Check for updates

PROF. ANURANJAN ANAND (Orcid ID : 0000-0001-9584-8730)

Author Manuscript

Article type : Original Article

Title: A linkage and exome study implicates rare variants of *KANK4* and *CAP2* in bipolar disorder in a multiplex family.

Running title: *KANK4* and *CAP2* variants in bipolar disorder.

Authors:

Ram Murthy Anjanappa[1, 2, #], Sourav Nayak[1], Nagaraj S Moily[2, 3], Vallikiran Manduva[2, 4], Ravi Kumar Nadella[2], Biju Viswanath[2], Yemmiganur Chandrashekar Janardhan Reddy[2], Sanjeev Jain[2] and Anuranjan Anand[1, 5*].

Affiliations:

Molecular Biology and Genetics Unit[1] and Neuroscience Unit[5], Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore.

Department of Psychiatry[2], National Institute of Mental Health and Neurosciences, Bangalore.

Present address- Centre for Human Genetics[#], Bangalore

Present address- Department of Biochemistry and Molecular Biology[3], The University of Melbourne, Australia

Present address- Department of Psychiatry[4], St. Johns hospital, Idukki, Kerala.

* Corresponding author: Professor Anuranjan Anand

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/BDI.12815

This article is protected by copyright. All rights reserved

Molecular Biology and Genetics Unit,
Jawaharlal Nehru Centre for Advanced Scientific Research,
Jakkur, Bangalore 560 064.
Ph: +91-080-2208 2804/2712
e-mail: anand@jncasr.ac.in

Author Manuscript

Acknowledgements

We thank all members from the family for their participation in the study. We also thank Janardhanan C Narayanaswamy, P. Chandrasekar, Lakshmi Narayan Kota and J. Muralidharan for their support during recruitment of the family. Authors too thank Professor Sharat Chandra for critical reading of the manuscript. RMA acknowledges receipt of senior research fellowship from Indian Council of Medical Research (ICMR), New Delhi and research associate fellowship from Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bangalore. RKN was funded by the Accelerator Program for Discovery in Brain disorders using stem cells (ADBS) (jointly funded by the Department of Biotechnology, Government of India, and the Pratiksha Trust).

Conflicts of interest

All authors report no competing financial interests or potential conflicts of interests.

Abstract

Objectives: Bipolar disorder (BPD) is a neuropsychiatric disorder with a complex pattern of inheritance. Although many genetic studies have been conducted on BPD, its genetic correlates remain uncertain. This study aimed to identify the genetic cause of the disorder in an Indian family, which is comprehensively evaluated clinically and is under follow-up for over 12 years.

Methods: We analysed a four-generation family with several of its members diagnosed for BPD, employing a combination of genetic *linkage* and *exome analysis*.

Results: We obtained suggestive LOD score for a chromosome 1 and a chromosome 6 marker (D1S410; LOD = 3.01, Θ = 0; and D6S289; LOD = 1.58, Θ = 0). Manual haplotyping of the regions encompassing these two markers helped

This article is protected by copyright. All rights reserved

delimit a critical genomic interval of 32.44 Mb (D1S2700-D1S435; chromosome 1p31.1-13.2) and another of 10.34 Mb (D6S470-D6S422; chromosome 6p22.3-22.2). We examined the exomic sequences corresponding to these two intervals and found rare variants, NM_181712.4: c.2461G>T (p.Asp821Tyr) in *KANK4* at 1p31.1-13.2; and NM_006366:c.-93G>A, in the 5' UTR of *CAP2* at 6p22.3-22.2.

Conclusions: Our study, suggests involvement of *KANK4* or *CAP2* or both in BPD in the family. Further analysis of these two genes in BPD patients and functional evaluation of the allelic variants identified are suggested.

Keywords: Bipolar disorder, KANK4, CAP2, Linkage analysis, Exome sequencing

Author Manuscript

Introduction

Bipolar disorder (BPD) is a severe and complex psychiatric condition characterised by alternating episodes of depression and mania. It has a lifetime prevalence of about 1-3% across populations.[1, 2] According to the Global Burden of Disease Study-2013, there are 48.8 million BPD cases globally and BPD is the 16th leading condition in years lived with disability.[3] Twin, adoption and family studies suggest 70-80% heritability in risk for BPD.[4] However, the genetic risk factors are complex, and have been difficult to identify and confirm. BPD genetics has been progressing in terms of identification of a number of genetic loci based on genome-wide linkage and association studies .[5-7] The genetic loci identified through linkage studies are spread across the whole genome, emphasizing the complexity of underlying mechanisms of the disorder.[8] The number of susceptibility loci involved, the disease risk conferred by each locus, the extent of genetic heterogeneity, and the degree of interaction among loci remain poorly understood. Hence, there is a need to continue to search for genetic signals, especially in BPD families with multiple affected individuals, from various geographical regions, which may help identify biologically important and high-impact gene variants underlying the disorder.

The study of pedigrees with Mendelian or near-Mendelian inheritance for a genetic disorder is a powerful way to overcome the difficulties of phenotypic and genetic heterogeneity.[9] Several BPD genetic loci have been identified using large-family studies and a few among these map to chromosomes 4p, 4q35, 16p13, 13q14, 20p11.2-q11.2 and Xq24-27.1.[10-15] There are instances where unexpected genetic heterogeneity has been observed within single large families,[16-22] suggesting that

This article is protected by copyright. All rights reserved

even in apparently monogenic families, the phenotypic manifestation may not be entirely due to a straight forward effect of a single major locus or gene.

Here, we report our findings of a combined genome-wide linkage and exome sequencing study of Family 2717 in which several members are affected with BPD. This study revealed rare alleles of *KANK4* and *CAP2* genes as potential causative alleles for the disorder.

