ELSEVIER

Journal of Affective Disorders 101 (2007) 159–168

JOURNAL OF AFFECTIVE DISORDERS

www.elsevier.com/locate/jad

Research report

Association of polymorphisms in P2RX7 and CaMKKb with anxiety disorders

Angelika Erhardt [a,*], Susanne Lucae [a], Paul G. Unschuld [a], Marcus Ising [a], Nikola Kern [b], Daria Salyakina [a], Roselind Lieb [a], Manfred Uhr [a], Elisabeth B. Binder [a,1], Martin E. Keck [a,2], Bertram Müller-Myhsok [a], Florian Holsboer [a]

[a] *Max Planck Institute of Psychiatry, Kraepelinstr. 2–10, D-80804 München, Germany*
[b] *Present address: King's College, Maudsley Hospital, London, UK*

Received 2 June 2006; received in revised form 17 November 2006; accepted 17 November 2006
Available online 2 January 2007

Abstract

Background: There is considerable evidence that genetic factors play an important role in the pathophysiology of affective disorders including bipolar disorder, major depressive disorder and anxiety disorders. Long-term follow up studies as well as drug treatment studies suggest that these clinical conditions share a number of pathophysiological commonalities including genetic variables. One possible candidate region is located on chromosome 12q24.31, originated from previous linkage and association studies with bipolar disorder and unipolar depression. This region contains two candidate genes for purinergic ligand-gated ion channels, P2RX7 and P2RX4, and one gene coding for calmodulin-dependent protein kinase kinase b (CaMKKb).
Methods: In the present study, we investigated the genetic associations between 15 SNPs in the candidate genes P2RX7, P2RX4 and CaMKKb on chromosome 12q24.31 in 179 patients with anxiety disorders and syndromal panic attacks versus 462 healthy controls.
Results: One nominal case–control association could be detected for a SNP in the 5′UTR region of P2RX4, which did not remain significant after correction for multiple testing. We found, however, a prominent association between severity of panic- and agoraphobia symptoms and an exonic SNP (rs3817190) in the CaMKKb gene and a trend for association with an exonic SNP in P2RX7 (rs1718119) with severity scores in the panic- and agoraphobia scale.
Conclusion: The locus 12q24.31 seems to be an important genetic region for anxiety, bipolar and unipolar disorders, suggesting a genetic overlap in the group of affective disorders. The specific contribution of the herein reported gene polymorphisms to the clinical condition is still unclear and warrants further analysis.

Keywords: Anxiety disorders; Bipolar disorder; P2RX7; CaMKKb; Association; SNP

Abbreviations: BP, bipolar disorder; CaMKKb, Calmodulin-dependent protein kinase kinase beta; CREB, c-AMP responsive element binding protein; GABA, Gamma-aminobutyric-acid; GAD, generalized anxiety disorder; HWE, Hardy–Weinberg-Equilibrium; LTM, long term memory; LTP, long term potentiation; MAF, minor allele frequency; MDD, major depressive disorder; P2RX, purinergic ligand-gated ion channels X; SCID I and II, Structured Clinical Interviews for DSM-IV; SD, standard deviation; SNP, single nucleotide polymorphism.

* Corresponding author. Tel.: +49 89 30622 420; fax: +49 89 30622 605.
E-mail address: erhardt@mpipsykl.mpg.de (A. Erhardt).
[1] Present address: Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine,Atlanta, GA 30322, USA.
[2] Present address: Division of Psychiatry Research, Psychiatric University Hospital, CH-8032 Zürich, Switzerland.

