p*-value**	OR_{dis} for C; 95% CI	OR_{dis} for T; 95% CI
DD sample (n=362)	259 (71.55%)	103 (28.45%)	*p*=0.11[2]	1.3; 0.9–1.8	0.7; 0.5–1.1
CG sample (n=298)	196 (65.77%)	102 (34.23%)			

1 Pearson´s χ^2 =2.84; df=2; effect size w=0.18; n=330; 1-β=0.82
2 Pearson´s χ^2 =2,55; df=1; effect size w=0.12; n=660; 1-β=0.87
OR_{dis} - Odds ratio of disease; DD – patients with recurrent depressive disorders; CG – control group

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Tab. 2. Correlations between selected clinical features of recurrent depressive disorders and the distribution of T/C KIBRA genotypes in the group of patients with DD. Significance of difference (*p*-value) was assessed with use of Kruskal-Wallis` test.

	Distribution of T/C genotypes KIBRA in the group of patients with DD
Age at onset (years)	H=0.559; p=0.756
Duration of the disease (years)	H=1.022; p=0.495
Number of episodes	H=1.914; p=0.384

DD – patients with recurrent depressive disorders

Gong *et al.* 2009). Our study was to evaluate the genetic role of the protein variant, important for the processes which control memory functions, including the function of episodic memory, in the development of recurrent DD. Our results, obtained from a study, performed on a uniform population group, confirm the distribution of genotypes to be convergent with the results of other studies performed on groups from the Caucasian population (Nacmias *et al.* 2008; Bates *et al.* 2009; Rodríguez-Rodríguez *et al.* 2009).

Our study has been the first one, evaluating the correlation between recurrent DD and SNP T/C of KIBRA encoding gene. The results of the study reveal no significant correlations between the studied polymorphism and recurrent DD in the Polish population. The results of the study contribute another element to the complex discussion on the role of KIBRA gene T/C polymorphism for the development of episodic memory disturbances and of neuropsychiatric diseases, which memory disorders accompany and/or predict. The lack of correlation between episodic memory dysfunction, which may be a prognostic factor for depression, and KIBRA T/C was demonstrated by Need *et al.* (2008). Such a result indicates that memory dysfunctions in depression need not be genetically determined. Therefore, it may be assumed that the cause of occurrence of episodic memory dysfunction in depression is determined by other factors, thus overtly prompting further studies on this issue. On the other hand, there are results which indicate a certain relationship between KIBRA gene T/C SNP with episodic memory functionality. In a clinical study, performed on healthy subjects in young, medium and elderly age, a certain importance of the T allele was observed, as of a factor protecting against working memory dysfunction (Papassotiropoulos *et al.* 2006; Schaper *et al.* 2007). Following the results of the studies, the presence of the T allele may be a protective factor against the development of neuropsychiatric diseases, characterised by episodic memory dysfunction. Nacmias *et al.* (2008) presented results, demonstrating the role of T/C SNP in the KIBRA gene. These

researches perceived the importance of the T allele in proper memory functioning, however, they did not indicate any differences in the degree of depression symptoms in groups, which differed among themselves by the presence of T or C allele. In turn, Bates at al. (2009), presented a modest influence, exerted by the KIBRA gene on the episodic memory.

Diverse results, regarding the role of the polymorphism, investigated in our study, are demonstrated in reports from studies on Alzheimer's disease (AD), that one of the main feature is memory dysfunction (Eustache 1992). Rodrigeuz *et al.* (2009), showed that the presence of the T allele is characteristic for the majority of patients with AD, being a risk factor for AD occurrence in later age. The mentioned study is burdened with certain flaws, which limit its conclusiveness and which are associated with small sample size, being also raised by Corneveaux *et al.* (2008). Contrary, these researches, in a multicentre study, performed on a large group of patients, demonstrated the protective role of the T allele, regardless of patient's age. They observed an increased risk of AD in later age for carriers of the C allele (Corneveaux *et al.* 2008). In turn, Almeida *et al.* (2008) observed a positive effect of the T allele on episodic memory functionality, however, they failed to find any evidence with mild cognitive impairment, which is a prognostic factor for AD (Visser *et al.* 2006). All those results, obtained by many researchers, indicate that the presence of various variants of the KIBRA T/C polymorphism influences episodic memory dysfunctions, however, it need not stimulate the disease for which episodic memory disorders are a poor prognostic factor.

The presence of the T allele, regarded to be the allele protecting against memory dysfunctions (Schaper *et al.* 2007), does not in reduce the development of DD; neither is the presence of the C allele to be associated with the development and course of the disease. Our study demonstrates a lack of significant genetic correlation between the episodic memory process controlling factor and recurrent DD. On the other hand, a certain association between genetic mutations of the memory modulating proteins and the risk of recurrent DD should not be totally disregarded. The study, performed by Izumi *et al.* (2008), points out a relationship between the occurrence of psychiatric diseases and SNP of neuropsin – a protein participating in disturbances of cognitive functions, including memory.

The authors are, however, aware of certain limitations of the study. A high risk of spurious genetic findings should be considered, when interpreting the results, evaluating SNP or candidate gene correlations (Sullivan 2007). When drawing conclusions from the performed study, the authors took into account the number of patients, rather small as for genetic study.

We demonstrated in our study that T/C polymorphism of the KIBRA gene does not influence for recurrent DD development. The obtained results show that,

despite the observed associations of the KIBRA gene with memory dysfunctions, those associations do not contribute to depression onset. Our results may also suggest that episodic memory dysfunctions are not selective or specific for recurrent DD, just as memory dysfunction need not be a genetically determined factor, predictive for development of DD. On the other hand, one should emphasize the genetic complexity of the development of neuropsychiatric diseases, as well as of their predisposing factors. Because of the relationship with episodic memory dysfunction, which may be a prognostic factor for depression, further studies should be undertaken on larger population groups, as well as on different ethnic groups, while also taking into account other genetic factors, responsible for the control of the cognitive functions.

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