Author Manuscript

Materials and Methods

Clinical Ascertainment: Family 2717 was ascertained through the clinical services of NIMHANS, Bangalore, through the index case, IV:4 (Figure 1). Individuals in the family were assessed following the clinical criteria prescribed in the Diagnostic Interview for Genetic Studies (DIGS)[23] and Family Interview for Genetic Studies (FIGS)[24]. The assessment reached at from the interview of each participant was supplemented by information from relatives. The case reports were written for the patients and were reviewed by three psychiatrists, to arrive at best-estimate diagnosis (Table 1). The key phenotypes comprised manic and major depressive episodes, including suicide attempts. The affection status of deceased individuals was provisionally assigned by clinical histories obtained from normal relatives. During the course of the last 12 years (August, 2006 to August, 2018), we collected information on 71 subjects -19 BPD, 1 one major depressive disorder, one recurrent depressive disorder, and 50 apparently unaffected individuals. The age of onset of BPD ranged from 16 to 30 years. Individual III:9, who was unaffected during recruitment developed depression and committed suicide at the age of 54. This was considered a major departure from the mean age of onset (20.06 ± 4.56) and the individual was not considered affected for the purpose of genetic linkage analysis. The controls were recruited following assessment using DIGS and FIGS for psychiatric illness and family history respectively. Those who have no psychiatric illness and no first-degree relatives with any axis I psychiatric disorders were recruited. The study was approved by the institutional ethics committee. After providing complete description of the study to the subjects, written informed consent was obtained, and 10 ml of venous blood was drawn from each participant.

***Genotyping and linkage analysis*:** Genomic DNA was isolated from whole blood of the participating individuals using modified salting-out method.[25] Twenty-eight individuals from the family including 11 BPD affected individuals, were genotyped

This article is protected by copyright. All rights reserved

(Figure 1). The initial genome scan was performed using 382 highly polymorphic fluorescent microsatellite markers (ABI PRISM® Linkage Mapping Set version 2.5 MD10) that have an average spacing of 10 cM covering the 22 autosomes (Thermo Fisher Scientific Inc., Waltham, MA, USA). Markers were amplified in individual PCR reactions and the amplified products were then pooled and electrophoresed on an ABI 3730 Genetic Analyzer (Thermo Fisher Scientific Inc.). Twenty-four additional markers were used for fine mapping at an average resolution of about 2 cM for chromosome 1. A reference individual, CEPH 1347-02, with known genotypes, was used as an internal control. Allele sizes were observed using Genescan v3.7 and assignment of genotypes was done using Genemapper v3.7 (Thermo Fisher Scientific Inc.). Parametric two-point LOD scores were computed using SuperLink v1.5 and multipoint analysis was performed using Simwalk v2.91 of easyLINKAGE v5.02.[26] Haplotypes were constructed manually, according to the order of the Marshfield linkage map. Data was analysed for dominant mode of inheritance with 80% and 90% penetrance values and phenocopy rates- 1% and 5%. The disease allele frequency was assumed to be 0.001.

***Whole-exome sequencing and data analysis*:** Whole-exome sequencing was carried out for the 11 affected members. Exome capture was performed according to the sample preparation protocol of NEXTERA Rapid Capture Expanded Exome kit, which covers 62 Mb of genomic sequence including microRNAs and UTRs (Illumina, San Diego, CA, USA). Sequencing (paired end 2x100 bp) was performed on an Illumina HiSeq2500 machine according to the recommended protocol (Illumina, CA, USA). Raw intensity files from HiSeq2500 were converted into readable paired-end sequence text files using bcl2fastq.pl program (http://support.illumina.com/downloads/bcl2fastq-conversion-software-v217.html). Each read-pair was examined for adapter contamination and adapter sequences present in reads were trimmed using Cutadapt (https://cutadapt.readthedocs.io/en/stable/). After trimming of low-quality bases (phred score < 20) from 3'-ends, sequence reads of at least 70% of bases with a minimum phred score of 20 were considered for alignment. These trimmed reads were aligned to human reference genome hg19 (UCSC Genome Browser) using Burrows-Wheeler Aligner (BWA) 0.7.5a.[27] Duplicate reads, potentially resulting from PCR artefacts were removed using SAMTools v0.1.19.[28] Further, local realignment surrounding known indel regions was performed, and the quality score for each read

This article is protected by copyright. All rights reserved

Author Manuscript

was recalibrated with GATK. Variants were called by GATK HaplotypeCaller and were annotated against the 1000 Genomes and dbSNP144 databases. To investigate the probable effects of each variant, annotation and functional effect prediction program SnpEff was used.[29] Variants filtered were confirmed and segregation in the family was affirmed by Sanger sequencing. Control-sample sequencing was also performed by Sanger method of sequencing.

Results

Chromosome 1p31.1-13.2 and chromosome 6p22.3-22.2 regions

In the two-point genome-wide linkage analysis, the highest LOD score (Z_{max}) of 2.37 (Θ = 0.05) was obtained for the marker D1S230 (chromosome 1p31.1; 95.31 cM) at 90% penetrance value and 5% phenocopy rate. Further, from among the 24 additional markers analysed for the 1p31.1 region, we obtained a two-point LOD score of 3.01 (Θ = 0) for D1S410 (100.39 cM). Five additional markers, D1S438, D1S219, D1S464, D1S2876 and D1S430, in the region had LOD scores higher than 2.0 (supplementary figure 1). Multipoint analysis produced a parametric LOD score of 2.99 in the region (supplementary figure 2). Manual examination of haplotypes enabled delimiting the critical genomic region in affected individuals of the family. A recombination event between D1S2700 and D1S2788 in affected individual IV:1 defined the distal boundary, whereas the proximal boundary was defined by a recombination event between D1S2627 and D1S435 in the individual IV:5 (Figure 2). The D1S2700-D1S435 interval corresponds to about 32.44 Mb of genome (Chr1:59,117,045-91,558,940; Human Genome Map Viewer Build 37.1 database, NCBI, NIH, USA) and was present in seven affected individuals of the family.