0165-0327/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jad.2006.11.016

1. Introduction

Anxiety disorders are among the most prevalent psychiatric conditions throughout the world, leading to a considerable morbidity and high functional impairment (Kessler et al., 1994). Family and twin studies suggest that genetic factors may explain more than 40% of the phenotypic variance in anxiety disorders (Hettema et al., 2001). Being related to complex genetic disorders, the identification of genes in anxiety disorders is complicated through non-mendelian inheritance, genetic heterogeneity and incomplete penetrance (Smoller and Tsuang, 1998). Furthermore, high comorbidity rates with mood diseases, such as bipolar (BP) and unipolar depressive disorder (MDD), point toward the possibility of a genetic overlap in the underlying pathophysiology. In BP, for instance, anxiety disorders occur at higher rates than in the general population (Kessler et al., 1998). For panic disorder, the lifetime prevalence among subjects with BP was found to reach 20.8% (Chen and Dilsaver, 1995). Epidemiologic evidence suggests that anxiety disorders are more common in BP than in MDD, indicating that, at the genetic level, anxiety disorders may be related closer to BP than to MDD (Chen and Dilsaver, 1995). As is the case in MDD, patients with comorbid anxiety and BP show greater symptom severity and chronicity, more suicidality (44% vs. 19%), and poorer treatment response to pharmacotherapy (Feske et al., 2000; Frank et al., 2002; Young et al., 1993). Concerning a possible genetic relationship, BP and comorbid anxiety were found to be associated with family history based linkage studies leading to the hypothesis that anxiety disorders are a marker of genetic heterogeneity in BP (MacKinnon et al., 1998; Rotondo et al., 2002). Overlapping genetic predisposition may play a role in BP and anxiety disorders, and SNPs involved, gene–gene interactions or epigenetic effects could explain differences in phenotypes seen under clinical conditions. Thus, susceptibility loci found in bipolar disorder could be of interest in patients with non-comorbid anxiety disorders.

Recent genetic linkage studies in families from the Saguenay-Lac-St.-Jean (SLSJ) region of Quebec led to the identification of a susceptibility locus for both BP and MDD in the region of chromosome 12q24.31 (Barden et al., 2006; Curtis et al., 2003; Shink et al., 2005). An independent study at the Max Planck Institute of Psychiatry in Munich revealed a case–control association of one marker on 12q24.31 with MDD (Lucae et al., 2006). The high comorbidity rate between MDD, bipolar and anxiety disorders suggest that this genomic region might present a common susceptibility locus in affective disorders. Several candidate genes, such as genes for ligand-gated purinergic ion channels P2RX7, P2RX4 and for the calmodulin-dependent protein kinase kinase b (CAMKKb), are located on 12q24.31. All of these genes share common features including expression in clinically relevant brain regions and play a role in Ca^{2+}-dependent signalling pathways. P2X receptor subunits have two transmembrane domains, a large extracellular loop containing the ATP binding site, and intracellular N and C terminal tails. In the central nervous system, P2RX7 appears to be limited to e.g. activated microglia, lymphocytes, astroglia, although, neuron-specific location was also reported, whereas P2RX4 immunoreactivity was evident in the cerebellum, cerebral cortex, hippocampus and thalamus (Di Virgilio et al., 1999; Ishii et al., 2003; Le et al., 1998). P2X channels have the ability to form a large pore and to change their cation selectivity during prolonged exposure to ATP which could have profound effects on the encoding properties of the synapses (review: Robertson et al., 2001). Preclinical studies suggest that ATP signalling via purinergic receptors is involved in the cross talk between neurons and glial cells and there is evidence that antidepressant compounds modulate the expression of P2RX7 in the central nervous system (review: Illes and Ribeiro, 2004). CaMKKb is ubiquitously expressed with the highest expression in the brain, especially in the cerebellar granule cell layer and to a lower extent in the cerebral cortex, hippocampal formation and amygdala (Anderson et al., 1998). Results from animal studies suggest that CaMKKb isoforms are important in hippocampus-dependent spatial long term memory formation, suggesting a possible role in fear conditioning processes (Peters et al., 2003). All three genes located in the suspected genomic region 12q24.31 could have functional roles in the pathophysiology of anxiety disorders. Therefore, we investigated a possible case–control association of SNPs in P2RX7, P2RX4 and CAMKKb in patients with anxiety disorders versus healthy individuals devoid of mood disorders.

2. Methods

2.1. Patients and controls

179 outpatients consecutively admitted to the Anxiety Disorders Clinic of the Max Planck Institute of Psychiatry for diagnosis and treatment of an anxiety disorder, mostly presenting with a panic disorder with agoraphobia (74.3%), panic disorder without agoraphobia (13.4%), social phobia (7.8%), generalized anxiety