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Epistatic interaction of *CREB1* and *KCNJ6* on rumination and negative emotionality

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Abstract

G protein-activated K+ channel 2 (GIRK2) and cAMP-response element binding protein (CREB1) are involved in synaptic plasticity and their genes have been implicated depression and memory processing. Excessive rumination is a core cognitive feature of depression which is also present in remission. High scores on the Ruminative Response Scale (RRS) questionnaire are predictive of relapse and recurrence. Since rumination involves memory, we tested the hypothesis that variation in the genes encoding GIRK2 (KCNJ6) and CREB1 mechanisms would influence RRS scores. GIRK2 and CREB1 polymorphisms were studied in two independent samples (n=651 and n=1174) from the general population. Strongly significant interaction between the TT genotype of rs2070995 (located in KCNJ6) and the GG genotype of rs2253206 (located in CREB1) on RRS were found in both samples. These results were validated in an independent third sample (n=565; individuals with personality disorders) showing significant main effect of the variants mentioned as well as significant interaction on a categorical diagnosis of Cluster C personality disorder (obsessional–compulsive, avoidant and dependent) in which rumination is a prominent feature. Our results suggest that genetic epistasis in post-receptor signaling pathways in memory systems may have relevance for depression and its treatment.

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1. Introduction

Rumination is a common cognitive symptom of depression which is characterized by the focusing of the patients' attention on problematic issues and their possible causes and consequences. Numerous studies suggest that ruminative response style may not only predict the onset of depressive symptoms, but may also be predictive of longer duration and more severe depressive symptoms (Nolen-Hoeksema, 1991; Lyubomirsky and Nolen-Hoeksema, 1993; Nolen-Hoeksema et al., 1993, 1994, 1997; Just and Alloy, 1997). As rumination is a relatively stable trait, regardless of the current depression level (Nolen-Hoeksema and Davis, 1999; Watkins, 2009), a genetic determination is probable. However, up to now only two studies have addressed this question. Hilt et al. reported that a functionally relevant BDNF polymorphism was significantly associated with rumination (Hilt et al., 2007). Beevers et al. (2009) confirmed this association, while 5-HTTLPR failed to show any significant relation to rumination neither alone nor in interaction with BDNF.

Regarding the neurocognitive background of rumination, clinical studies have shown that rumination can be regarded as a mediator between overgeneralization of memories — the tendency to retrieve autobiographical memories as categories rather than specific instances — and depression (Debeer et al., 2009; Kuyken and Moulds, 2009). Memory processes have been associated with several molecules such as BDNF, G protein-activated inwardly rectifying potassium channels (GIRK) and cAMP-response element binding protein CREB (Chung et al., 2009a,b; Koppel and Goldberg, 2009), making these attractive candidates to explore in relation to rumination.

GIRK channels are localized on dendritic spines near excitatory synapses and regulate neuronal excitability by mediating inhibitory effects of G protein-coupled receptors (GPCRs) for neurotransmitters and neuromodulators, including $GABA_B$, NMDA, serotonin, DRD2, M2 and adenosine A1 receptors (Mark and Herlitze, 2000; Chung et al., 2009a,b). Neuronal GIRK channels are predominantly heteromultimers composed of GIRK1 and GIRK2 subunits (Kobayashi and Kawakami, 1995; Lesage et al., 1995; Karschin et al., 1996; Liao et al., 1996). GIRK2 null mutation or GIRK channel blockade abolished depotentiation of long-term potentiation in cultured hippocampal neurons demonstrating that GIRK channels are critical for excitatory synaptic plasticity which is considered a cellular correlate of learning and memory (Chung et al., 2009a,b).

CREB1 is a member of the leucine zipper family of DNA-binding proteins (Sands and Palmer, 2008). The cAMP signal transduction pathway is activated through ligand binding to G-protein-coupled receptors and culminates with phosphorylation of the CREB protein potentiating its transcriptional activity (Mamdani et al., 2008; Sands and Palmer, 2008). CREB is also implicated in memory, as it plays a crucial role in several intracellular events linked to cognitive processes like long-term memory (LTP) and synaptic plasticity (Bito et al., 1996; Impey et al., 1996; Segal and Murphy, 1998; Barco et al., 2005; Josselyn and Nguyen, 2005; Alberini, 2009; Zhou et al., 2009). Pharmacological and genetic studies in mice and rats demonstrate that CREB is required for a variety of complex form of memory, including spatial and social learning indicating that CREB may be a universal modulator of memory formation (Silva et al., 1998). In addition, recent data suggest that CREB regulation is more related to emotionally-relevant memory as discussed earlier (Radulovic and Tronson).