The second genetic location of potential interest was chromosome 6p22.3-22.2. At this location, LOD score of 1.58 (θ = 0) was observed for D6S289 (29.23 cM) at 90% penetrance value and 5% phenocopy rate. The score from parametric multipoint analysis was lower than the two-point results in the region, but remained positive and provided support with a LOD score of 0.58 (supplementary figure 2). Manual haplotype analysis narrowed down the region to 10.34 Mb (Chromosome 6:10,025,785-20,370,120 bp; Human Genome Map Viewer Build 37.1 database, NCBI, NIH, USA). Recombination events in III:6 and IV:4, between markers D6S470 and D6S289, defined the upper boundary, and recombination in III:3 and IV:5, between D6S289 and D6S422, defined the lower boundary. The chromosome

This article is protected by copyright. All rights reserved

Author Manuscript

6p22.3-22.2 region is concordant in 10 of the 11 affected members with one of the individuals, IV:10, being homozygous for the haplotype (Figure 3).

KANK4 and CAP2 variants

From the whole-exome sequencing data (supplementary table, for alignment details), we examined sequences of the gene transcripts from the critical intervals 1p31.1-13.2 (Chromosome 1: 59,117,045 - 91,558,940 bp) and 6p22.3-22.2 (Chromosome 6:10,025,785 - 20,370,120 bp). The 32.44 Mb region on 1p31.1-13.2 harbours 437 known genes, of which 131 are protein coding. A total of 641 sequence variants were found in the region, of which 238 were heterozygous. Among these five (two SNVs and three InDels) were novel and 233 (204 SNVs and 29 InDels) were known. No novel coding variant was found. In the 6p22.3-22.2 region, a total of 179 genes are present in the 10.34 Mb genomic region, among which 47 are protein coding. In this region, 45 variants were detected, of which 25 were heterozygous. We filtered out variants with allele frequency of more than 1% either in the dbSNP144, Exome Aggregation Consortium (ExAC), 1000 Genomes or Exome Variant Server (EVS) databases. From among the variants with allele frequency below 1%, we focused on the ones in the 5'-UTR regions and those that were non-synonymous, splice-site, stop-gain and stop-loss single-nucleotide variants as well as frame-shift InDels.

We found a rare non-synonymous heterozygous variant, NM_181712.4:c.2461G>T, in *KANK4* (*KN motif and ankyrin repeat domains 4*), segregating in 7 out of 11 affected individuals in the family (Figure 1 and Figure 4A) at 1p31.1-13.2. The two-point LOD score for this variant was 2.04. This variant results in substitution of an aspartic acid at amino acid residue 821 with tyrosine, p.D821Y. The c.2461G>T variant is reported in ExAC (allele frequency: 0.000008269; 1/120928), gnomAD (0.0001) and TOPMED (0.0001). It is reported in dbSNP144 with allele frequency unknown (rs776739426) and is absent in 1000 Genomes and EVS databases. The c.2461G>T variant was not detected in 576 control chromosomes from the southern Indian population. Aspartic acid at the 821 amino acid position is found to be conserved across species: *Homo sapiens* (NP_859063.3), *Pan troglodytes* (XP_003949478.1), *Macaca mulatta* (XP_001084958.1), *Canis lupus* (XP_003434656.1), *Bos taurus* (NP_001095523.2), *Mus musculus* (NP_766460.2),

This article is protected by copyright. All rights reserved

Author Manuscript

Rattus norvegicus (NP_001101417.1), *Gallus gallus* (XP_422516.3) and *Xenopus tropicalis* (XP_002931658.2) (Figure 4B). The p.D821Y substitution is predicted to be deleterious by SIFT (damaging, with a score of 0),[30] polyphen 2 (probably damaging, with a score of 1),[31] mutation taster (disease causing, with a probability 0.9999)[32] and PROVEAN (deleterious, with a score of -8.255).[33]

For 6p22.3-22.2, we considered those variants that were homozygous in IV:10, and heterozygous among other members sharing the critical haplotype. Among the 45 variants found in the region, one variant, NM_006366.2:c.-93G>A, residing in the 5'-UTR of *CAP2* (*Cyclase associated actin cytoskeleton regulatory protein 2*), was found to meet these criteria. Variant c.-93G>A is present among 10 affected members in the family (Figure 3 and Figure 4A). Two-point LOD score for c.-93G>A was 1.67. This variant has been deposited in dbSNP144 with no information about its frequency (rs910586667) while a frequency of 0.00002 is reported in TOPMED. Its frequency has not been reported in ExAC, 1000 Genomes, EVS or gnomAD. It is absent in the reference set of 576 control chromosomes mentioned earlier.

Discussion

We describe results of a genetic analysis of a BPD family from south India. Two-point linkage analysis was carried out in the initial stage of the analysis, followed by exome-based analysis. Our observations suggest potential involvement of NM_181712.4:c.2461G>T (*KANK4*), NM_006366.2:c.-93G>A (*CAP2*), or both, in Family 2717. These two variants segregate in the majority of affected members in the family. Neither variant was seen in the ethnically matched controls examined.

We found suggestive evidence of linkage at 1p31.1-13.2 and 6p22.3-22.2 considering an arbitrary cut-off of greater than 1.5 for LOD score. Earlier studies have reported linkage signals at 1p31.1-13.2. Rice and colleagues, reported MOD (Model LOD score) score of 1.94 under broad diagnostic model which included schizoaffective bipolar type, BPD, and recurrent major depressive disorder, for D1S1648 (101.48 cM; 1p22.2).[34] A report by Ewald et al. provided suggestive evidence at D1S216 (104.79 cM; 1p22.1) with a combined LOD score of 2.75 in two Danish Caucasian families consisting of members with bipolar disorder, single-episode mania or schizoaffective disorder, and depression.[35] Individual family analysis showed that a major proportion of the LOD score of 2.14 was generated by

This article is protected by copyright. All rights reserved

Author Manuscript

a single family (Family 4). Haplotype analysis suggested disease haplotype of 7.3 cM (9.4Mb) in Family 4. Both these reported regions, 1p22.2 and 1p22.1, overlap with the linkage peak on chromosome 1p31.1-13.2 that we have observed in this study (Figure 4C).

