Imaging Genetics: The Example of Schizophrenia

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State of Gene Discovery Efforts

- The glass half full
  - Several replicated linkage findings (e.g., 22q11, 6p22, 8p12-21, 1q21-22, 1q42, 13q32-34, 12q24, 14q22-32)
  - Candidate genes identified in each region with varying degrees of biological plausibility (e.g., COMT, DTNBP1, NRG1, RGS4, DISC1, G72, DAAO, Akt1)
  - Some of these genes have been implicated in GWAS of rare CNVs
- The glass half empty
  - Markers and haplotypes associating with the syndrome are not consistent across studies
  - Functionally significant variants have not been identified (other than for COMT)
  - Very few (and none of these candidates) significant in GWAS of common SNPs despite large N's

Today's Talk

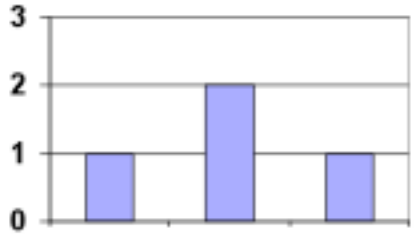
- Dissecting phenotypic & genetic issues that limit the power of *case-control* GWAS
  - What phenotype(s) do the genes encode?
  - Heterogeneity of risk alleles
- A translational strategy based on endophenotypes
  - Validation of endophenotypic traits in discordant twins
  - Association of genetic variants with endophenotypes
  - Validation of genetic associations in mutant mice
  - Examples: DISC1, Dysbindin

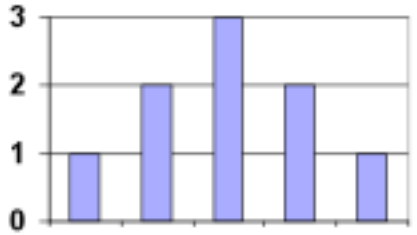
Phenotype Strategy	**Genotype Strategy**	
	Whole-Genome	Candidate Gene
Qualitative	Case-Control GWAS	Case-Control custom panel
Quantitative	QTL GWAS	QTL custom panel

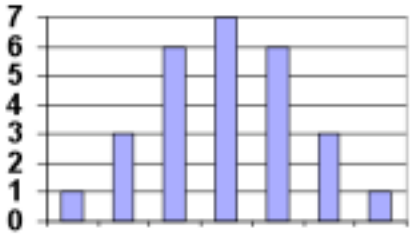
Is schizophrenia truly a *categorical phenotype*?

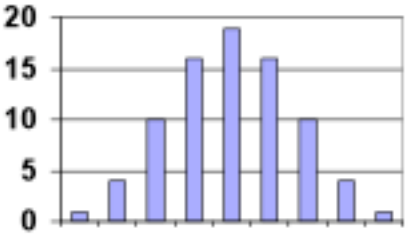
- Multiplicity of 'small effect' risk factors (genetic and environmental), and absence of 'major effect' risk factors, predicts continuum of risk

1 Gene	2 Genes	3 Genes	4 Genes
→ 3 Genotypes	→ 9 Genotypes	→ 27 Genotypes	→ 81 Genotypes
→ 3 Phenotypes	→ 5 Phenotypes	→ 7 Phenotypes	→ 9 Phenotypes