Rumination as a trait is also associated with personality disorders (PD) characterized by neuroticism, like Cluster C. Furthermore, rumination is considered possible mediating variable between neuroticism and symptoms of anxiety and depression(Roelofs et al., 2008).. Given previous data that rumination is predictive of neuroticism(Wupperman and Neumann, 2006), we hypothesized an effect of the tested polymorphisms on neuroticism. As Cluster B PD is reflected by impulsive behaviors, while Cluster C PDs are characterized by anxious, fearful and obsessive–compulsive traits more reminiscent of rumination, we predicted that Cluster C PD would be associated with the interrogated markers.

These considerations led us to test the clinical implication that genetic variation in CREB and GIRK2 would influence rumination. We analyzed separate and interactive effects of two polymorphisms located in the *KCNJ6* (encoding GIRK2) and *CREB1* genes on rumination (RRS) scores in two independent European samples. The findings were further investigated in a third sample consisting of patients suffering from personality disorders with respect to their impact on several personality traits.

2. Methods

2.1. Samples

In the Hungarian cohort 651 unrelated volunteers, 527 women and 124 men were included in the study. Subjects were not specifically screened for psychiatric disorders. All subjects were Hungarian and of Caucasian origin and they gave written informed consent before entering the study.

We independently carried out the analyses with data from 1174 individuals (349 men and 841 women) from Manchester. Participants were recruited from the practices of general practitioners and university students through online advertisements and a website. Inclusion was independent of self-reported psychiatric disorders. All participants provided written informed consent. The descriptive data of the study populations are shown in Table 1.

The third sample consisted of 565 adult in- and outpatients (238 men and 327 women) meeting DSM-IV criteria for personality disorders, previously described in detail (Hess et al., 2009). Inclusion criteria were the presence of personality disorder (PD) and age between 18 and 60 years. Exclusion criteria were medical conditions and lifetime diagnosis of schizophrenia or other psychotic disorders. The SCID-II was used to diagnose PDs, and PDs were also allocated to cluster B (n=448) and C (n=218).

Demographic information on all samples is detailed in Table 1.

Studies were approved by local ethical committees and complied with the Declaration of Helsinki.

2.2. Phenotype measures

Rumination was measured by Ruminative Response Scale (RRS) of the Response Style Questionnaire (Nolen-Hoeksema, 1991). We used the 10 Brooding items of the original RRS (which contains both Brooding and Reflection items) and the analyses were performed with mean scores (sum of scores divided with the number of items completed).

The background questionnaire was adapted from the version developed by the Epidemiology Unit of the University of Manchester. This well-structured self-rating questionnaire consists of 22 items and collects detailed information about medical history including

Table 1 Characterization of the study populations.

		Budapest sample	Manchester sample	Würzburg sample
N		651	1174	565
Age		30.0 ± 10.69	34.17 ± 10.43	34.98 ± 12.67
Sex	*Males*	124 (19.0%)	349 (29.3%)	238 (42.1%)
	Females	527 (81.0%)	841 (70.7)	327 (57.9%)
Prevalence of depression		131 (20.1%) (LT prevalence)	527 (44.3%) (LT prevalence)	84 (14.9%) (P prevalence)
RRS scores		1.981 ± 0.455	2.246 ± 0.613	–
Cluster C PD		–	–	218 (38.6%)
NEO neuroticism		–	–	106.18 ± 27.89 [91 ± 23]
NEO extraversion		–	–	99.49 ± 22.31 [111 ± 20]
NEO openness		–	–	109.75 ± 18.87 [124 ± 20]
NEO agreeableness		–	–	117.91 ± 15.63 [113 ± 17]
NEO conscientious.		–	–	109.25 ± 21.8 [114 ± 20]
TPQ NS		–	–	15.19 ± 5.56 [17 ± 5]
TPQ HA		–	–	19.1 ± 7.55 [15 ± 6]
TPQ RD		–	–	18.28 ± 4.54 [19 ± 4]

Difference between means of RRS score of Bp and MN samples was significant by t-test ($p<0.001$). NS, Novelty Seeking; HA, Harm Avoidance; RD, Reward Dependence. German reference values (NEO-PI: Ranseen et al., 1998; TPQ:Weyers et al., 1995) for personality scores are given in brackets. LT prevalence, lifetime prevalence; P prevalence, point prevalence.

psychiatric history and medications, family psychiatric history and socio-economic background.

The Würzburg sample was psychometrically assessed using the NEO-PI-R and TPQ personality questionnaires as described previously (Jacob et al., 2004, 2005). Details on NEO-PI-R and TPQ scores, along with German reference values, are given in Table 1.

