Ankyrin 3 Genetic Association Studies of Bipolar Disorders

Wade Berrettini, MD, PhD
The Karl E. Rickels Professor of Psychiatry and Director of the Center for Neurobiology and Behavior,
Department of Psychiatry
University of Pennsylvania
215-898-0092; wadeb@mail.med.upenn.edu
Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder


Genome-wide statistical significance was obtained for SNPs in two genes, ANK3 (ankyrin 3) and CACNA1C, an L-type calcium channel subunit gene. The SNPs do not obviously change gene/protein function. Confirmation for ANK3 was obtained in two independent samples.
rs7098008  
\[ p = 0.0084 \]

CHR 10: 61,500,000  61,700,000  61,900,000  62,100,000

rs4582919  
rs7910492

rs10994357  
rs7903441  
\[ p = 0.00038 \]

rs1934795  
\[ p = 0.0077 \]

p = 2 \times 10^{-9} for a haplotype of the 3 blue SNPs (Ferreira et al, 2008); red SNPs with nominal significance in GAIN EA BPD sample (Smith); black SNP \( p = 0.0001 \) in 745 German cases and 830 controls (Schulze)

Schulze rs9804160 = 3 \times 10^{-6}

rs10994336  
Brain-specific isoforms

Fig 1: ANK3 SNPs in LD with Bipolar Disorder

Serine-rich brain-specific exon
ANK3: A Spectrum of Associated Alleles

- When multiple alleles (in several haplotype blocks) are associated with a phenotype, at high levels of statistical significance, so that the results are not likely to be false positive, then the most probable explanation is multiple uncommon alleles in the implicated gene.

- Many of these alleles may be absent from the databases because they are rare, but they may have large effects on disease risk (examples of BRCA1 and BRCA2).
ANK3 Serine-rich Domain

A single exon encodes this ~ 2500 AA fragment of the ANK3 protein. The 5’ end of this domain is shown below.

It is unique in the genome, with very little homology to the other two ANK genes. Its function is unknown; its expression is brain-specific.

1501 RPYQSWTTAPITVPGPASKGFTSLSSSSSNTPSASPLKSIWSTSPSPIKSTLGASTTSS
1561 VKSISDVASPISRFTMSSPIKTVVSQPYNIQVSSGTLARAPAVTEATPLKGLASNSTF
1621 SSRTSPVTAGSLLERSSITMTPPASPKNINMYSSLPLFKSIITSAAPLIISSPLKSVVS
1681 PVKSAVDVIASSAKITMASSLSSPVKQMPGHAEEAVNGSIGSPLKYPSSSTLINGCKATAT
1741 LQEKSATNSSVSSVSSAATDTVEKVFSTTAMPFSPLRSYVSAAPSAFQLRTPSASAL
1801 YTSGLSSISATTSSVTSSITVPVYSVVNLPEPALKKLPDSSFTKSAAALLSPIKTLT
1861 TETHPQPFHFSRTSSPVKSSLFLAPSALKLSTPSSLSSQEIJKDVAEMKEDLMRMTAILQ
Sequencing of ANK3 SRD Exon

Focus on SRD because it is a brain-specific exon with unique sequence in the genome.

BPD is a brain-specific disease. Affected individuals do not have symptoms referable to any other organ system apart from the CNS.

DNA from ~400 BPI individuals used to amplify the 10 kb SRD exon, then 16 pools, each containing 24 DNAs, were constructed to take advantage of bar-coded PCR primers, using ABI SOLiD sequencing.
SOLiD Workflow

**RNA**
- Application specific sample preparation

**DNA**
- Emulsion PCR & substrate preparation

**Sequencing chemistry**

**Imaging & basecalling**
- Application specific Data analysis
ANK3 SRD SAMPLE PREPARATION FOR SOLiD SEQUENCING

Long Distance PCR using SequalPrep LD PCR Kit (non-biotinylated primers)

Check amplicon purity by gel electrophoresis
  if “clean”, then ExoSAPit to get rid of primers, dNTPs
  if “extra bands”, then gel purify SRD amplicon

Further clean ExoSAPit’d amplicons by PureLink columns

Quantify amplicons by Pico-Green Quant-it Assay with fluorescent plate reader

Make 16 Pools of 25 ng/individual X 24 individuals = 600 ng total/pool for 384 individuals