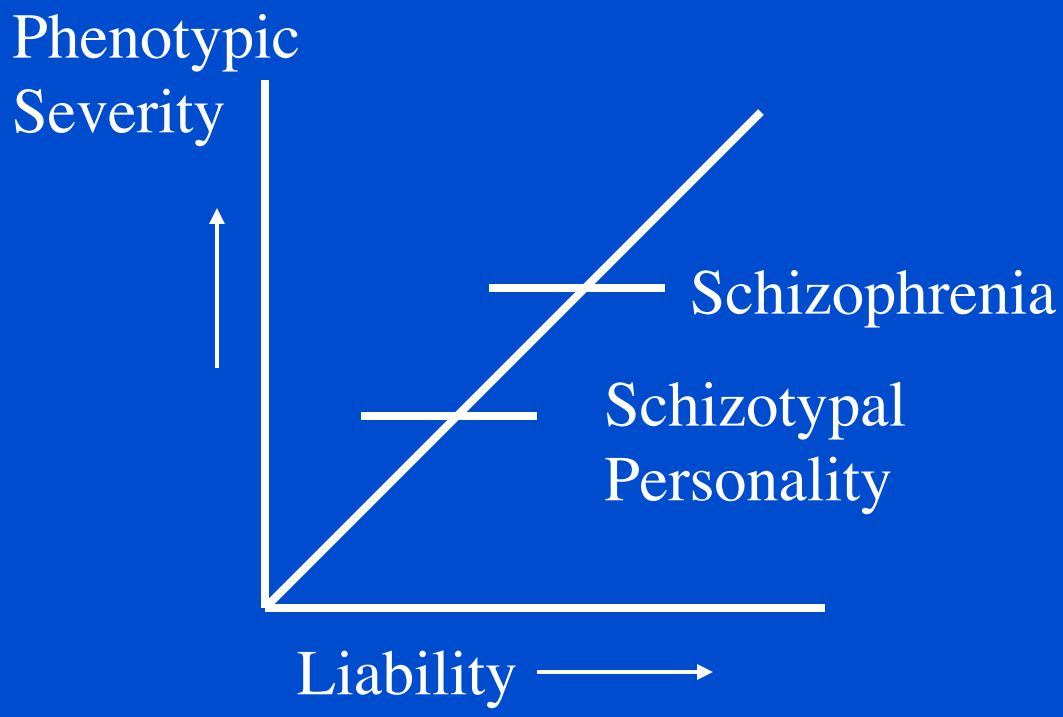






Liability-Threshold Model

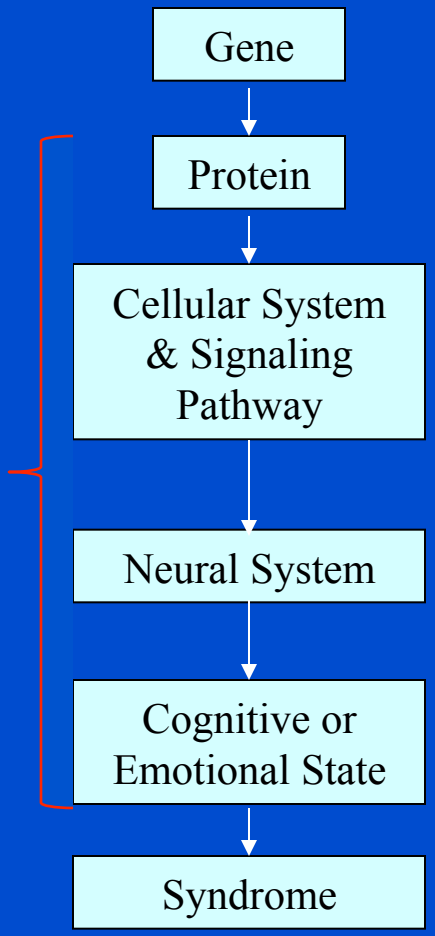
- Liability to disorder is distributed continuously in population
- Phenotypic severity derives from liability continuum
- Threshold for disorder defined on functional/ pragmatic grounds
- Sub-syndromal degrees of affection are common