2.3. Genotyping

Buccal mucosa samples were collected from each subject and genomic DNA was extracted according to a protocol published by Freeman et al (Freeman et al., 2003). DNA quality and quantity was determined with NanoDrop B-100 spectrophotometer, and all samples were diluted to a DNA concentration of 20 ng/μl. One SNP in *CREB1* gene promoter *(rs2253206*) and another synonymous (165 P/P) polymorphism located in exon 3 of *KCNJ6* gene (*rs2070995)* were genotyped at Centre for Integrated Genomic Medical Research at The University of Manchester using the Sequenom® MassARRAY technology (Sequenom Inc., San Diego, CA, USA). The iPLEX™ assay, based on post-PCR single base primer extension, was performed according to manufacturer's instructions. Genotyping was blinded with regard to phenotype. All laboratory work was performed under the ISO 9001:2000 quality management requirements. For the Würzburg sample, DNA as extracted from blood collected into EDTA was used. Genotyping was carried out at the Department of Psychiatry, University of Würzburg, using the same platform in the case of the *KCNJ6* variant, while CREB1 *rs2253206* was genotyped using routine PCR followed by MseI digestion and visualization by gel electrophoresis. Details can be obtained upon request from the corresponding author. All SNPs were in HWE ($p>0.05$) in each sample.

2.4. Statistical analyses

Budapest and Manchester samples: Descriptive statistics including Hardy–Weinberg equilibrium, minimal allele frequency were computed using Haploview 4.0 software (Barrett et al., 2005). Single marker association studies were performed under additive model using generalized linear models (GLM) in the 'SNPassoc' R-package (Gonzalez et al., 2007). All analyses were adjusted to age and gender. Gene–gene interaction based on the polymorphisms (G × G) was tested with generalized linear models using SPSS 15.0 for Windows.

Würzburg sample: Chi-square test was used to test for deviation of the observed genotype frequencies from Hardy–Weinberg equilibrium. Single marker association studies were performed under additive model for quantitative traits (traits of NEO-PI-R and TPQ) and under log-additive model for binary traits (Cluster C personality disorders and depression) using generalized linear models (GLM) implemented in the R function 'glm.' For gene–gene interaction analysis we included both genetic and multiplicative gene–gene interaction main effect of the T allele of rs2070995 and the G allele rs2253206 into a regression model using GLM again. To avoid multiple testing, the gene–gene interaction model was only selected if it explained the data better than the model without the interaction term, i.e. if the R function 'anova.glm' provided a p value <0.05. All analyses were adjusted for age and gender. We assumed an allele-dose effect of the putative risk alleles mentioned earlier, i.e. a genotype of an individual was coded to 0, 1 or 2 depending on how many copies of the considered allele does this individual have.

Due to exploratory characteristics of the analyses performed in the third samples we reported here nominal two-sided p values. Given the dependence between the considered traits, a Bonferroni correction of the nominal p values may be too conservative. Furthermore, it is difficult to assess the total number of the tests as it was not ascertainable at the beginning, but resulted from the model selection step.

3. Results

3.1. Descriptive statistics

Table 1 contains descriptive statistics of our three study samples for sex, age, comorbid disorders, rumination and personality traits. Individuals from the Manchester cohort scored significantly higher on RRS than subjects from Budapest ($p<0.001$). Gender showed a significant effect on rumination in both population with females scoring significantly higher on RRS compared with males in Budapest ($Mean_{male}=1.862\pm0.447$, $Mean_{female}=2.01\pm0.445$; $p=0.001$) and Manchester ($Mean_{male}=2.047\pm0.587$, $Mean_{female}=2.329$; $p<0.001$). Genotype frequencies of rs2070995 and rs2253206 of all three study samples are displayed in Table 2.

Table 2 Descriptive statistics of studied polymorphisms.

SNP (Alleles)	Location	MAF		
		Bp	MN	Wü
rs2070995 (C/T)	Exon 3 in *GIRK2* gene	22.6%	21.4%	19.0%
rs2253206 (G/A)	Promoter of *CREB1* gene	48.7%	48.6%	44.2%

MAF, minimal allele frequency; Bp, Budapest sample; MN, Manchester sample; Wü, Würzburg sample.

3.2. Genetic association analyses

Individual effects of rs2070995 and rs2253206 were not significant on rumination in either the Budapest or the Manchester sample. Interaction between rs2070995 and rs2253206 was significant on RRS score in both Budapest and Manchester samples (Tables 3a and b; Fig. 1). Individuals carrying the TT genotype of rs2070995 and the GG genotype of rs2253206 scored significantly higher on the RRS scale compared to other genotype carriers ($p_{Bp}=0.0009$; $p_{MN}=0.0027$). Interaction results remained significant if the model was adjusted for age and sex. When Brooding and Reflection items were analyzed separately, no significant association became evident in both populations.

We examined the initial findings in a third sample using a different phenotype (personality disorders and traits). While *KCNJ6* rs2070995 alone was not associated with any of the tested personality domains (results not shown), the *CREB1* rs2253206 G allele was associated with decreased NEO Agreeableness (beta = −2.44, se = 0.89, $p=0.006$), decreased NEO Conscientiousness (beta = −3.54, se = 1.2, $p=0.004$) and increased TPQ Novelty Seeking (beta = 0.85, se = 0.31, $p=0.006$) as compared to the A allele. Furthermore, a regression model including both polymorphisms and their multiplicative interaction revealed main effects of genotypes as well as an interaction effect on Cluster C personality disorder (OR, 95% confidence interval and *p* value for rs2070995 T allele: 0.72, 0.54–0.96, 0.02; for rs2253206 G allele: 0.40, 0.21–0.74, 0.004; for interaction term: 1.94, 1.24–3.03; 0.003).