There are additional studies showing evidence of linkage at chromosome 6p. Genome-wide significance has been obtained in a non-parametric analysis (NPL score of 9.86, P-value <0.005) for a family from eastern Cuba with BPD and recurrent depression.[18] This linkage peak covers a region from 28.75 to 42.88 cM (13.1-22 Mb; 6p23-21.1), which overlaps with the 6p22.3-22.2 region identified in this study. A study that tested linkage to 6p24-22 in an eastern Quebec population has found LOD scores of 2.49 for D6S296 (14.07 cM; 8.8 Mb; 6p24.1) and 2.15 for D6S277 (14.61 cM; 8.5 Mb; 6p23) in a single family (Pedigree 151) using both BPD and schizophrenia in affection status.[36] This linkage peak is 2.2 Mb away from the telomeric boundary of the region at 6p22.3-22.2.

We identified one rare variant each in the critical regions 1p31.1-13.2 and 6p22.3-22.2. The 1p31.1-13.2 region harbours a missense mutation (p.D821Y) in *KANK4* which is present in seven affected members of the family and 6p22.3-22.2 harbours a 5'-UTR mutation in *CAP2* at present in 11 affected members.

KANK proteins (KANK1-KANK4) are characterized by a KANK N-terminal (KN) motif in the N-terminal region, coiled-coil motifs, and ankyrin repeats in the C-terminal region. The KN motif is known to contain a potential nuclear localization signal and a nuclear export signal.[37] *KANK4* encodes a protein with five ankyrin repeat domains, a KN motif and a coiled-coiled motif.[38] KANK4 is synthesized in brain as well as in other tissues like colon, kidney, liver, lung and skeletal muscle, and plays a role in the regulation of actin stress fibre formation.[37] Recently, a homozygous mutation, c.2401T>C (p.Y801H) in *KANK4* has been identified in a European-origin family with steroid-resistant nephrotic syndrome (SRNS) with two of its affected members manifesting Intellectual disability.[39] KANK1, a paralog of KANK4, has been implicated in cerebral palsy, spastic quadriplegia-2 [40] and also in SRNS.[39]

The other gene of interest, *CAP2*, encodes one of the cyclase-associated proteins (CAPs), a family of conserved actin monomer binding proteins found in eukaryotes and known to be involved in regulating actin dynamics.[41] CAP was originally identified in yeast as an adenylyl-cyclase-associated protein that is involved in Ras-

This article is protected by copyright. All rights reserved

Author Manuscript

cAMP signalling and plays a role in nutritional responses and actin distribution, and is required for normal cellular morphology.[42] In mammals, CAP2 is predominantly synthesized in testis, brain, heart, skin, cardiac muscle and skeletal muscle.[43] At the subcellular level, CAP2 is found in the cytoplasm and, interestingly, also in the nucleus .[43] Loss of function of CAP2 in mice leads to microphthalmia and cardiac disease including cardiac conduction disorder.[44] CAP2 has been shown to be involved in neuronal development and neuronal actin dynamics.[45] We propose screening *KANK4* and *CAP2* in additional BPD families and functional evaluation of the variants identified in the family studied here.

Author Manuscript

References

1. Merikangas KR, Jin R, He JP, et al. Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. *Arch Gen Psychiatry*. 2011;68:241-251.

2. Grande I, Berk M, Birmaher B, Vieta E. Bipolar disorder. *Lancet*. 2016;387:1561-1572.

3. Ferrari AJ, Stockings E, Khoo JP, et al. The revalence and burden of bipolar disorder: findings from the Global Burden of Disease Study 2013. *Bipolar Disord*. 2016;18:440-450.

4. Shih RA, Belmonte PL, Zandi PP. A review of the evidence from family, twin and adoption studies for a genetic contribution to adult psychiatric disorders. *Int Rev Psychiatry*. 2004;16:260-283.

5. Serretti A, Mandelli L. The genetics of bipolar disorder: genome 'hot regions,' genes, new potential candidates and future directions. *Mol Psychiatry*. 2008;13:742-771.

6. Kerner B. Genetics of bipolar disorder. *Appl Clin Genet*. 2014;7:33-42.

7. Goes FS, Pirooznia M, Parla JS, et al. Exome Sequencing of Familial Bipolar Disorder. *JAMA Psychiatry.* 2016; **73**: 590-597.

8. Barnett JH, Smoller JW. The Genetics of bipolar disorder. *Neuroscience*. 2009;164:331-343.

This article is protected by copyright. All rights reserved

9. Kohn Y. Focus on rare genetic variants in bipolar disorder: how outliers help understand complex disorders. *Int J Europsychopharmacol.* 2005;8:491-493.

10. Blackwood DH, He L, Morris SW, et al. A locus for bipolar affective disorder on chromosome 4p. *Nat Genet*. 1996;12:427-430.

11. Adams LJ, Mitchell PB, Fielder SL, Rosso A, Donald JA, Schofield PR. A Susceptibility Locus for Bipolar Affective Disorder on Chromosome 4q35. *Am J Hum Genet*. 1998;2:1084-1091.

12. Jones I, Hamshere M, Nangle JM, et al. Bipolar affective puerperal psychosis: genomewide significant evidence for linkage to chromosome 16. *Am J Psychiatry.* 2007;164:1099-1104.

13. Badenhop RF, Moses MJ, Scimone A, et al. A genome screen of a large bipolar affective disorder pedigree supports evidence for a susceptibility locus on chromosome 13q. *Mol Psychiatry*. 2001;6:396-403.

14. Radhakrishna U, Senol S, Herken H, et al. An apparently dominant bipolar affective disorder (BPAD) locus on chromosome 20p11.2–q11.2 in a large Turkish pedigree. *Eur J Hum Genet*. 2001;9:39-44.

