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Data supplement

Method

Sample characteristics

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, schizophreniform disorder *n*=19 and schizoaffective disorder *n*=45) (SZ group) and 279 healthy controls. The patients were diagnosed according to the Structural Clinical Interview for DSM-III-R (SCID).[8] Healthy controls were randomly recruited from the same catchment area as the patients, and underwent an interview where demographic and clinical information was obtained. Our sample consisted of White Northern European participants (mainly Norwegians), which have previously been demonstrated to be genetically homogeneous.[1,2] For 735 out of 779 individuals (94.4%), Norway was the country of origin for both parents. Clinical evaluation of the patients and healthy controls participating in this study is described in details in a previous report.[3] Demographical data are reported in Table DS1.

Based on previous reports of *ANK3* gene variants and bipolar disorder,[4–9] we investigated the association between 2398 imputed *ANK3* single nucleotide polymorphisms (SNPs) and mRNA levels in a subset of 685 individuals (BD group *n*=201, SZ group *n*=245 and healthy controls *n*=239).

RNA measurement

Blood samples were collected using Tempus Blood RNA Tubes (Life Technologies Corporation, Carlsbad, California, USA). Total RNA was extracted with ABI PRISM 6100 Nucleic Acid PrepStation (Life Technologies Corporation, Carlsbad, California, USA) and TEMPUS 12-port RNA Isolation Kit according to manufacturer's protocol. High-Capacity cDNA Reverse Transcription Kit (Life Technologies Corporation, Carlsbad, California, USA) was used for reverse transcription of 1 µg RNA. Quantitative reverse transcriptase polymerase chain reaction was performed on ABI PRISM 7900HT Sequence Detection System by using TaqMan Gene Expression Assays (*ANK3*: Hs00241738_m1; Life Technologies Corporation,

Carlsbad, California, USA) and the gene *GUSB* (Hs99999908_m1; Life Technologies Corporation, Carlsbad, California, USA) was used as endogenous control. Instruction protocol from manufacturer was strictly followed. The results were processed in RQ Manager 1.2.1. *ANK3* mRNA ΔCt (cycling threshold) in each individual was calibrated against mean *ANK3* mRNA ΔCt in the healthy control group. The following formula was used for statistical analyses in this report: $2^{(-\Delta\Delta Ct)}$, as described previously.[3]

Genotyping and SNP selection

The sample was genotyped at Expression Analysis (Durham, North Carolina, USA) using the Affymetrix Genome-Wide Human SNP array 6.0 (Affymetrix Inc, Santa Clara, California, USA). Quality control was performed using PLINK (version 1.07; http://pngu.mgh.harvard.edu/purcell/plink/).[10] Overall, significant plate-specific markers were set to missing (in that plate). One of two duplicates, one of two relatives (identity by descent >0.1875), samples with a reported gender differing from that determined by X chromosome marker homozygosity, mixup-samples (as calculated by pairwise genomewide identity by state), samples with non-European ancestry (as calculated with HapMap3 and multidimensional scaling) and samples with individual genotyping below 95%, were excluded. SNPs were excluded based on minor allele frequency <1%, low yield (<95%) and deviation from Hardy–Weinberg equilibrium (P<0.001).

MACH Imputed data

Candidate SNPs were imputed with MACH,[11] using the European samples available in the Phase I release of the 1000 Genomes project (www.sph.umich.edu/csg/abecasis/MACH/download/1000G-PhaseI-Interim.html) after the quality control described above. In addition, all SNPs not present in the 1000 Genomes reference, as well as all SNPs where strand alignment was ambiguous (A/T and G/C SNPs), were removed from the sample data-sets. Imputations were carried out in a three stage process taking advantage of the ChunkChromosome (http://genome.sph.umich.edu/wiki/ChunkChromosome), MACH (http://www.sph.umich.edu/csg/abecasis/MaCH/download/), and minimac programs (http://genome.sph.umich.edu/wiki/Minimac). First, the data-sets were broken into 2500 SNP pieces, with 500 SNP overlap using ChunkChromosome. Second, each piece was phased using MACH (40 rounds, 400 states). Third, each phased piece was imputed to the 1000

Genomes European reference panel using minimac (20 rounds, 400 states). Minimac provides an estimated r^2 score that provides a quality metric for each imputed SNP. All SNPs with r^2 less than 0.5 were excluded from further analysis leaving 9,584,802 SNPs. 2398 imputed *ANK3* SNPs based on UCSC coordinates ± 20 kb (hg19) were extracted for inclusion in the current analyses.