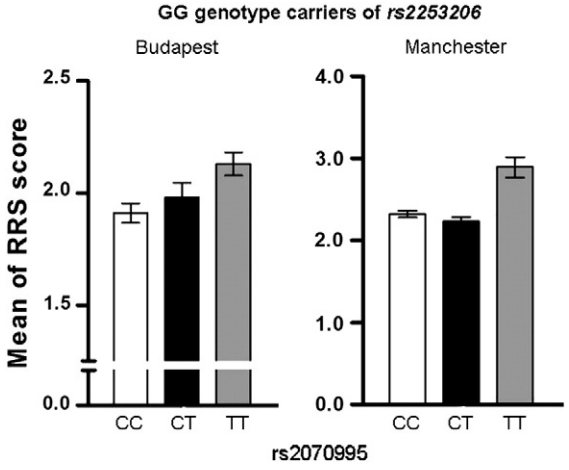

Fig 1 Highest RRS score depends on rs2253206 and rs2070995 carrier status in both the Budapest sample and the Manchester sample. Individuals carrying GG genotype of rs2253206 and TT genotype of rs2070995 scored significantly higher on RRS in both Budapest sample ($p_{int}=0.00099$) and in Manchester sample ($p_{int}=0.0027$) compared to other genotypes. RRS, Ruminative Response Scale.

3.3. Putative transcription factor binding profile analysis of CREB1

We performed an *in silico* data analyses for putative transcription factor binding profile of rs2253206. An allele-specific binding site was detected with the G allele resulting in a binding site for the GR-alpha transcription factor whereas the A allele results in binding for HNF1A, HNF1B and HNF1C factors with a similarity of more than 90% based on TRANSFAC database, using the PROMO web-based software (Messeguer et al., 2002).

4. Discussion

We here describe an epistatic interaction of *CREB1* and *KCNJ6* gene on rumination in two independent European samples. These predicted findings corroborate the

Table 3 Interaction between rs2070995 and rs2253206 on RRS score in Budapest sample (a) and Manchester sample (b).

a) Means ± S.D. of RRS in Budapest sample				
$p_{int}=0.0009$		rs2070995 *(KCNJ6)*		
		CC	CT	TT
rs2253206 (*CREB1*)	GG	1.91 ± 0.044 (n = 96)	1.98 ± 0.065 (n = 50)	2.13 ± 0.052 (n = 9)
	GA	1.94 ± 0.030 (n = 192)	2.10 ± 0.049 (n = 102)	2.00 ± 0.075 (n = 20)
	AA	2.08 ± 0.050 (n = 86)	1.82 ± 0.0622 (n = 48)	2.06 ± 0.143 (n = 8)
b) Means ± S.D. of RRS in Manchester sample				
$p_{int}=0.0027$		rs2070995 *(KCNJ6)*		
		CC	CT	TT
rs2253206 (*CREB1*)	GG	2.32 ± 0.043 (n = 187)	2.23 ± 0.051 (n = 116)	2.89 ± 0.122 (n = 9)
	GA	2.26 ± 0.032 (n = 358)	2.22 ± 0.043 (n = 207)	2.07 ± 0.159 (n = 22)
	AA	2.15 ± 0.044 (n = 169)	2.32 ± 0.070 (n = 93)	2.12 ± 0.189 (n = 13)

RRS, Ruminative Response Scale; p_{int}, *p* value of interaction.

hypothesized role of memory mechanisms in rumination. Furthermore, the same genotypes interacted to increase the risk of the Cluster C PD, 'anxious or fearful disorders' characterized by high levels of depression, anxiety, worry and social avoidance. Such symptoms are known to be associated high trait-levels of rumination in clinical and non-clinical samples (Smith et al., 2006). The findings in three independent samples are compatible with the theory that the tendency to ruminate is a continuously distributed vulnerability trait in the population with a unified molecular–neural–cognitive architecture which contributes to risk of mood/anxiety disorders. However, while the genetic association with rumination in the population samples suggests that this mediates the association with Cluster C PD, this remains uncertain in the absence of direct measures of rumination in the PD sample. This requires further study as does the related issue of whether *CREB1*×*KCNJ6* increases rumination scores in depressed patients. Nevertheless, the results suggest that rumination may be an informative endophenotype for genetic studies investigating not only depression but more generally negative emotionality.