Vacuum dry each 600 ng of pooled amplicon DNA (to adjust volume for sonication)

Resuspend pooled amplicon DNA in 100 µl nuclease-free water

Proceed to Library Construction
Create library of DNA fragments: 2 methods for DNA

Fragment Library (directed resequencing, ChIP-Seq, …)

Mate Pair Library (whole genome sequencing, structural variation)
Fragment library

Complex sample
- e.g. Genomic DNA, TAG library, Concatenated PCR products

Fragment sample, Randomly or Targeted
- e.g. sonication, mechanical, enzymatic digestion

Ligate P1 and P2 Adaptors

P1 adapter + DNA fragment + P2 adapter

~110 Bases
Multiplex Libraries

Pool 1
- 1µm bead
- P1 adapter
- DNA fragment
- Internal Adapter
- Barcode 1
- P2 adapter

Pool 2
- 1µm bead
- P1 adapter
- DNA fragment
- Internal Adapter
- Barcode 2
- P2 adapter

Pool 16
- 1µm bead
- P1 adapter
- DNA fragment
- Internal Adapter
- Barcode 16
- P2 adapter

10 bases
5 are read
Quantification and pooling of barcoded ANK3 SRD pools

Agilent chip to check for purity and rough fragment size estimates

Quantify DNA with Quant-it High Sensitivity dsDNA Assay

Pooled 40 ng of each barcoded pool into “mega”-pool

Size selected barcoded DNA fragments of 150-300 bp length by gel electrophoresis

Gel purified pooled libraries for use in emPCR
Emulsion PCR (emPCR): Amplification of the Library Fragments onto beads

- Use PCR to amplify library fragments using P1 oligos
  - This attaches templates (library fragments) to the beads

- Beads containing templates will be used for sequencing process on Instrument
  - Sequence on each bead will be read as an independent sequence or read
  - Each read will be matched to the reference sequence
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Mis-sense T1861M in ANK3
Sequence read for individual 00C01859 with rare variant T1861M (1000 Genomes)
PolyPhen-2 report for Q12955 T1861M

Query

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Results

+ Prediction/Confidence

HumDiv

This mutation is predicted to be **POSSIBLY DAMAGING** with a score of **0.628** (sensitivity: **0.85**, specificity: **0.88**)

HumVar

This mutation is predicted to be **POSSIBLY DAMAGING** with a score of **0.221** (sensitivity: **0.89**, specificity: **0.65**)

Details

- Multiple sequence alignment

  UniProtKB/UniRef100 Release 2010_11 (02-Nov-2010)
Deletion of E1926 in ANK3 SRD

Sequence read for rare variant $\Delta$E1926 in proband 90C02533
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**Prediction**

**This variant is predicted to be benign**

<table>
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<th>Prediction</th>
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<th>Substitution effect</th>
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**Details**

**SEQUENCE FEATURES OF THE SUBSTITUTION SITE**

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**PSIC PROFILE SCORES FOR TWO AMINO ACID VARIANTS**

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<th>Diagnostics</th>
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**MAPPING OF THE SUBSTITUTION SITE TO KNOWN PROTEIN 3D STRUCTURES**

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**Fragment of multiple alignment around position 1926:**

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0
1 ref[XF_657919_1] PREDICTED: similar to ankyrin 3 isoform 1 isofo...
2 ref[XP_001372192_1] PREDICTED: similar to ankyrin 3 (Monodelphis...  
3 ref[XP_421546_2] PREDICTED: similar to ankyrin 3 (Gallus gallus)
4 ref[XP_658171_1] PREDICTED: similar to ankyrin 3 isoform 1 isofo...
5 ref[XP_698324_2] PREDICTED: similar to ankyrin 3 (Danio rerio)
6 ref[XP_001029156_1] ankyrin 3, epithelial isoform 2 (Rattus norv...
7 ref[XP_700106_2] PREDICTED: similar to ankyrin 3 (Danio rerio)
8 ref[XP_001324644_1] PREDICTED: similar to ankyrin 3 (Danio rerio)
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```
Mis-sense S2043N in ANK3 SRD

Sequence read for individual 01C06617 with rare variant S2043N
PolyPhen-2 report for Q12955 S2043N

Query

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Results

+ Prediction/Confidence

HumDiv

This mutation is predicted to be **POSSIBLY DAMAGING** with a score of **0.377** (sensitivity: **0.88**, specificity: **0.86**)