Statistical analysis

Analysis of covariance (ANCOVA) was selected to investigate ANK3 mRNA level differences between the BD, SZ and healthy control groups in the IBM SPSS software package for Windows, version 20. ANK3 mRNA was entered as dependent variable and diagnostic spectrum (bipolar disorder, schizophrenia and healthy controls) as independent. Six individuals were removed due to deviant ANK3 mRNA levels defined as 3 standard deviations from the mean. Potential covariates were explored. Age was significantly correlated with mRNA level (Pearson r=–0.152, P=6.1 x 10^{-5}). Independent sample *t*-tests revealed significant association between gender and mRNA level ($t(777)$=8.55, P=9.1 x 10^{-17}), but not between dichotomous medication variables (medicated (yes/no), antipsychotics, lithium, antidepressants, hypnotics or psychostimulants) and mRNA level. An overall effect of anti-epileptics was found in the combined patient sample, but this effect disappeared when gender and age were included in the model. Thus, age and sex were selected as covariates in the current analyses. To further elucidate the differences in mRNA level between the BD, SZ and healthy control groups, logistic regression between two and two groups was performed with mRNA, age and gender as predictors. Additionally, ANCOVA analyses were performed in males and females separately for main diagnostic spectrums, as well as in the total sample for the diagnostic subcategories.

Association analyses between 2398 imputed *ANK3* SNPs and mRNA levels in a subset of 685 participants were performed with a linear regression model using PLINK (version 1.07; http://pngu.mgh. harvard.edu/purcell/plink/).[11] These analyses were conducted in the total subset and in BD, SZ and health control groups separately, with gender and age as covariates, as well as diagnostic category in the total subset.

Results

ANK3 mRNA levels differed significantly between the BD, SZ and healthy control groups (ANCOVA, $F(2, 774)$=8.276, P=2.8 x 10^{-4}; Fig. 1 and Table DS2). The effect size (partial eta

squared) was 0.02 for diagnostic group and 0.13 for the total corrected model (Table DS2). The *post hoc* logistic regression tests showed significantly lower mRNA levels in the BD group (P=9.0 x 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). The full regression models for bipolar disorder *v*. healthy controls (χ^2 (3, N=506)=33.05, P=3.2 x 10^{-7}), schizophrenia *v*. controls (χ^2 (3, N=552)=10.68, P=0.01) and bipolar disorder *v*. schizophrenia (χ^2 (3, N=500)=31.55, P=6.5 x 10^{-7}) were significant and the models explained 8.5%, 2.6% and 8.2% (Nagelkerke R squared) of the variance respectively. Results from the *post hoc* logistic regression analysis are presented in Table DS3. The results remained largely the same when analysed in males and females separately (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 with healthy controls, and significantly higher mRNA levels in bipolar type 2 disorder than in schizophrenia and schizophreniform disorder (online Table DS6, Table DS7 and Fig. DS1). *ANK3* SNPs close to one of the transcription start sites were significantly associated with *ANK3* mRNA levels in the total subsample as well as in the BD, SZ and health control groups separately (Table DS8 and Fig. DS3).

Additional references

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Table DS1. Demographics and clinical characteristics.