A link between depression and GIRK2 on the one hand and CREB1 on the other hand has already been suggested by several lines of evidence. In pharmacogenetic studies, fluoxetine and paroxetine had inhibitory effects on GIRK channels although fluvoxamine, zimelidine and citalopram had slight or no effects on GIRKs (Kobayashi et al., 2003, 2004, 2006). Antidepressants affect expression of CREB1 and reduce its ability to initiate transcription (Sulser, 2002). Both GIRK2 and CREB1 genes were associated with affective disorders in whole genome association or linkage studies (Zubenko et al., 2002; Hamshere et al., 2009) although replications are warranted.

In line with preliminary data showing that genetic variation in *BDNF*, which is a crucial regulator of neuroplasticity, is associated with rumination (Hilt et al., 2007; Beevers et al., 2009), the two genes analyzed in our study are also implicated in synaptic plasticity. GIRK2 has crucial role in long-term potentiation (LTP), which is important process in the synaptic plasticity via regulating hyperpolarization by mediating inhibitory effects of several neurotransmitters and neuromodulators (Chung et al., 2009a,b). CREB1 is also known as a significant factor in LTP and memory (Han et al., 2007; Zhou et al., 2009). A large body of data suggests that CREB-dependent transcription is essential for both long-lasting forms of synaptic plasticity and long-term memory (Silva et al., 1998; Shaywitz and Greenberg, 1999; Mayr and Montminy, 2001; Lonze and Ginty, 2002; Carlezon et al., 2005).

The connection between GIRK2, CREB1 and memory functioning has been demonstrated in an experimental study. Zhou et al. reported that hyperpolarization mediated by GIRK receptors is realized only in those hippocampal cells where CREB is expressed (Zhou et al., 2009). This suggests that LTP is dependent on interaction between GIRK and CREB. While the interaction at the molecular level is not exactly known, there are numerous molecular points where both proteins interact with each other (Fig. 2). One commonality is the mode of activation. Besides being activated by G-protein βγ subunits, GIRKs are regulated by cAMP-dependent protein kinase (PKA) (Mullner et al., 2009), and interestingly, phosphorylation of CREB — which is the final step of its activation — is also mediated by PKA.

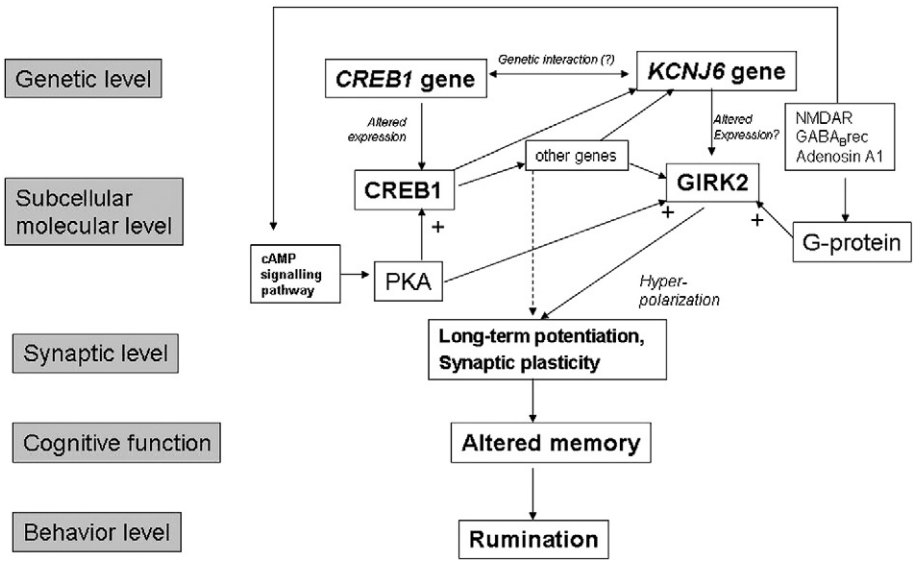

Fig 2 Interaction between GIRK2 and CREB1 at different biological levels. Interaction between GIRK2 and CREB1 is associated with rumination, which is related to altered memory function. Interaction might occur directly (by the influence of the transcription factor CREB1 on the expression of *KCNJ6*) or indirectly via expressional regulation of other genes related to GIRK2. Transcription binding profile analysis revealed that allelic variation of the *CREB1* promoter polymorphism rs2253206 can be associated with altered gene expression. There is no data about molecular functionality of rs2070995 yet it is likely in linkage with variants involved in *KCNJ6* gene expression. CREB1 and GIRK2 are activated by common neurotransmitters (e.g. NMDA) via the same signaling pathways (cAMP, PKA). Both molecules are reported to be crucial components of long-term potentiation and, accordingly, synaptic plasticity. Finally, hyperpolarization mediated by GIRK can only be accomplished in CREB positive hippocampal cells. PKA, cAMP depending protein kinase; +, activation.