HumVar

This mutation is predicted to be **POSSIBLY DAMAGING** with a score of **0.277** (sensitivity: **0.88**, specificity: **0.67**)

Details

- Multiple sequence alignment

QUERY

| sp|UP100017P055A#1| ASQRSPLERFYQVEKAASEEEDYMTEKIDYLTNEIGS SSLTELKTEFEAK----DGEEFQRKVLKFAALQCB |
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| sp|UP1000001390A#1| ASHAPSPLERFYQVEKAASEEEDYMTEKIDYLTNEIGS SSLTELKTEFEAK----DGEEFQRKVLKFAALQCB |
| sp|B5x757#1| T-----HAFHKVE1KTTRLSEKDYMTEKIDYLTNEIGS SSLTELKTEFEAK----DGEEFQRKVLKFAALQCB |
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| sp|UP100001CFA35A#1| STYPRTYVEPQVKEKASDEKPLKVKVDTYLTNEIGS SSLSKIAAEKKPTEAREGKDGEEFQRKVLKFAALQCB |
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| sp|UP100000S206C3#1| ------------------------AKSKSPDEDEKPLKVKVDTYLTNEIGS SSLSKIAAEKKPTEAREGKGEEFQRKVLKFAALQCB |
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UnProtKB/UnRef100 Release 2010_11 (02-Nov-2010)
Mis-sense D2319N in ANK3 SRD

Two BPI probands have the same rare variant: D2319N
PolyPhen-2 report for Q12955 D2319N

Query

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Results

**HumDiv**

This mutation is predicted to be **POSSIBLY DAMAGING** with a score of **0.794** (sensitivity: **0.82**, specificity: **0.89**).

**HumVar**

This mutation is predicted to be **POSSIBLY DAMAGING** with a score of **0.538** (sensitivity: **0.62**, specificity: **0.75**).

Details

- Multiple sequence alignment

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UniProtKB/UniRef100 Release 2010_11 (02-Nov-2010)
Mis-sense F2375V in ANK3 SRD

Sequence for Rare Variant F2375V in Proband 90C04288
### Query

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### Results

#### Prediction/Confidence

**HumDiv**

This mutation is predicted to be **BENIGN** with a score of **0.004** (sensitivity: **0.97**; specificity: **0.56**)

#### HumVar

This mutation is predicted to be **BENIGN** with a score of **0.017** (sensitivity: **0.96**; specificity: **0.44**)

### Details

#### Multiple sequence alignment

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|-------|----------|---------------|---------------|-------------|
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*UniProtKB/UnRef100 Release 2010_11 (02-Nov-2010)*
Mis-sense S2409P in ANK3 SRD

RFLP validation for proband 90C03337 with rare variant S2409P
Mis-sense N2643S in ANK3 SRD

Sequence reads for individual 00C02088 with rare variant N2643S
Mis-sense R2719T in ANK3 SRD

Sequence trace for proband 90C04387 with rare variant R2719T
PolyPhen-2 report for Q12955 R2719T

Query

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Results

+++ Prediction/Confidence

HumDiv

This mutation is predicted to be **PROBABLY DAMAGING** with a score of **0.889** (sensitivity: **0.79**, specificity: **0.91**)

HumVar

This mutation is predicted to be **POSSIBLY DAMAGING** with a score of **0.783** (sensitivity: **0.76**, specificity: **0.80**)

Details

- Multiple sequence alignment: UniProtKB/UniRef100 Release 2010_11 (02-Nov-2010)
Mis-sense S3521G in ANK3 SRD

Sequence for rare variant S3521G in proband 90C02533
PolyPhen-2 report for Q12955 S3521G

Query

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Results

Prediction/Confidence

HumDiv

This mutation is predicted to be POSSIBLY DAMAGING with a score of 0.632 (sensitivity: 0.85, specificity: 0.88)

HumVar

This mutation is predicted to be POSSIBLY DAMAGING with a score of 0.367 (sensitivity: 0.86, specificity: 0.70)

Details

Multiple sequence alignment

UniProtKB/UniRef100 Release 2010_11 (02-Nov-2010)
Mis-sense E3563G in ANK3 SRD.
Sequence read for proband 02C09444 with rare variant E3563G.
PolyPhen-2 report for Q12955 E3563G

