A roadmap for discovery and validation of mechanisms and targets in psychiatric disorders

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www.libd.org
PGC 2 – 70,000 subjects, over 80 GWAS “significant” loci...

How can we translate noncoding variation into mechanisms of Illness?

From: S Ripke
Noncoding variations affect gene function via diverse mechanisms, but they all read out in the transcriptome.

Mechanisms:
- splicing
- miRNA
- 5’ UTR variations
- noncoding RNA
- promoter effects
- enhancer effects
- looping
- methylation
- chromatin state

RNA sequencing in brain:
- splicing
- novel exons
- TSS’s
- UTR’s
- abundance
A roadmap for new target discovery

**Gene(s) of interest**

- RNA sequencing in brain
- Transcript associated with illness state
- Transcript associated with genetic risk

**Molecular mechanisms of association**

- Cell models based on molecular mechanisms
- Animal models based on molecular mechanisms

**CLINICAL STUDIES**
Two examples of new CNS target development and validation

1. GRM3 modulation
2. KCNH2 modulation
GRM3 (7q21.12): Genetic association with “psychosis”

Exons 1

5’

SNPs M1

M7

M2

M3

M4

M5

M6

three marker haplotype
p<.0001 in cbdb dataset*

Egan et al 2004*

Marti et al 2002

Fujii et al 2003

Falling et al 2005

Sklar et al 2008

Green et al 2006

fMRI

M4 is associated with inefficient prefrontal processing during WM in normal subjects

A allele homozygotes (N=42) >
G allele carriers (N=35)

*Egan et al Proc Natl Acad Sci (USA) 2004
PGC2: Association with GRM3
GRM3dE4 may encode a soluble receptor isoform
rs2228595 is associated with expression of GRM3dE4 in prefrontal cortex

SNPs analyzed

<table>
<thead>
<tr>
<th>SNP</th>
<th>dbSNP rs#</th>
<th>Location</th>
<th>SCZ assoc.</th>
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<tr>
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<td>intron 2</td>
<td>Egan et al, 2004</td>
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<td>6</td>
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<td>exon 3</td>
<td>Marti et al, 2002</td>
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<td>7</td>
<td>rs1468412</td>
<td>intron 3</td>
<td>Fuji et al, 2005</td>
</tr>
<tr>
<td>8</td>
<td>rs7804100</td>
<td>intron 4</td>
<td></td>
</tr>
</tbody>
</table>

ANCOVA: p=0.006

Sartorius et al Neuropsychopharm 2007
A Roadmap for Genes to Drugs

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CLINICAL STUDIES
Detection of Possible Alternative Splicing / New TSS in GRM3

R2954 (CONT, CAUC,M, 47)

R3541 (SCZ, CAUC,M, 48)

R3932 (CONT, CAUC,M, 54)

R4366 (SCZ, CAUC,M, 50)
BrainCloud is a freely-available, biologist-friendly, stand-alone application for exploring the temporal dynamics and genetic control of transcription in the human prefrontal cortex across the lifespan. BrainCloud was developed through collaboration between the Lieber Institute and NIMH.
Association with *KCNH2*: Some important details

Chromosome 7:

Resequenced Regions

- KCNH2-1A
- KCNH2-1B
- KCNH2-1C

Genotyped SNPs

- M1
- M2
- M3
- M4
- M15
- M16
- M17
- M18
- M19
- M20
- M21
- M22
- M23
- M24
- M25
- M26
- M27
- M28
- M29
- M30
- M31
- M32
- M33
- M34
- M35
- M36
- M37
- M38
- M39
- M40
- M41
- M42

Association Signal

- Custom Assay

QPCR Assays

- Hs00165120_m1

Affymetrix Probesets (U133 set)

- 205242_s_at
- 210036_s_at
- 205581_s_at
KCNH2 5’-RACE

novel exon 1
1.15 Kb extension

PCR-1

PCR-2

(Ladder)
Characterization of KCNH2 currents and firing patterns in cortical neurons expressing KCNH2-1A and Isoform 3.1

Expression of KCNH2 isoform 3.1 is increased in schizophrenic brain and is associated with risk genotype.

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CLINICAL STUDIES
Blockade of HERG human $K^+$ channels and $I_{Kr}$ of guinea-pig cardiomyocytes by the antipsychotic drug clozapine

So-Young Lee, Young-Jin Kim, Kyong-Tai Kim, Han Choe & Su-Hyun Jo

Department of Life Science, Pohang University of Science and Technology, Pohang, Korea; Department of Physiology, Research Institute for Biomacromolecules, Ulsan University College of Medicine, Seoul 138-736, Korea and Department of Physiology, Cheju National University College of Medicine, Jeju 690-756, Korea
Objective: Antidopaminergic drugs bind to hERG1 potassium channels encoded by the gene KCNH2, which accounts for the side effect of QT interval prolongation. KCNH2 has also been associated with schizophrenia risk, and risk alleles predict increased expression of a brain-selective isoform, KCNH2 3.1, that has unique physiological properties. The authors assessed whether genetic variation associated with KCNH2 3.1 expression influences the therapeutic effects of antipsychotic drugs.

Method: The authors performed a pharmacogenetic analysis of antipsychotic treatment response in patients with schizophrenia using data from two independent studies: a National Institute of Mental Health (NIMH) double-blind, placebo-controlled inpatient crossover trial (N=54) and the multicenter outpatient Clinical Antipsychotic Trials in Intervention Effectiveness (CATIE) study (N=364). The KCNH2 genotype that was previously associated with increased expression of KCNH2 3.1 in the brain was treated as a predictor variable. Treatment-associated changes in symptoms were evaluated in both groups with the Positive and Negative Syndrome Scale. The authors also analyzed time to discontinuation in the olanzapine arm of the CATIE study.

Results: In the NIMH study, individuals who were homozygous for the KCNH2 3.1 increased expression-associated T allele of rs1036145 showed significant improvement in positive symptoms, general psychopathology, and thought disturbance, while patients with other genotypes showed little change. In the CATIE study, analogous significant genotypic effects were observed. Moreover, individuals who were homozygous for the T allele at rs1036145 were one-fifth as likely to discontinue olanzapine.

Conclusions: These consistent findings in two markedly different treatment studies support the hypothesis that hERG1-mediated effects of antipsychotics may not be limited to their potential cardiovascular side effects but may also involve therapeutic actions related to the brain-specific 3.1 isoform of KCNH2.
Confirmed compounds with >10-fold difference in potency between 3.1 and 1A
Black = 3.1
Blue = 1A

80-fold separation
Risk associated alleles in *KCNH2* predict inefficient hippocampal engagement in normal subjects

Thus, this is a **biomarker of the CNS action of a KCNH2 3.1 modulator**

![Episodic memory fMRI Paradigm](image)

- N= 79, matched for demographics and performance, \( p < .05 \) FWE corrected

Huffaker et al *Nature Medicine* 2009
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