ANK3 gene expression in bipolar disorder and schizophrenia

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Summary

ANK3 gene variants have consistently been associated with bipolar spectrum disorder and schizophrenia spectrum disorder. However, the relevance of its encoded protein, ankyrin-3, in these disorders remains elusive. Here, we show that ANK3 gene expression in blood is significantly increased in bipolar disorder and schizophrenia compared with healthy controls. Additionally, we identified potential cis-acting expression quantitative trait loci located close to the transcription start site of one of the isoforms of the gene. These findings suggest that ANK3 mRNA is an interesting marker for further investigation of the underlying mechanisms in psychotic disorders.

Declaration of interest

None.

ANK3 is among the most frequently reported risk genes in bipolar disorder,1 with further evidence for association with schizophrenia,2 suggesting a common genetic mechanism in these two clinically related disorders. ANK3 sequence variants have been associated with clinical characteristics of bipolar disorder and schizophrenia, including impaired cognitive function3 and mood dysregulation.4 Gene variants are also associated with disturbed brain connectivity,5 and ankyrin-3 is expressed throughout the brain,6 with functions related to clustering of sodium and potassium channels, axodendritic polarity and action potential generation. Despite indications of altered gene expression,7 it is still unclear whether ANK3 mRNA levels are altered in bipolar disorder and schizophrenia. Here we determined ANK3 mRNA levels in peripheral blood in individuals with bipolar disorder, with schizophrenia and in healthy controls. We also investigated potential cis-acting expression quantitative trait loci (eQTLs) in the ANK3 gene.

Method

Our sample (total \( n = 779\)) comprised 227 patients with bipolar disorder (type 1 \( n = 147\), type 2 \( n = 65\) and bipolar disorder not otherwise specified \( n = 15\) (BD group), 273 patients with schizophrenia (schizophrenia \( n = 209\), schizoaffective disorder \( n = 19\) and schizoaffective disorder \( n = 45\) (SZ group)) according to the Structured Clinical Interview for DSM-III-R (SCID),8 as well as 279 healthy controls. All participants were of White Northern European ethnicity. See online data supplement and Table DS1 for further details.

Quantitative reverse transcription polymerase chain reaction was performed using TaqMan Gene Expression Assays (ANK3: Hs00241738_m1; Life Technologies Corporation, Carlsbad, California, USA) and the gene GUSB (glucuronidase beta) as an endogenous control. Genotyping was performed in a subset of 685 participants with Affymetrix Genome-Wide Human SNP array 6.0 (Affymetrix Inc., Santa Clara, California, USA) and additional imputation conducted with MACH using the European samples available in the Phase 1 release of the 1000 Genomes project (www.sph.umich.edu/csg/abecasis/MACH/download/1000G-Phase1-Interim.html). Further information on RNA measurement, genotyping procedures and imputation is presented in the online supplement.

Analysis of covariance (ANCOVA) was selected to investigate ANK3 mRNA level differences between participants with bipolar disorder, those with schizophrenia and healthy controls in the IBM SPSS software package for Windows, version 20. ANK3 mRNA was entered as a dependent variable and diagnostic spectrum (bipolar disorder, schizophrenia and healthy controls) as an independent variable, with age and gender as covariates. To further elucidate the differences in mRNA level between participants with bipolar disorder, those with schizophrenia and healthy controls, logistic regression was performed with mRNA, age and gender as predictors. Additionally, ANCOVA analyses were performed in males and females separately for main diagnostic spectra, as well as in the total sample for the diagnostic subcategories. Association analyses between 2398 imputed ANK3 single nucleotide polymorphisms (SNPs) and mRNA levels in a subset of 685 participants were performed with a linear regression model, with gender and age as covariates, using PLINK (version 1.07; http://pngu.mgh.harvard.edu/purcell/plink/). Additional information is presented in the online data supplement.

Results

The mRNA levels differed significantly between the BD, SZ and healthy control groups (\( P = 2.8 \times 10^{-4}\); Fig. 1 and online Fig. DS1). The effect size (partial eta squared) was 0.021 for diagnostic spectrum (online Table DS2). The post hoc logistic regression tests showed significantly higher mRNA levels in the BD group (\( P = 9.0 \times 10^{-5}\), odds ratio (OR) = 2.29) and the SZ group (\( P = 0.010\), OR = 1.73) compared with the healthy control group, but there was no significant difference between the BD and SZ group (\( P = 0.13\), OR = 1.37). Results from the post hoc logistic regression analysis are presented in online Table DS3. The results remained largely the same when analysed in males and females separately (online Table DS4, Table DS5 and Fig. DS2). With respect to the diagnostic subcategories, there were significantly higher mRNA levels in bipolar type 1 disorder, bipolar type 2 disorder and schizophrenia compared with healthy controls, and significantly higher mRNA levels in bipolar type 2 disorder than in schizophrenia and schizoaffective disorder (online Table DS6, Table DS7 and Fig. DS1). ANK3 SNPs close to one of the

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transcription start sites were significantly associated with ANK3 mRNA levels in the total sample as well as in the BD, SZ and healthy control groups separately (online Table DS8 and Fig. DS3).

Discussion

We found ANK3 gene expression levels to be significantly increased in the BD and SZ groups compared with healthy controls. This effect was more pronounced for bipolar disorder than schizophrenia, with higher ANK3 expression in the affective end (bipolar type 2 disorder) than in the schizophrenia end of the psychosis spectrum (schizophrenia and schizophreniform disorder) (Table DS6, Table DS7 and Fig. DS1). Additionally, potential cis-acting eQTLs were identified. These results are in line with sequence variant findings, as ANK3 SNPs have been associated with both bipolar disorder and schizophrenia, albeit with stronger evidence in the former. Thus, increased ANK3 mRNA might be involved in a common pathophysiological mechanism underlying these disorders, supporting a continuum model of psychosis. ANK3 mRNA levels in peripheral blood in bipolar disorder, 7 although post-mortem brain findings of down-regulation of ANK3 mRNA in superior temporal gyrus in individuals with schizophrenia (n = 46) have also been reported. 3

Measurement of ANK3 mRNA in peripheral blood may not correlate with central nervous system (CNS) expression, but there is evidence for correlation between levels of transcripts expressed in blood and multiple CNS tissues. 9

The effect sizes for SNP associations with psychiatric disorders are very low (OR < 1.2), providing no clinical relevance. The current effect sizes for ANK3 mRNA level in bipolar disorder (OR = 2.29) and schizophrenia (OR = 1.73) were larger, indicating that mRNA level is a more robust marker than sequence variants for further assessment of underlying mechanisms in psychotic disorders. ANK3 mRNA level might be useful for investigations of the relevance of the ANK3 gene in brain networks, pharmacological interventions and clinical phenotypes in bipolar disorder and schizophrenia.

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