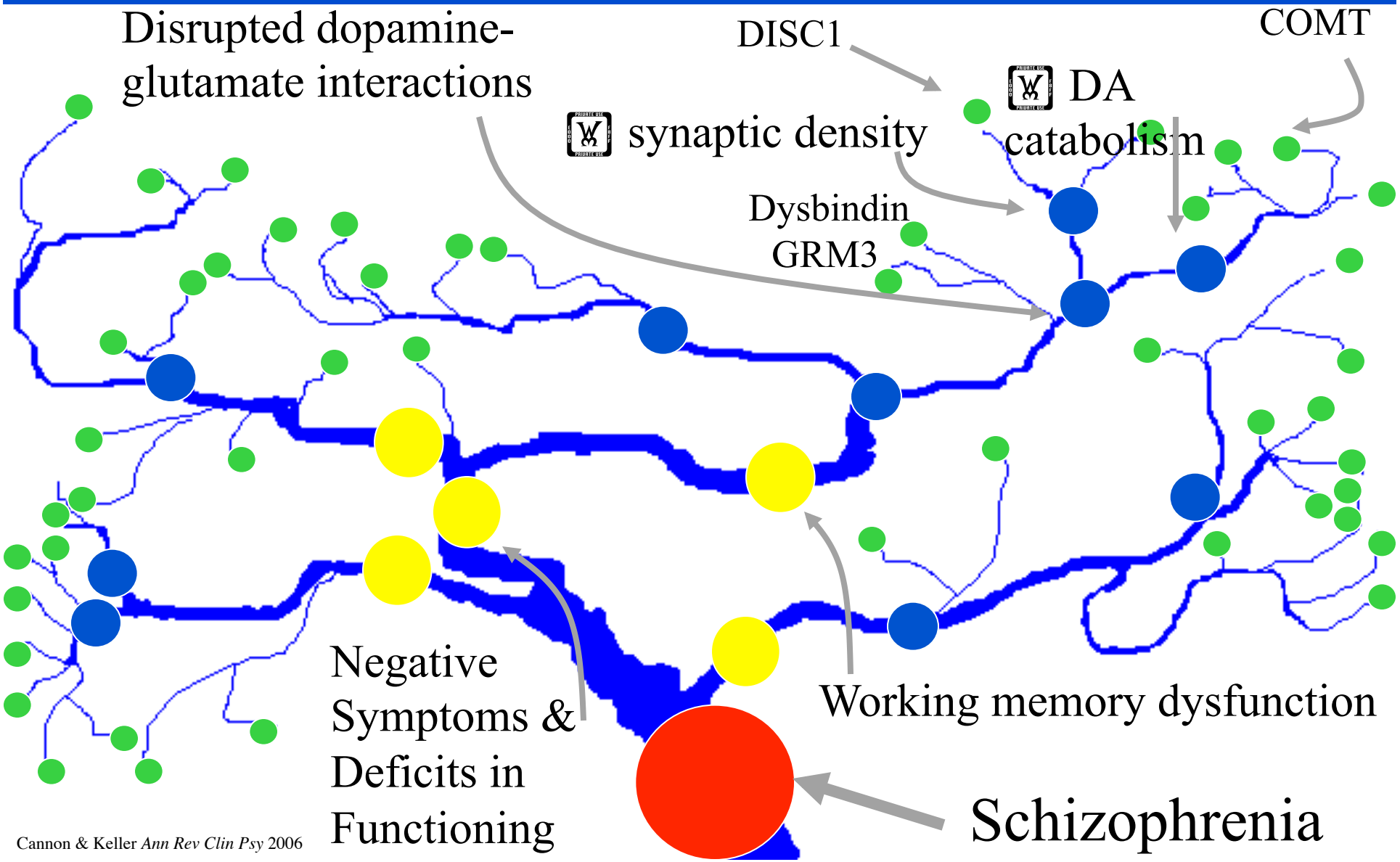
$$L_i = A_i + B_i + X_i + (A_i \times B_i) \ldots + C_i$$

What phenotype(s) do the genes that predispose to schizophrenia code for? (part 2)

- Schizophrenia does not boil down to a *single* symptom, cognitive or emotional state, neural system, cell signaling pathway, protein, or gene
- Rather, schizophrenia is a syndrome involving many different symptom combinations
- Any given case thus reflects an aggregation of disruptions in multiple neural systems, cell signaling pathways, proteins, and genes
- Though there should be some common profiles, different cases would be expected to differ in the particular combinations of neural systems, cell signaling pathways, proteins, and genes involved