15. Pekkarinen P, Terwilliger J, Bredbacka PE, Lonnqvist J, Peltonen L. Evidence of a predisposing locus to bipolar disorder on Xq24-q27.1 in an extended Finnish pedigree. *Genome Res*. 1995;5:105-115.

16. Morissette J, Villeneuve A, Bordeleau L, et al. Genome-wide search for linkage of bipolar affective disorders in a very large pedigree derived from a homogeneous population in Quebec points to a locus of major effect on chromosome 12q23-q24. *Am J Med Genet*. 1999;88:567-587.

17. Shink E, Morissette J, Barden N. Genetic heterogeneity in a very large bipolar affective disorder pedigree from Quebec. *Am J Med Genet B Neuropsychiatr Genet*. 2003;119B:65-68.

18. Marcheco-Teruel B, Flint TJ, Wikman FP, et al. A genome-wide linkage search for bipolar disorder susceptibility loci in a large and complex pedigree from the Eastern part of Cuba. *Am J Med Genet B Neuropsychiatr Genet*. 2006;141B:833-843.

Author Manuscript

This article is protected by copyright. All rights reserved

19. Kaneva R, Milanova V, Angelicheva D, et al. Bipolar disorder in the Bulgarian Gypsies: genetic heterogeneity in a young founder population. *Am J Med Genet B Neuropsychiatr Genet*. 2009;150B:191-201.
20. Collins AL, Kim Y, Szatkiewicz JP, et al. Identifying bipolar disorder susceptibility loci in a densely affected pedigree. *Mol Psychiatry*. 2013;18:1245-1246.
21. Georgi B, Craig D, Kember RL, et al. Genomic view of bipolar disorder revealed by whole genome sequencing in a genetic isolate. *PLoS Genet*. 2014;10:e1004229.
22. Mateus Jose Abdalla Diniz, Andiara Calado Saloma Rodrigues, Ary Gadelha, et al. Whole genome linkage analysis in a large Brazilian multigenerational family reveals distinct linkage signals for bipolar disorder and depression. *bioRxiv* 106260;doi: https://doi.org/10.1101/106260.
23. Nurnberger JI Jr, Blehar MC, Kaufmann CA, et al. Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH Genetics Initiative. *Arch. Gen. Psychiatry* 1994;51, 849-859.
24. Maxwell ME. Family Interview for Genetic Studies (FIGS): a manual for FIGS. Clinical Neurogenetics Branch, Intramural Research Program, National Institute of Mental Health, Bethesda, MD. 1992.

25. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;16:1215.

26. Lindner TH, Hoffmann K. easyLINKAGE: A PERL script for easy and automated two-multipoint linkage analyses. *Bioinformatics*. 2005;21:405-407.
27. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25:1754-1760.

28. Li H, Handsaker B, Wysoker A, Fennell T, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009;25:2078-2079.

29. Cingolani P, Platts A, Wang le L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. *Fly (Austin)*. 2012;6:80-92.

30. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc*. 2009;4:1073-1081.

This article is protected by copyright. All rights reserved

Author Manuscript

31. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7:248-249.

32. Schwarz JM, Cooper DN, Schuelke M, Seelow D. **MutationTaster2: mutation prediction for the deep-sequencing age.** *Nat Methods*. 2014;11:361-362.

33. Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*. 2015;31:2745-2747.

34. Rice JP, Goate A, Williams JT, et al. Initial genome scan of the NIMH genetics initiative bipolar pedigrees: chromosomes 1, 6, 8, 10, and 12. *Am J Med Genet.*1997;74:247-253.

35. Ewald H, Flint T, Kruse TA, Mors O. A genome-wide scan shows significant linkage between bipolar disorder and chromosome 12q24.3 and suggestive linkage to chromosomes 1p22-21, 4p16, 6q14-22, 10q26 and 16p13.3. *Mol Psychiatry*. 2002;7:734-744.

36. Maziade M, Bissonnette L, Rouillard E, et al. 6p24-22 region and major psychoses in the Eastern Quebec population. Le Groupe IREP. *Am J Med Genet*. 1997;74:311-318.

37. Zhu Y, Kakinuma N, Wang Y, Kiyama R. Kank proteins: A new family of ankyrin-repeat domain-containing proteins. *Biochim Biophys Acta*. 2008;1780:128-133.

38. Kakinuma N, Roy BC, Zhu Y, Wang Y, Kiyama R. Kank regulates RhoA-dependent formation of actin stress fibers and cell migration via 14-3-3 in PI3K-Akt signaling. *J Cell Biol*. 2008;181:537-549.

39. Gee HY, Zhang F, Ashraf S, et al. KANK deficiency leads to podocyte dysfunction and nephrotic syndrome. *J Clin Invest*. 2015;125:2375-2384.

40. Lerer I, Sagi M, Meiner V, Cohen T, Zlotogora J, Abeliovich D. **Deletion of the ANKRD15 gene at 9p24.3 causes parent-of-origin-dependent inheritance of familial cerebral palsy.** *Hum Mol Genet*. 2005;14:3911-3920.

41. Hubberstey AV, Mottillo EP. Cyclase-associated proteins: CAPacity for linking signal transduction and actin polymerization. *FASEB J*. 2002;16:487-499.

This article is protected by copyright. All rights reserved

Author Manuscript

42. Shima F, Okada T, Kido M, et al. Association of yeast adenylyl cyclase with cyclase-associated protein CAP forms a second Ras-binding site which mediates its Ras-dependent activation. *Mol Cell Biol*. 2000;20:26-33.

43. Peche VS, Holak TA, Burgute BD, et al. Ablation of cyclase-associated protein 2 (CAP2) leads to cardiomyopathy. *Cell Mol Life Sci*. 2013;70:527-543.

44. Field J, Vojtek A, Ballester R, et al. Cloning and characterization of CAP, the *S. cerevisiae* gene encoding the 70 kd adenylyl cyclase-associated protein. *Cell*. 1990;61:319-327.

