

Akt2 Gene is Associated with Anxiety and Neuroticism in Humans

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Abstract

Background: The exact cellular and molecular mechanisms underlying the pathophysiology, successful treatment and prevention of the highly associated anxiety and depressive disorders have not been identified. *Akt2* is a key protein in the Phosphatidylinositol-3 (PI3K)/ Glycogen Synthase 3 kinase (GSK3) signaling pathway. This pathway is involved in Brain-Derived Neurotrophic Factor (BDNF) signaling, fear memory, mood stabilization and action of several antidepressant drugs. In this study, we examined whether *Akt2* Single Nucleotide Polymorphisms (SNPs) are associated with anxiety and depression associated personality traits.

Methods: Four hundred and sixty-three healthy participants completed a self-rating scale for anxiety traits (Spielberger Trait-Anxiety Inventory, STAI) and depressive personality traits (NEO-FFI). Four SNPs of the *Akt2* gene (rs7247515, rs3730256, rs892118, rs11671439) were examined.

Results: The ANCOVA showed that the dependent variable anxiety trait score was significantly affected by all four genotypes. The anxiety state score was a significant covariate in three genotypes. Neuroticism was influenced by three of the four examined genotypes.

Conclusion: We found a connection between different genotypes of the *Akt2* gene and personality traits concerning anxiety and depression. These findings may be of importance for the understanding of the pathophysiology of depressive and anxiety disorders. Furthermore, *Akt2* might be a potential novel therapeutic target in the treatment of those devastating mood disorders.

Keywords: Anxiety; Behavior; Depression; *Akt2*; PI3K; GSK3; BDNF

Introduction

The *Akt* protein, also known as protein kinase B, is a downstream target of the very important Brain-Derived Neurotrophic Factor (BDNF), Phosphatidylinositol-3 (PI3K), Glycogen Synthase 3 Kinase (GSK3) signaling pathway that has been shown to be crucial for the pathophysiology of various mood disorders. BDNF has been implicated in bipolar disorder, depression, depression-related personality traits and anxiety behaviour [1-5]. Decreased expression of BDNF contributes to stress-related mood disorders. The upregulation of BDNF on the other hand plays a role in the actions of different antidepressant treatments [6]. Accordingly, alterations of BDNF concentrations have been observed after antidepressant and mood stabilizing treatment, physical exercise and electroconvulsive therapy [6]. The Phosphatidylinositol-3 (PI3) kinase has further been shown to mediate the BDNF-dependent spatial memory formation in rats [7]. Phosphoinositide dependent kinase PDK1 and PDK2, the downstream targets of the PI3K, include *Akt* [8]. *Akt* phosphorylates and thus inhibits glycogen synthase 3 GSK3. The PI3K-*Akt* pathway is involved in the signaling of hormones associated with anxiety and depression such as estrogen [9] or thyroid hormones [10,11].

Lithium, valproate, olanzapine and clozapine are at least in part effective via PI3K-*Akt* signaling [12-15]. Moreover, PI3K is involved

in behavioural sensitization to cocaine [16], the extinction of fearful memories and hippocampal plasticity [17]. Disruption of GSK3 phosphorylation by *Akt* decreased anxiety and reduced proneness to depression in mice [18,19]. Conversely, decreased expression of PI3K PDK1, leads to increased anxiety [20].

Little is known about the differential role of the *Akt* isoforms *Akt1*, *Akt2* and *Akt3* involved in the PI3K/PDK1/*Akt*/GSK3 dependent human behaviour. To this end, behavioural studies have been performed in *Akt2* knockout mice and their wild type littermates [21], showing the *Akt2* gene to be associated with depression and anxiety. The present study thus aimed to better understand the role of the isoform *Akt2* in human behaviour. We explored four SNPs of the *Akt2* gene on their possible role on anxiety behaviour [22,23]. In the present study we examined whether there is a connection between the four SNPs of the *Akt2* gene and personality traits. We hypothesized to find anxiety and depression related personality traits to be influenced by genotype.