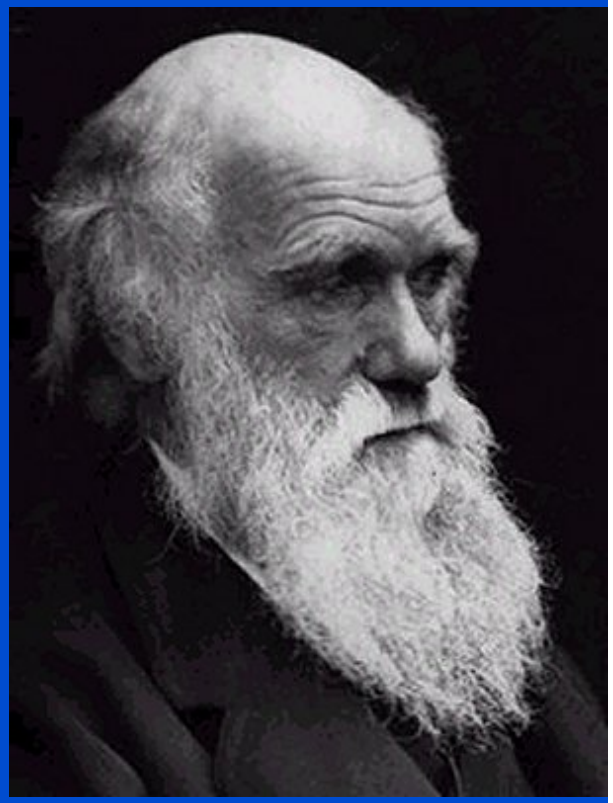
Psychiatric Genetics: Populating the branches