Furthermore, the phosphorylation of CREB can be triggered by a variety of signaling processes, including an increase of intracellular Ca^{2+} via activation of voltage- or ligand-gated channels such as NMDA receptors, or an increase in cAMP via activation of G protein-coupled receptors (Lonze and Ginty, 2002). This suggests activation of GIRK2 and CREB by common first messengers. On the other hand, increased CREB expression or activity changes the expression of several ion channels and second messenger systems that modulate those channels (Zhou et al., 2009). Our results suggest that this interaction can occur at genetic level, which can result in a measurable phenotypic difference. As two variants (G/A) of the analyzed polymorphism in CREB1 promoter showed absolutely different transcription binding profile this suggests that different allele variants of rs2253206 influence the promoter activity in the opposite way. Regarding multiple shared points of the regulation of these two molecules, it is possible that higher or lower expression of CREB1 can alter the balance of this complex system. Further molecular biological analyses are required to clarify this possible effect of promoter on CREB1 function. On the other hand, as rumination was proved to be a trait-like feature, it is likely that genetic interaction between CREB1 and GIRK2 influences neuronal function during development of central nervous system in early ages. Due to this genetic alteration learning and memory process, which are crucial for organizing a well functioning behavior, will vulnerable and in the critical periods of CNS maturing less effective cognitive styles like rumination will develop.

Our findings that rumination is determined by a genetic interaction calls attention on a trait marker of anxiety–depression which can be appropriate for further biological association studies to discover more specific pharmacologic target molecules.

Our results of three independent European samples characterized by different depression prevalence showed that GIRK2 and CREB1 genes play crucial role in cognitive vulnerability for rumination which is a common symptom of depressive phenotype. As Cluster C personality disorders are characterized by anxiety, worrying, and predominantly negative emotionality, with rumination as a key component, we propose that the categorical gene×gene association finding is also driven by an effect on this psychological construct, making it an attractive endophenotype for further studies as it is also accessible to imaging studies.

Role of the funding source

Financial supports (named in the Acknowledgements section) were used only for scientific works.

Contributors

Judit Lazary collected the Budapest genetic samples, performed statistical analyses of Budapest and Manchester data and prepared the manuscript. Gabriella Juhasz collected Manchester samples, coordinated genotpying of Budapest and Manchester samples, checked the manuscript. Ian M. Anderson advised on study design. Christien P. Jacob genotyped Würzburg genetic samples. T. Trang Nguyen performed statistical analyses of Würzburg data. Klaus–Peter Lesch advised on study design. Andreas Reif assisted in the preparation of manuscript and advised on study design. J.F. William Deakin advised on study design. Gyorgy Bagdy advised on study design and assisted in the preparation of manuscript.

Conflict of interest

All authors reported no biomedical financial interests or potential conflicts of interest.

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References

Alberini, C.M., 2009. Transcription factors in long-term memory and synaptic plasticity. Physiol. Rev. 89, 121–145.

Barco, A., Patterson, S., Alarcon, J.M., Gromova, P., Mata-Roig, M., Morozov, A., Kandel, E.R., 2005. Gene expression profiling of facilitated L-LTP in VP16-CREB mice reveals that BDNF is critical for the maintenance of LTP and its synaptic capture. Neuron 48, 123–137.

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265.

Beevers, C.G., Wells, T.T., McGeary, J.E., 2009. The BDNF Val66Met polymorphism is associated with rumination in healthy adults. Emotion 9, 579–584.

Bito, H., Deisseroth, K., Tsien, R.W., 1996. CREB phosphorylation and dephosphorylation: a Ca(2+)- and stimulus duration-dependent switch for hippocampal gene expression. Cell 87, 1203–1214.

Carlezon Jr., W.A., Duman, R.S., Nestler, E.J., 2005. The many faces of CREB. Trends Neurosci. 28, 436–445.

Chung, H.J., Ge, W.P., Qian, X., Wiser, O., Jan, Y.N., Jan, L.Y., 2009a. G protein-activated inwardly rectifying potassium channels mediate depotentiation of long-term potentiation. Proc. Natl. Acad. Sci. USA 106, 635–640.

Chung, H.J., Qian, X., Ehlers, M., Jan, Y.N., Jan, L.Y., 2009b. Neuronal activity regulates phosphorylation-dependent surface delivery of G protein-activated inwardly rectifying potassium channels. Proc. Natl. Acad. Sci. USA 106, 629–634.

Debeer, E., Hermans, D., Raes, F., 2009. Associations between components of rumination and autobiographical memory specificity as measured by a Minimal Instructions Autobiographical Memory Test. Memory 17, 892–903.

Freeman, B., Smith, N., Curtis, C., Huckett, L., Mill, J., Craig, I.W., 2003. DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. Behav. Genet. 33, 67–72.

Gonzalez, J.R., Armengol, L., Sole, X., Guino, E., Mercader, J.M., Estivill, X., Moreno, V., 2007. SNPassoc: an R package to perform whole genome association studies. Bioinformatics 23, 644–645.