45. Kumar A, Paeger L, Kosmas K, Kloppenburg P, Noegel AA, Peche VS. Neuronal Actin Dynamics, Spine Density and Neuronal Dendritic Complexity Are Regulated by CAP2. *Front Cell Neurosci*. 2016;10:180.

Author Manuscript

This article is protected by copyright. All rights reserved

Table 1 Clinical details of affected members in Family 2717.

Sample ID	AAO	AAA	Diagnosis	DM	HTN	HypoT	Seizures	DSH attempt	Suicide	Comments
I:A	20	-	BPD	No	No	No	No	No	No	One episode of depression and mania in her 20's and died at age of 75 years
II:A	-	-	BPD	No	No	No	No	Yes	Yes	Multiple episodes of mania
II:8	25	58	BPD	Yes	Yes	No	No	No	No	Multiple episodes of mania and depression
III:2	30	61	BPD	Yes	No	No	No	No	No	Multiple episodes of mania
III:3	27	61	BPD	No	No	No	No	No	No	Two episodes of mania
III:6	18	55	BPD	No	No	No	No	No	No	Episodic illness with postpartum onset, episodes are s/o mania
III:A	16	-	BPD	No	No	No	No	No	No	Episodes of mania and died due to renal failure at age of 17
III:9	54	54	MDD	No	No	No	No	Yes	Yes	One episode of depression at 54 years and committed suicide
III:11	17	50	BPD	No	No	No	No	No	No	Episodes of depression and manic polarity along with psychotic symptoms
III:12	25	48	BPD+ NDS+ ADS	No	No	No	No	No	No	Both depressive and manic episodes
III:B	-	46	BPD	No	No	No	No	No	No	Both depressive and mania episodes
III:C	-	40	BPD	No	No	No	No	No	No	Multiple episodes of mania
III:D	-	38	BPD	No	No	No	No	No	No	Multiple episodes of mania
III:E	17	38	RDD	No	No	No	No	Yes	No	Two episodes of depression
IV:A	24	24	BPD	No	No	No	No	No	No	One episode of mania
IV:B	18	-	BPD	No	No	No	No	Yes	Yes	Multiple episodes of mania, committed suicide at 21 years
IV:1	17	39	BPD	No	No	No	No	Yes	No	Both depressive and mania episodes
IV:4	17	34	BPD	No	No	No	No	No	No	Multiple episodes of mania and no depressive episodes

Author Manuscript

This article is protected by copyright. All rights reserved

IV:5	18	33	BPD	No	No	No	No	No	No	Multiple episodes of mania
IV:8	16	33	BPD	No	No	No	No	No	No	Episodes of both mania and depression
IV:10	16	32	BPD	No	No	No	No	No	No	Multiple episodes of mania

AAO- Age at Onset; AAA- Age at Assessment; DM- Diabetes Mellitus; HTN- Hypertension; *HypoT- Hypothyroidism;* DSH- *Deliberate Self Harm; BPD- Bipolar Disorder; MDD-* Major Depressive Disorder; NDS- Nicotine Dependence Syndrome; ADS- Alcohol Dependence Syndrome; RDD- Recurrent Depressive Disorder.

Figure legends:

Figure 1 Pedigree chart of Family 2717. Open symbols indicate unaffected individuals; complete-filled ones diagnosed with BPD, half-filled major depressive disorder, and grey-shaded represent recurrent depressive disorder. Crossed symbols represent deceased individuals. Double horizontal lines represent consanguineous marriage. Asterisk indicates members for whom DNA was available and Arabic-numbered individuals are those included for linkage analysis. Individuals with both, asterisk and Arabic-number were genotyped. An alphabetic label denotes individuals for whom detailed clinical evaluation could not be done, but provisional diagnosis was made from information given by other family members, except for III:E. The arrow indicates the proband.

Figure 2 Critical haplotye on chromosome 1p region. Alleles at the risk haplotype are shaded in grey. Alleles of unassigned phase are shown in parenthesis. Only the risk haplotype from the homologous chromosomes is shown in individuals carrying it. Arrows indicate key recombination events in individuals IV:1 and IV:5. Microsatellite markers with their genetic position according to Marshfield maps and Z_{max} are shown.

This article is protected by copyright. All rights reserved

Author Manuscript

Figure 3 Critical haplotype on chromosome 6p region showing the probable disease haplotype shaded in grey. Only the risk haplotype from the homologous chromosomes is shown in individuals carrying it. Arrows indicate key recombination events in individuals III:6 and IV:5. Microsatellite markers with their genetic position and Z_{max} are shown.

Figure 4 **A.** Sequence chromatograms showing heterozygous changes, c.2461G>T (p.D821Y) in *KANK4* and c.-93G>A in *CAP2*. **B.** Amino acid alignment showing evolutionary conservation of the aspartic acid residue (D) at position 821 of KANK4. **C.** Schematic representation of chromosome 1 and 6 showing relative positions of 1p31.1-13.2 and 6p22.3-22.2 loci, respectively, along with previously reported linkage signals.