	BD (n=227)	SZ (n=273)	CTR (n=279)	ANOVA/ independent t-test/ Chi square analysis		
				$F/t/\chi^2$	**p**	**Post hoc**
Gender (% male)	38.8	57.5	54.5	χ^2=19.6	<0.001	-
Age [1]	35.2 (12.1)	31.8 (10.3)	33.3 (9.4)	F=6.6	0.001	(SCZ<BD)
Age of onset [2]	22.8 (9.6)	23.9 (8.4)	-	-	-	-
GAF symptom	55.2 (11.7)	41.8 (11.0)	-	t=-13.2	<0.001	(SCZ<BD)
GAF function	52.5 (12.5)	42.6 (10.3)	-	t=-9.7	<0.001	(SCZ<BD)
PANSS positive	10.2 (3.8)	15.5 (5.5)	-	t=12.2	<0.001	(SCZ>BD)
PANSS negative	10.4 (3.7)	15.8 (6.3)	-	t=11.3	<0.001	(SCZ>BD)
PANSS total	46.7 (11.2)	63.9 (17.3)	-	t=12.8	<0.001	(SCZ>BD)
IDS	18.0 (12.5)	18.3 (11.8)	-	-	-	-
YMRS	3.6 (4.9)	5.3 (5.1)	-	t=3.6	<0.001	(SCZ>BD)
Medication status (%)[3]						
Medicated	87.2	90.5				
Antipsychotics	54.6	83.9				
Lithium	18.5	1.8				
Antiepileptics	46.3	19.4				
Antidepressants	38.3	28.9				
Hypnotics	12.8	15.0				
Psychostimulants	1.3	0.4				

Means (standard deviation) are reported unless otherwise specified.

BD, bipolar spectrum disorder. SZ, schizophrenia spectrum disorder. CTR, healthy controls.

ANOVA, analysis of variance. GAF: Global assessment of functioning. PANSS: Positive and negative syndrome scale. IDS, Inventory of Depressive Symptomatology. YMRS, young mania rating scale.

[1] Age at blood sampling.

[2] Age of onset was defined as first affective episode (depression, hypomania or mania) for BD and fist psychotic episode for SZ.

[3] Information regarding medication status was lacking for three patients with BD and two patients with SZ.

Table DS2. Results from the ANCOVA analysis for diagnostic spectrum groups.

Variable	F	df	p	η^2
Age	16.993	1	$4.2x10^{-5}$	0.021
Gender	65.753	1	$2.0x10^{-15}$	0.078
Diagnosis	8.276	2	$2.8x10^{-4}$	0.021
Corrected model	28.005	4	$9.6x10^{-22}$	0.126

η^2, partial eta squared.

Table DS3. Results from the logistic regression analysis for diagnostic spectrum groups.

					95% CI for OR	
	β	**Wald**	**P**	**OR**	**Lower**	**Upper**
BD vs CTR						
ANK3 mRNA	0.828	15.338	$9.0x10^{-5}$	2.288	1.512	3.462
Age	0.015	2.784	0.095	1.015	0.997	1.032
Gender	0.445	5.388	0.020	1.560	1.072	2.272
Constant	-1.917	24.545	$7.3x10^{-7}$	0.147		
SZ vs CTR						
ANK3 mRNA	0.546	6.679	0.010	1.726	1.141	2.610
Age	-0.020	5.162	0.023	0.980	0.963	0.997
Gender	-0.245	1.830	0.176	0.783	0.549	1.116
Constant	0.140	0.169	0.681	1.151		
BD vs SZ						
ANK3 mRNA	0.317	2.346	0.126	1.373	0.915	2.059
Age	0.026	9.571	0.002	1.027	1.010	1.044
Gender	0.678	12.177	$4.8x10^{-4}$	1.969	1.346	2.882
Constant	-1.796	24.333	$8.1x10^{-7}$	0.166		

BD, bipolar spectrum disorder. CTR, healthy controls. SZ, schizophrenia spectrum disorder. OR, odds ratio. CI, confidence interval.

Table DS4. Results from the ANCOVA analyses for diagnostic spectrum groups in males and females separately.