Hamshere, M.L., Green, E.K., Jones, I.R., Jones, L., Moskvina, V., Kirov, G., Grozeva, D., Nikolov, I., Vukcevic, D., Caesar, S., Gordon-Smith, K., Fraser, C., Russell, E., Breen, G., St Clair, D., Collier, D.A., Young, A.H., Ferrier, I.N., Farmer, A., McGuffin, P., Holmans, P.A., Owen, M.J., O'Donovan, M.C., Craddock, N., 2009. Genetic utility of broadly defined bipolar schizoaffective disorder as a diagnostic concept. Br. J. Psychiatry 195, 23–29.

Han, V.Z., Zhang, Y., Bell, C.C., Hansel, C., 2007. Synaptic plasticity and calcium signaling in Purkinje cells of the central cerebellar lobes of mormyrid fish. J. Neurosci. 27, 13,499–13,512.

Hess, C., Reif, A., Strobel, A., Boreatti-Hummer, A., Heine, M., Lesch, K.P., Jacob, C.P., 2009. A functional dopamine-beta-hydroxylase gene promoter polymorphism is associated with impulsive personality styles, but not with affective disorders. J. Neural Transm. 116, 121–130.

Hilt, L.M., Sander, L.C., Nolen-Hoeksema, S., Simen, A.A., 2007. The BDNF Val66Met polymorphism predicts rumination and depression differently in young adolescent girls and their mothers. Neurosci. Lett. 429, 12–16.

Impey, S., Mark, M., Villacres, E.C., Poser, S., Chavkin, C., Storm, D.R., 1996. Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. Neuron 16, 973–982.

Jacob, C.P., Strobel, A., Hohenberger, K., Ringel, T., Gutknecht, L., Reif, A., Brocke, B., Lesch, K.P., 2004. Association between allelic variation of serotonin transporter function and neuroticism in anxious cluster C personality disorders. Am. J. Psychiatry 161, 569–572.

Jacob, C.P., Muller, J., Schmidt, M., Hohenberger, K., Gutknecht, L., Reif, A., Schmidtke, A., Mossner, R., Lesch, K.P., 2005. Cluster B personality disorders are associated with allelic variation of monoamine oxidase A activity. Neuropsychopharmacology 30, 1711–1718.

Josselyn, S.A., Nguyen, P.V., 2005. CREB, synapses and memory disorders: past progress and future challenges. Curr. Drug Targets CNS Neurol. Disord. 4, 481–497.

Just, N., Alloy, L.B., 1997. The response styles theory of depression: tests and an extension of the theory. J. Abnorm. Psychol. 106, 221–229.

Karschin, C., Dissmann, E., Stuhmer, W., Karschin, A., 1996. IRK(1–3) and GIRK(1–4) inwardly rectifying K+ channel mRNAs are differentially expressed in the adult rat brain. J. Neurosci. 16, 3559–3570.

Kobayashi, M., Kawakami, K., 1995. ATF-1CREB heterodimer is involved in constitutive expression of the housekeeping Na, K-ATPase alpha 1 subunit gene. Nucleic Acids Res. 23, 2848–2855.

Kobayashi, T., Washiyama, K., Ikeda, K., 2003. Inhibition of G protein-activated inwardly rectifying K+ channels by fluoxetine (Prozac). Br. J. Pharmacol. 138, 1119–1128.

Kobayashi, T., Washiyama, K., Ikeda, K., 2004. Inhibition of G protein-activated inwardly rectifying K+ channels by various antidepressant drugs. Neuropsychopharmacology 29, 1841–1851.

Kobayashi, T., Washiyama, K., Ikeda, K., 2006. Inhibition of G protein-activated inwardly rectifying K+ channels by the antidepressant paroxetine. J. Pharmacol. Sci. 102, 278–287.

Koppel, J., Goldberg, T., 2009. The genetics of episodic memory. Cogn. Neuropsychiatry 14, 356–376.

Kuyken, W., Moulds, M.L., 2009. Remembering as an observer: how is autobiographical memory retrieval vantage perspective linked to depression? Memory 17, 624–634.

Lesage, F., Guillemare, E., Fink, M., Duprat, F., Heurteaux, C., Fosset, M., Romey, G., Barhanin, J., Lazdunski, M., 1995. Molecular properties of neuronal G-protein-activated inwardly rectifying K+ channels. J. Biol. Chem. 270, 28,660–28,667.

Liao, Y.J., Jan, Y.N., Jan, L.Y., 1996. Heteromultimerization of G-protein-gated inwardly rectifying K+ channel proteins GIRK1 and GIRK2 and their altered expression in weaver brain. J. Neurosci. 16, 7137–7150.

Lonze, B.E., Ginty, D.D., 2002. Function and regulation of CREB family transcription factors in the nervous system. Neuron 35, 605–623.