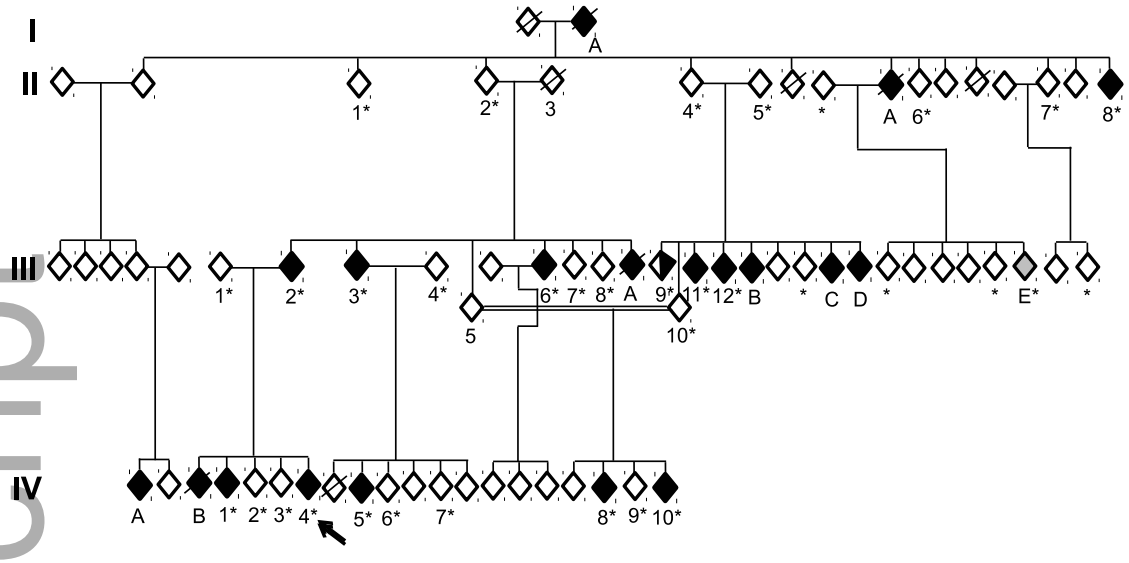
Supplementary figure 1: Genome-wide parametric two-point LOD scores for the family analysed.

Supplementary figure 2: A graphical representation of parametric multipoint LOD scores across the genome for Family 2717.