Males:	**Variable**	**F**	**df**	**p**	**η^2**
	Age	6.263	1	0.013	0.016
	Diagnosis	2.824	2	0.061	0.014
	Corrected model	4.442	3	0.004	0.033
Females:	**Variable**	**F**	**df**	**p**	**η^2**
	Age	10.515	1	0.001	0.027
	Diagnosis	5.434	2	0.005	0.028
	Corrected model	7.359	3	$8.3x10^{-5}$	0.055

η2, partial eta squared.

Table DS5. Results from the post hoc Tukey test for pairwise comparisons of diagnostic spectrum groups in males and females separately.

					95% Confidence Interval for Difference	
(I) group	**(J) group**	**Mean Difference (I-J)**	**Std. Error**	**P**	**Lower Bound**	**Upper Bound**
Males						
CTR	**SZ**	-0.062	0.045	0.169	-0.149	0.026
	BD	-0.123*	0.053	0.020	-0.227	-0.019
SZ	**CTR**	0.062	0.045	0.169	-0.026	0.149
	BD	-0.062	0.053	0.247	-0.166	0.043
BD	**CTR**	0.123*	0.053	0.020	0.019	0.227
	SZ	0.062	0.053	0.247	-0.043	0.166
Females						
CTR	**SZ**	-0.122*	0.062	0.048	-0.244	-0.001
	BD	-0.193*	0.059	0.001	-0.309	-0.077
SZ	**CTR**	0.122*	0.062	0.048	0.001	0.244
	BD	-0.071	0.061	0.244	-0.190	0.049
BD	**CTR**	0.193*	0.059	0.001	0.077	0.309
	SZ	0.071	0.061	0.244	-0.049	0.190

*, The mean difference is significant at the 0.05 level.

Table DS6. Results from the ANCOVA analysis in diagnostic subcategories.

Variable	F	df	P	η2
Age	15.226	1	$1.0x10^{-4}$	0.019
Gender	62.458	1	$9.4x10^{-15}$	0.075
Diagnostic subcategory	3.492	6	0.002	0.026
Corrected Model	14.558	8	$6.5x10^{-20}$	0.131

η^2, partial eta squared.

Table DS7. Results from the post hoc Tukey test for pairwise comparisons of diagnostic subcategories.

(I) group	(J) group	Mean Difference (I-J)	Std. Error	P	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
CTR	**schizophrenia**	-0.089*	0.040	0.027	-0.168	-0.010
	schizophreniform	0.010	0.104	0.920	-0.194	0.215
	schizoaffective	-0.130	0.071	0.065	-0.269	0.008
	bipolar I	-0.122*	0.045	0.006	-0.210	-0.034
	bipolarNOS	-0.225	0.116	0.053	-0.452	0.003
	bipolar II	-0.229*	0.061	$1.7x10^{-4}$	-0.348	-0.110
schizophrenia	**CTR**	0.089*	0.040	0.027	0.010	0.168
	schizophreniform	0.100	0.105	0.343	-0.107	0.306
	schizoaffective	-0.041	0.073	0.571	-0.184	0.101
	bipolar I	-0.033	0.048	0.488	-0.127	0.061
	bipolarNOS	-0.135	0.117	0.248	-0.365	0.095
	bipolar II	-0.140*	0.063	0.027	-0.264	-0.016
schizophreniform	**CTR**	-0.010	0.104	0.920	-0.215	0.194
	schizophrenia	-0.100	0.105	0.343	-0.306	0.107
	schizoaffective	-0.141	0.120	0.242	-0.377	0.096
	bipolar I	-0.133	0.107	0.216	-0.344	0.078
	bipolarNOS	-0.235	0.151	0.121	-0.532	0.062
	bipolar II	-0.240*	0.115	0.038	-0.466	-0.013
schizoaffective	**CTR**	0.130	0.071	0.065	-0.008	0.269
	schizophrenia	0.041	0.073	0.571	-0.101	0.184
	schizophreniform	0.141	0.120	0.242	-0.096	0.377
	bipolar I	0.008	0.074	0.915	-0.138	0.154
	bipolarNOS	-0.094	0.130	0.470	-0.350	0.161
	bipolar II	-0.099	0.085	0.244	-0.265	0.068
bipolar I	**CTR**	0.122*	0.045	0.006	0.034	0.210
	schizophrenia	0.033	0.048	0.488	-0.061	0.127
	schizophreniform	0.133	0.107	0.216	-0.078	0.344
	schizoaffective	-0.008	0.074	0.915	-0.154	0.138
	bipolarNOS	-0.102	0.118	0.388	-0.335	0.130
	bipolar II	-0.107	0.065	0.102	-0.235	0.021
bipolarNOS	**CTR**	0.225	0.116	0.053	-0.003	0.452
	schizophrenia	0.135	0.117	0.248	-0.095	0.365
	schizophreniform	0.235	0.151	0.121	-0.062	0.532
	schizoaffective	0.094	0.130	0.470	-0.161	0.350
	bipolar I	0.102	0.118	0.388	-0.130	0.335
	bipolar II	-0.005	0.125	0.971	-0.250	0.241