Patients and Methods

Patients

A total of 463 healthy participants, all of whom were unrelated individuals of German descent (Caucasians; 239 male, 224 female, age: 38.4 ± 8) were recruited for this study through newspaper

advertisement. The study was approved by the ethics committee of the Charité University Medicine Berlin, Campus Mitte. Exclusion criteria were psychiatric Axis-I or Axis-II disorder, Axis-I disorder of first-degree relatives or the intake of psychotropic drugs. In order to detect psychiatric comorbidity the Mini-International Neuropsychiatric Interview (M.I.N.I.) was applied by an experienced psychiatrist [23]. The M.I.N.I. is a short structured diagnostic interview for DSM-IV and ICD-10 psychiatric disorders [24]. Axis-I or axis-II disorders, axis-I diagnosis of first degree relatives and psychotropic drug intake led to exclusion of the study as detailed elsewhere [25].

The self-ratable State-Trait Anxiety Inventory (STAI), which enables anxiety to be quantified as a comparatively stable personality trait has been performed in all subjects [26].

The participants also completed the German version of the NEO-Five Factor Inventory (NEO-FFI), which consists of 60 items and allows reliable and valid assessment of personality traits along the dimensions neuroticism, extraversion and openness to experiences, agreeableness and conscientiousness [27]. Levels of neuroticism strongly predict the risks for both lifetime and new-onset major depression [28].

DNA genotyping

Genomic DNA was extracted from anti-coagulated venous blood samples by using a salting out method [29]. Allelic discrimination of the *Akt2* SNPs (rs7247515, rs3730256, rs892118, rs11671439) was performed using a TaqMan 5' exonuclease assay [30] according to the recommendation of the manufacturer (Applied Biosystems, Foster City, CA; Assay-on-Demand SNP product: C_11592758_10).

Statistical analysis

Between-group and between-genotype comparisons were performed with one-way analysis of variance (ANOVA) or χ^2 test. Genotype effects on personality variables were computed with an ANOVA including age as a covariate (ANCOVA). Further details are given in the Results section. All tests were performed with a two-sided $p < 0.05$.

Results

Subjects

We analyzed DNA samples of four SNPs (rs7247515, rs3730256, rs892118, rs11671439) from 463 subjects in the *Akt2* prodomain. The genotype frequency was CC (n=398; 86%) and CT (n=65; 14%) for rs7247515. The genotype frequency was AG (n=86; 19%) and GG (n=375; 81%) for rs3730256. The genotype frequency was AA (n=16; 3%) and AG (n=124; 26%) and GG (n=321; 70%) for rs892118. The genotype frequency was CT (n=70; 15%) and TT (n=392; 84%) for rs11671439.

The ANCOVA showed that the dependent variable STAI trait score was significantly affected by all four genotypes. The state score was a significant covariate in three genotypes. The NEO-FFI data showed that neuroticism was influenced by three different of the examined genotypes. No gender and age differences have been observed concerning all examined SNPs except one gender covariate in one genotype. All results are shown in Table 1.

| | Age | Gender | STAI (trait score) | STAI (state score) | NEO-FFI |
|------------|----------------------------|----------------------------|---------------------------|----------------------------|---------------------------|
| rs7247515 | F=0.988 df=1 p<0.321 | F=3.74 df=1 p<0.054 | F=4.2 df=1 p<0.039 | F=4.10 df=1 p<0.043 | F=8.96 df=1 p<0.003 |
| rs3730256 | F=1.15 df=1 p<0.284 | F=5.303 df=1 p<0.022 | F=7.58 df=1 p<0.006 | F=5.55 df=1 p<0.019 | F=7.33 df=1 p<0.007 |
| rs892118 | F=1.079 df=1 p<0.299 | F=1.301 df=1 p<0.255 | F=7.14 df=1 p<0.008 | | F=1.48 df=1 p<0.285 |
| rs11671439 | F=0.993 df=1 p<0.320 | F=3.28 df=1 p<0.071 | F=7.14 df=1 p<0.008 | F=6.198 df=1 p<0.013 | F=10.7 df=1 p<0.001 |

Table 1: Association between behavioural and epidemiological data and genotype

Discussion

We found neuroticism and trait anxiety to be influenced by all studied polymorphisms of the *Akt2* gene. Therefore our hypothesis can be confirmed, that genetically different preconditions in the PI3K/Akt signaling pathway might influence vulnerability for anxiety and depression. In the past decades evidence has been gathered that the BDNF/PI3K/*Akt*/GSK3 pathway might be connected with mood disorders. Since *Akt2* is activated by phosphorylation through PI3K which is again activated by the neurotropic BDNF [7], BDNF may therefore signal through the increase of Akt2. BDNF is a well-known neurotropic for which much evidence exists for a connection with anxiety and depression [31]. In previous studies we found a connection between BDNF genotype, BDNF serum concentrations and the personality traits neuroticism and anxiety [2-4]. There is also much evidence for a connection between anxiety and depressive disorders and serotonergic neurotransmission in respect of BDNF [1-5].