Author Manuscript

This article is protected by copyright. All rights reserved

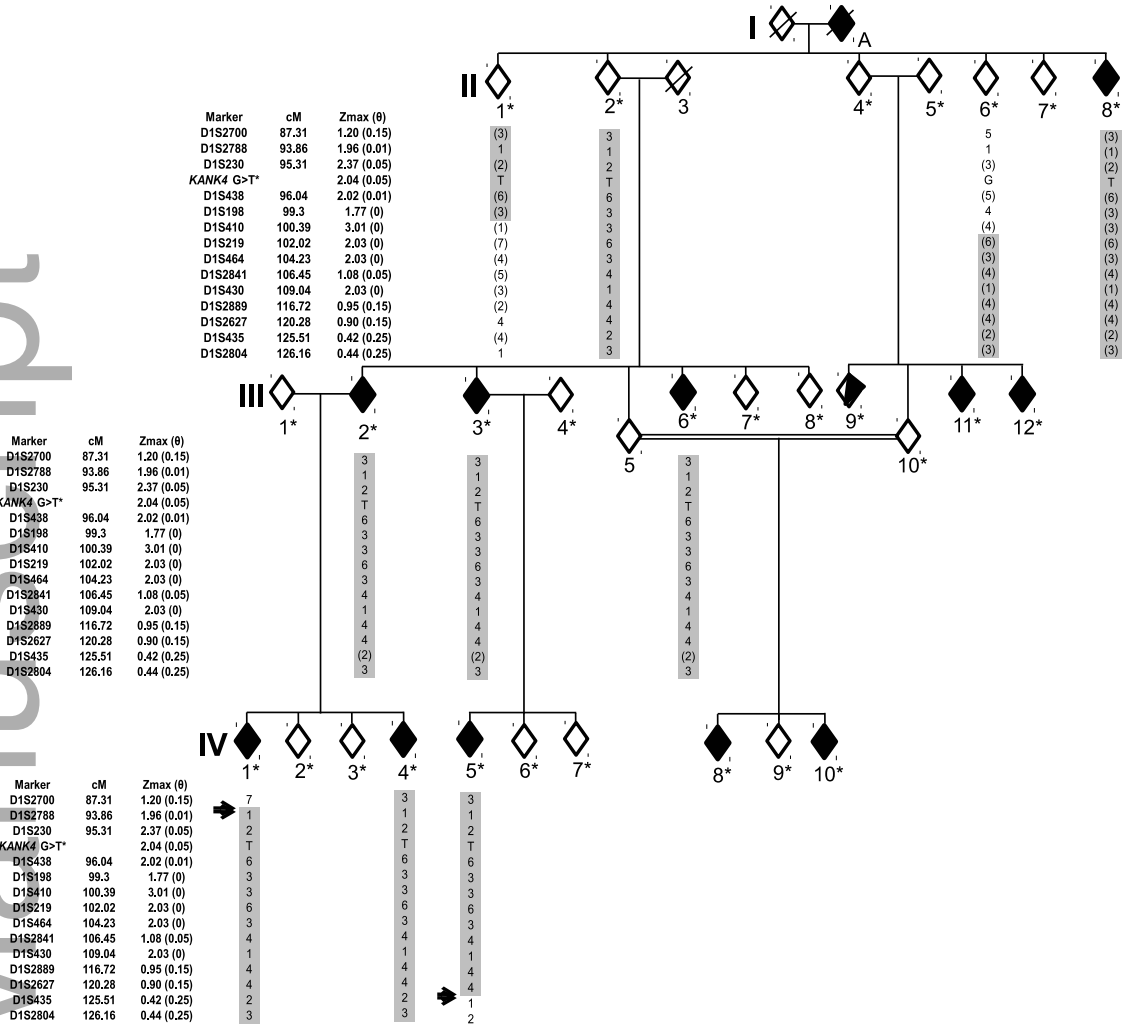
Author Manuscript



bdi_12815_f1.eps

This article is protected by copyright. All rights reserved

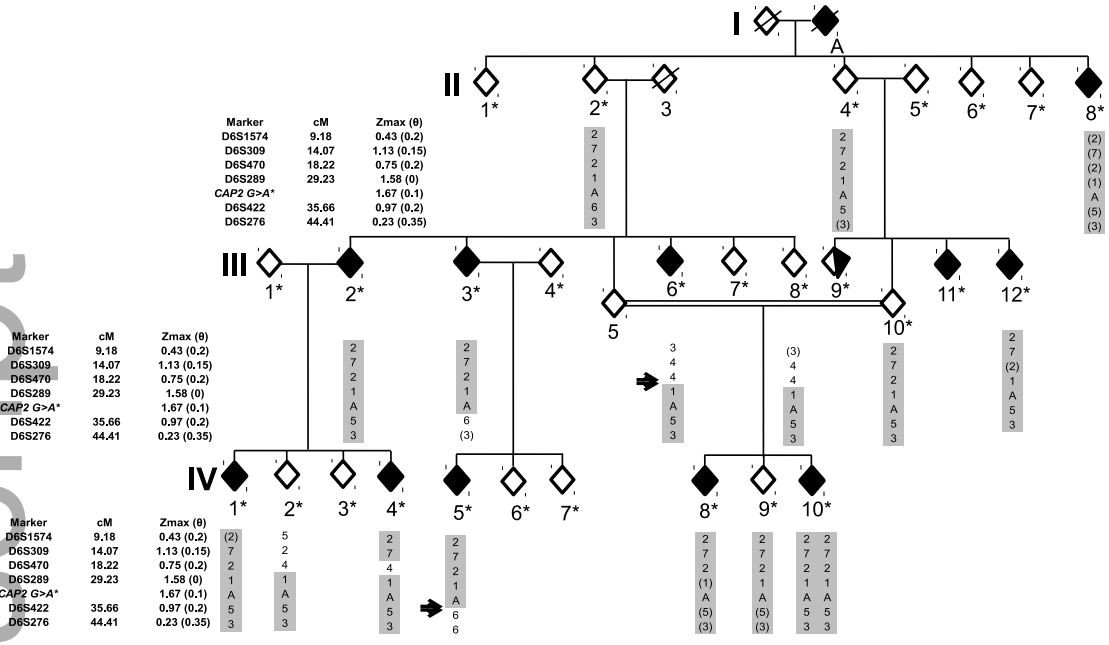
Author Manuscript



bdi_12815_f2.eps

This article is protected by copyright. All rights reserved

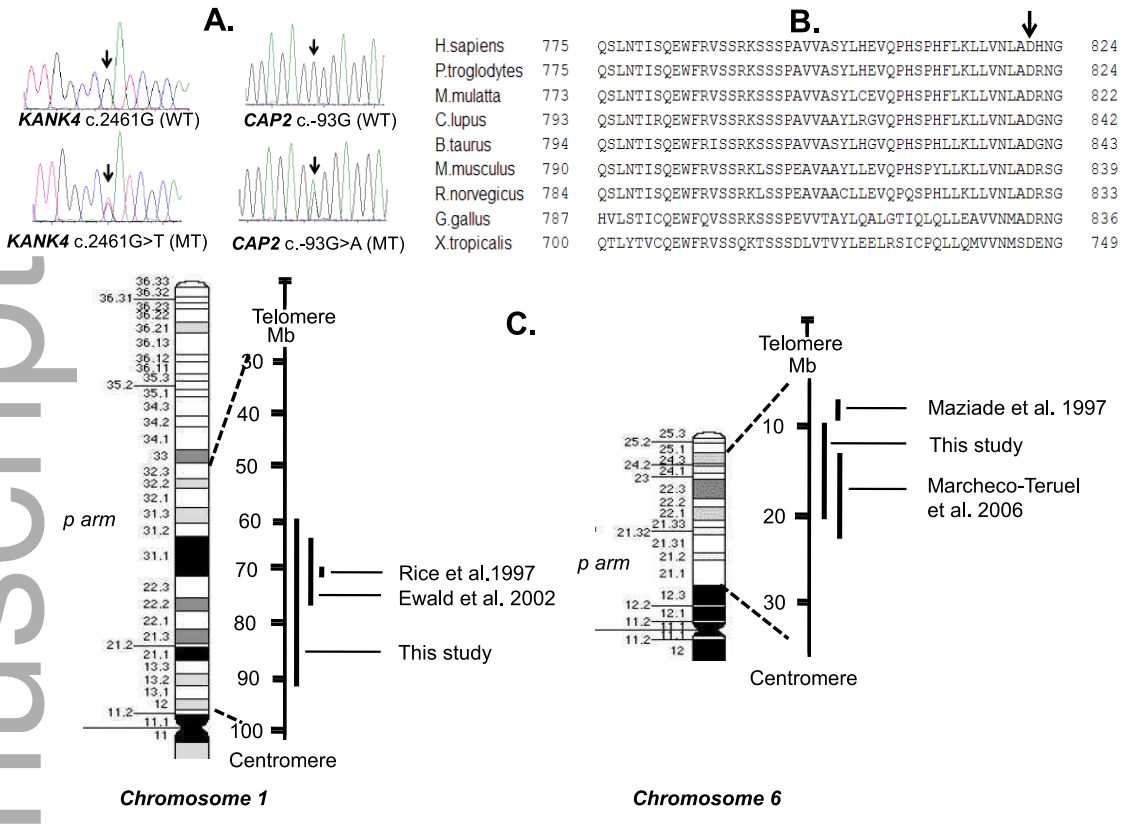
Author Manuscript



bdi_12815_f3.eps

This article is protected by copyright. All rights reserved

Author Manuscript



bdi_12815_f4.eps

This article is protected by copyright. All rights reserved

University Library

A gateway to Melbourne's research publications

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Anjanappa, RM;Nayak, S;Moily, NS;Manduva, V;Nadella, RK;Viswanath, B;Reddy, YCJ;Jain, S;Anand, A

Title:
A linkage and exome study implicates rare variants of KANK4 and CAP2 in bipolar disorder in a multiplex family

Date:
2019-08-21

Citation:
Anjanappa, R. M., Nayak, S., Moily, N. S., Manduva, V., Nadella, R. K., Viswanath, B., Reddy, Y. C. J., Jain, S. & Anand, A. (2019). A linkage and exome study implicates rare variants of KANK4 and CAP2 in bipolar disorder in a multiplex family. BIPOLAR DISORDERS, 22 (1), pp.70-78. https://doi.org/10.1111/bdi.12815.

Persistent Link:
http://hdl.handle.net/11343/286320