bipolar II	**CTR**	0.229*	0.061	$1.7x10^{-4}$	0.110	0.348
	schizophrenia	0.140*	0.063	0.027	0.016	0.264
	schizophreniform	0.240*	0.115	0.038	0.013	0.466
	schizoaffective	0.099	0.085	0.244	-0.068	0.265
	bipolar I	0.107	0.065	0.102	-0.021	0.235
	bipolarNOS	0.005	0.125	0.971	-0.241	0.250

*, The mean difference is significant at the 0.05 level.

Table DS8. Results from the association analysis between imputed *ANK3* SNPs and *ANK3* mRNA (see separate Excel spreadsheet).

Fig. DS1. *ANK3* mRNA expression level (mean value ± one standard error) according to diagnostic subcategory.

BDNOS, bipolar disorder not otherwise specified; BD2, bipolar type 2 disorder; BD1, bipolar type 1disorder; SZA, schizoaffective disorder; SZ, schizophrenia; SZF, schizophreniform disorder; CTR, healthy controls.

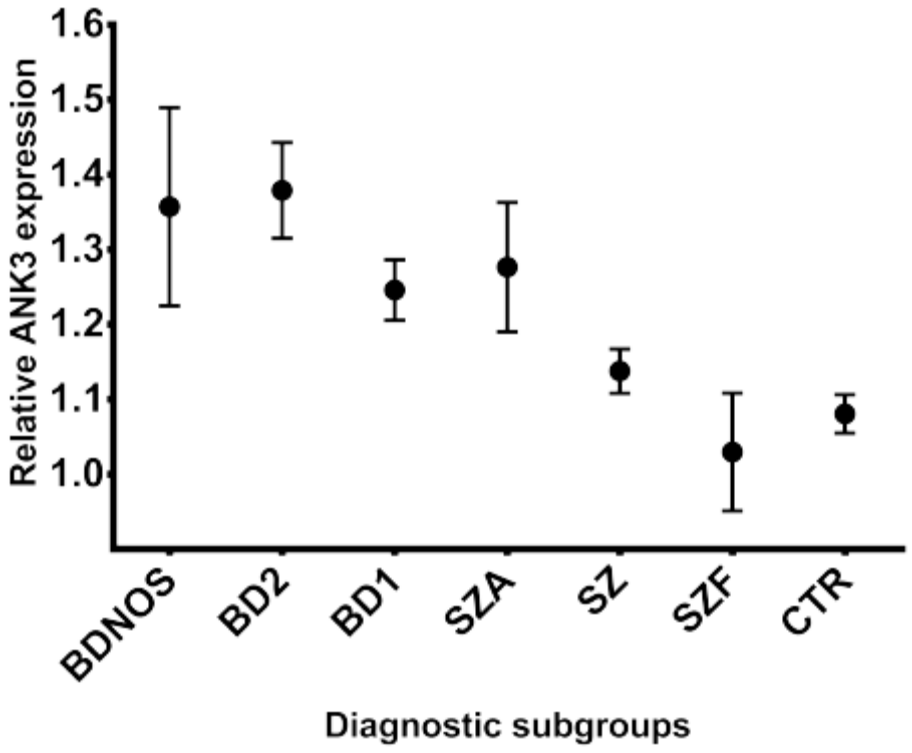

Fig. DS2. *ANK3* mRNA expression level (mean value ± one standard error) according to diagnostic group, for males and females separately.