The downstream target of *Akt2* is GSK3. Several recent advances have been reviewed on the involvement of the signaling molecules Akt and GSK3 in the regulation of behavior by the monoamine neurotransmitters dopamine and serotonin [32]. A large variety of pharmacological and molecular approaches for manipulating GSK3 are discussed, the results of which strongly support the proposal that inhibition of GSK3 reduces both depression-like and manic-like behaviors [33]. Studies in human postmortem brain and peripheral cells also have identified correlations between alterations in GSK3 and mood disorders. Evidence showed that depression may be associated with impaired inhibitory control of GSK3, and mania by hyperstimulation of GSK3. GSK3 and PI3K inhibition have been shown to counteract antidepressant-like effects of folic acid in the forced swimming test [34]. A similar effect has been observed on antidepressant effects of ghrelin, whereby PI3K inhibitors selectively inhibited ghrelin-induced antidepressant effects [35]. Accordingly, inhibition of PI3K decreases activity and memory while increasing insulin resistance, depression, and anxiety [36]. Attenuation of kinase activity of *Akt* in depressed suicide victims has shown to be connected with dysregulation of PI3K [37]. In this study, the effect on PI3K

signaling was associated with major depression rather than with suicide per se [37]. A temporary suppression of contextual fear was associated with blunted synaptic activity in the basal amygdala and decreased PI3K signaling in the hippocampus [17]. On the other hand, treatment with a PI3K activating transduction peptide is able to promote synaptogenesis and spinogenesis in primary cultures of rat hippocampal neurons, as well as in CA1 hippocampal neurons in vivo [38].

Taken together, we found a connection between the *Akt2* gene and human behaviour traits concerning anxiety and depression. This finding is in line with an overwhelming evidence for a connection between the BDNF/PI3K/Akt/GSK3 pathway and mood disorders. Changes in Akt phosphorylation might be a possible therapeutic target for the treatment and prevention of anxiety disorders and depression (Figure 1).

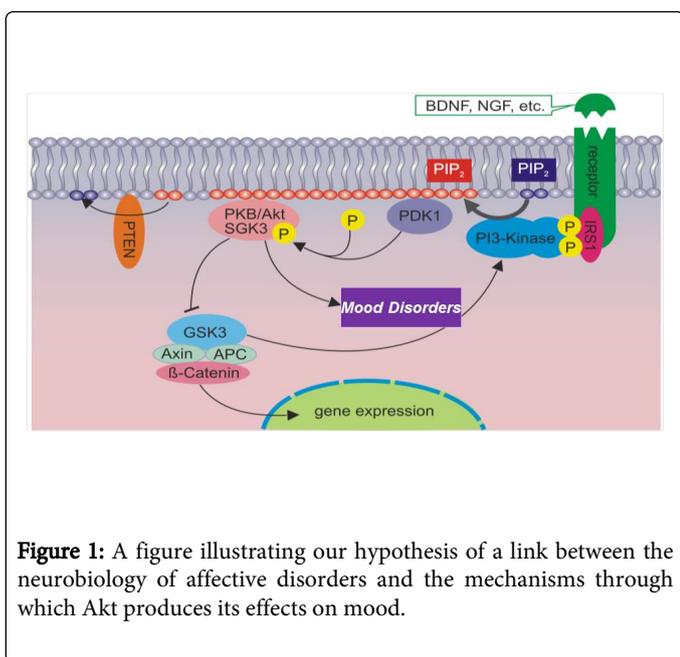


Figure 1: A figure illustrating our hypothesis of a link between the neurobiology of affective disorders and the mechanisms through which Akt produces its effects on mood.

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