Query

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Results

Prediction/Confidence

HumDiv

This mutation is predicted to be **BENIGN** with a score of **0.670** (sensitivity: **0.93**; specificity: **0.80**)

HumVar

This mutation is predicted to be **BENIGN** with a score of **0.082** (sensitivity: **0.93**; specificity: **0.57**)

Details

Multiple sequence alignment

QUERY

sp | UP100017FC55A1 | sp | UP1000028G93A1 | sp | UP1000228C64A1 | sp | UP1000088257F1 | sp | UP100008EB66D1 | sp | UP10001A2DE8E1


UniProtKB/UniRef100 Release 2010_11 (02-Nov-2010)
Mis-sense S3697C in ANK3 SRD

Sequence read for proband 90C03337 with rare variant S3697C
PolyPhen-2 report for Q12955 S3697C

Query

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Results

**Prediction/Confidence**

**HumDiv**

This mutation is predicted to be **BENIGN** with a score of 0.011 (sensitivity: 0.96, specificity: 0.73)

**HumVar**

This mutation is predicted to be **BENIGN** with a score of 0.030 (sensitivity: 0.95, specificity: 0.48)

Details

**Multiple sequence alignment**

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Mis-sense N3789S in ANK3 SRD

Sequence read for individual 90C04292 with rare variant N3789S
PolyPhen-2 report for Q12955 N3789S

Query

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Results

+ Prediction/Confidence

HumDiv

This mutation is predicted to be **POSSIBLY DAMAGING** with a score of 0.814 (sensitivity: 0.81; specificity: 0.90)

HumVar

This mutation is predicted to be **POSSIBLY DAMAGING** with a score of 0.684 (sensitivity: 0.79; specificity: 0.78)

Details

- Multiple sequence alignment

QUERY

sp|UP100017P055A#1 GQGLENETYVIASNTASLSGFPQKHEKPQKDPFDPPHH MNNLDSSSTTVTDMNSNVLTETHSAPCTCTKEKDFVVEYS
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UniProtKB/UniRef100 Release 2010_11 (02-Nov-2010)
Mis-sense K3942R in ANK3 SRD

Sequence trace of rare variant K3942R in proband 90C00165
PolyPhen-2 report for Q12955 K3942R

Query

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Results

**Prediction/Confidence**

**HumDiv**

This mutation is predicted to be **BENIGN** with a score of **0.000** (sensitivity: **1.00**, specificity: **0.00**)

**HumVar**

This mutation is predicted to be **BENIGN** with a score of **0.002** (sensitivity: **0.99**, specificity: **0.14**)

Details

**Multiple sequence alignment**

UniProtKB/UniRef100 Release 2010_11 (02-Nov-2010)
Mis-sense I3117V in ANK3 SRD

Sequence trace of SNP rs28932171: I3117V
Mis-sense K3123R in ANK3 SRD

Sequence trace of SNP rs10821668: K3123R
Mis-sense T3046S in ANK3 SRD

Sequence trace of SNP rs74153183: T3046S
Mis-sense Q2996H in ANK3 SRD

Sequence trace of SNP rs41274672: Q2996H
Mis-sense E3352G in ANK3 SRD

RFLP validation of SNP rs61845768: E3352G

Multiz Alignments of 44 Vertebrates

Simple Nucleotide Polymorphisms (dbSNP build 130)
Mis-sense T3333S in ANK3 SRD

RFLP validation for probands 02C09444 & 90C01744 with rare variant T3333S
PolyPhen-2 report for Q12955 T3333S

Query

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Results

**HumDiv**

This mutation is predicted to be **POSSIBLY DAMAGING** with a score of 0.814 (sensitivity: 0.81; specificity: 0.90)

**HumVar**

This mutation is predicted to be **POSSIBLY DAMAGING** with a score of 0.684 (sensitivity: 0.79; specificity: 0.78)

Details

**Multiple sequence alignment**

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Other confirmed “Synonymous” variants in ANK3 SRD

Common: SOLiD detected, in databases
  C>T : S3895S : rs7923682
  A>G : Q3461Q : Seattle
  T>C : A2965A : rs10740006
  C>T : S2671S : rs41274674
  C>T : H2380H : rs3802696
  C>T : V2161V : rs3134609

Rare: SOLiD detected; NOT in databases
  C>T : A3708A
## Current Tally for SNP Calls and Validation

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### SNP Calls Validation

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