BDNOS, bipolar disorder not otherwise specified; BD2, bipolar type 2 disorder; BD1, bipolar type 1disorder; SZA, schizoaffective disorder; SZ, schizophrenia; SZF, schizophreniform disorder; CTR, healthy controls.

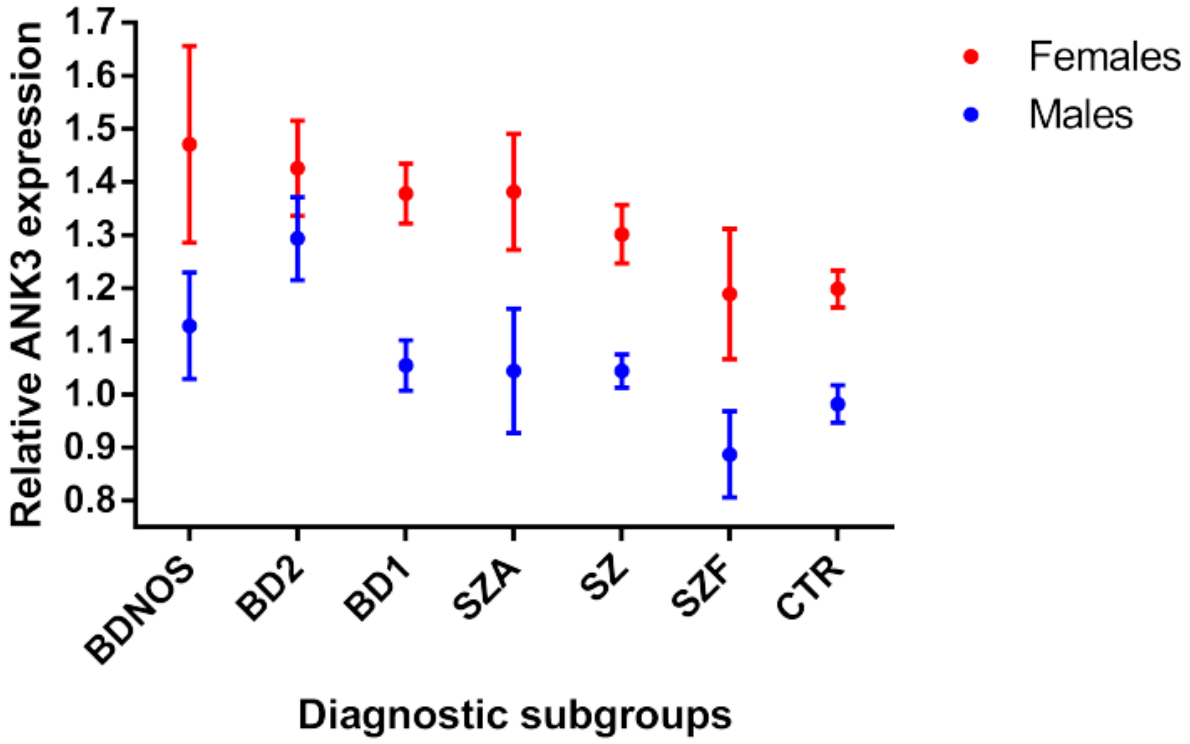

Fig. DS3. Heatmap plot for association between *ANK3* SNPs and *ANK3* mRNA in the total sample, as well as in bipolar disorder, schizophrenia and healthy controls separately. Association analyses are performed with linear regression and gender and age as covariates (and diagnostic category in the total sample). Gradient from grey (high *P*-value) to black (low *P*-value).