Lyubomirsky, S., Nolen-Hoeksema, S., 1993. Self-perpetuating properties of dysphoric rumination. J. Pers. Soc. Psychol. 65, 339–349.

Mamdani, F., Alda, M., Grof, P., Young, L.T., Rouleau, G., Turecki, G., 2008. Lithium response and genetic variation in the CREB family of genes. Am. J. Med. Genet. B Neuropsychiatr. Genet. 147B, 500–504.

Mark, M.D., Herlitze, S., 2000. G-protein mediated gating of inward-rectifier K+ channels. Eur. J. Biochem. 267, 5830–5836.

Mayr, B., Montminy, M., 2001. Transcriptional regulation by the phosphorylation-dependent factor CREB. Nat. Rev. Mol. Cell Biol. 2, 599–609.

Messeguer, X., Escudero, R., Farre, D., Nunez, O., Martinez, J., Alba, M.M., 2002. PROMO: detection of known transcription regulatory elements using species-tailored searches. Bioinformatics 18, 333–334.

Mullner, C., Steinecker, B., Gorischek, A., Schreibmayer, W., 2009. Identification of the structural determinant responsible for the phosphorylation of G-protein activated potassium channel 1 by cAMP-dependent protein kinase. FEBS J. 276, 6218–6226.

Nolen-Hoeksema, S., 1991. Responses to depression and their effects on the duration of depressive episodes. J. Abnorm. Psychol. 100, 569–582.

Nolen-Hoeksema, S., Davis, C.G., 1999. "Thanks for sharing that": ruminators and their social support networks. J. Pers. Soc. Psychol. 77, 801–814.

Nolen-Hoeksema, S., Morrow, J., Fredrickson, B.L., 1993. Response styles and the duration of episodes of depressed mood. J. Abnorm. Psychol. 102, 20–28.

Nolen-Hoeksema, S., Parker, L.E., Larson, J., 1994. Ruminative coping with depressed mood following loss. J. Pers. Soc. Psychol. 67, 92–104.

Nolen-Hoeksema, S., McBride, A., Larson, J., 1997. Rumination and psychological distress among bereaved partners. J. Pers. Soc. Psychol. 72, 855–862.

Radulovic, J. and Tronson, N.C., 2010. Molecular specificity of multiple hippocampal processes governing fear extinction. Rev. Neurosci. 21 (1), 1–17.

Ranseen, J.D., Campbell, D.A., Baer, R.A., 1998. NEO PI–R profiles of adults with attention deficit disorder. Assessment 5 (1), 19–24.

Roelofs, J., Huibers, M., Peeters, F., Arntz, A., van Os, J., 2008. Rumination and worrying as possible mediators in the relation between neuroticism and symptoms of depression and anxiety in clinically depressed individuals. Behav. Res. Ther. 46, 1283–1289.

Sands, W.A., Palmer, T.M., 2008. Regulating gene transcription in response to cyclic AMP elevation. Cell. Signal. 20, 460–466.

Segal, M., Murphy, D.D., 1998. CREB activation mediates plasticity in cultured hippocampal neurons. Neural Plast. 6, 1–7.

Shaywitz, A.J., Greenberg, M.E., 1999. CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu. Rev. Biochem. 68, 821–861.

Silva, A.J., Kogan, J.H., Frankland, P.W., Kida, S., 1998. CREB and memory. Annu. Rev. Neurosci. 21, 127–148.

Smith, J.M., Grandin, L.D., Alloy, L.B., Abranson, L.Y., 2006. Cognitive vulnerability to depression and axis II personality dysfunction. Cognitive Therapy and Research 30, 609–621.

Sulser, F., 2002. The role of CREB and other transcription factors in the pharmacotherapy and etiology of depression. Ann. Med. 34, 348–356.

Watkins, E.R., 2009. Depressive rumination and co-morbidity: evidence for brooding as a transdiagnostic process. J. Ration. Emot. Cogn. Behav. Ther. 27, 160–175.

Weyers, P., Krebs, H., Janke, W., 1995. Reliability and construct validity of a German version of Cloninger's tridimensional personality questionnaire. Pers. Ind. Diff. 19, 853–861.

Wupperman, P., Neumann, C.S., 2006. Depressive symptoms as a function of sex-role, rumination, and neuroticism. Pers. Individ. Differ. 40, 189–201.

Zhou, Y., Won, J., Karlsson, M.G., Zhou, M., Rogerson, T., Balaji, J., Neve, R., Poirazi, P., Silva, A.J., 2009. CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. Nat. Neurosci. 12, 1438–1443.

Zubenko, G.S., Hughes III, H.B., Maher, B.S., Stiffler, J.S., Zubenko, W.N., Marazita, M.L., 2002. Genetic linkage of region containing the CREB1 gene to depressive disorders in women from families with recurrent, early-onset, major depression. Am. J. Med. Genet. 114, 980–987.