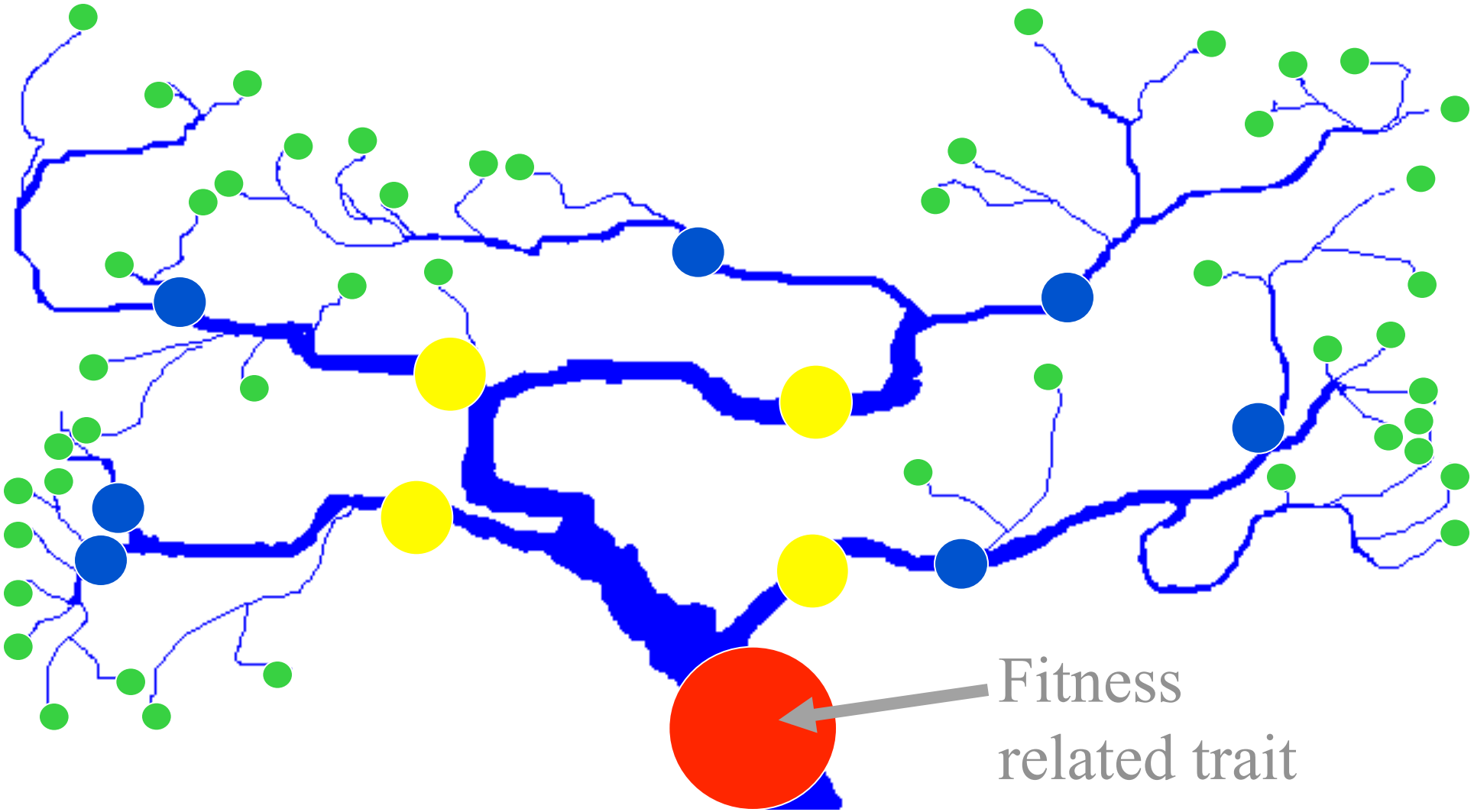
Cannon & Keller *Ann Rev Clin Psy* 2006

Evolutionary paradox of schizophrenia

- Schizophrenia is associated with reduced reproductive success, but is also maintained at relatively high prevalence in the population
  - E.g., Fertility Ratio (FR)=0.39; 95% CI=0.35-0.44 (Stahl & McCabe 2011)
- If schizophrenia decreased Darwinian fitness in ancestral environments, why hasn't natural selection eliminated the alleles that predispose to it?



Furthest downstream / more broadly defined processes



Common vs. Rare Variants

- **Common variants**
  - For diseases that are highly heritable and relatively common, genetic susceptibility is thought to involve common variations of many genes, each of small effect
  - Typically, these are measured by SNPs, reflecting single-base pair mutations
  - Of the 0.2% genetic variation in humans, .08% is by SNPs
- **Rare variants**
  - Cytogenetic abnormalities and copy number variants (CNVs) are rarer, but potentially more highly penetrant for the trait than the common variants (SNPs)
  - These involve mutations of larger segments (typically > 100 kb)
  - Of the 0.2% genetic variation in humans, .12% is by CNVs

Evolutionary Implications

- For common variants, low selection pressure against any one gene
  - Still, any non-zero degree of selection pressure will gradually eliminate risk alleles from population
  - For polygenic traits, this process is slow, so so proportion of genetic variation due to old mutations is larger than the proportion due to new mutations
  - Each person contains 500-2000 old slightly deleterious coding mutations†
  - For CNVs, if they do have a moderate to large effect on illness, these are likely to be under substantial negative selection
  - These mutations are therefore rare and more frequently de novo
  - 2-3 *new* non-lethal but deleterious coding mutations per person*
- Against the backdrop of a relatively large pool of mutations from ancestral populations, the constant introduction of new mutations (both SNPs and CNVs) ensures heterogeneity in the particular risk alleles in genes associated with fitness-reducing phenotypes

†Fay et al, 2001; Sunayaev et al, 2001 *Keighley & Eyre-Walker, 2000

Genetic Risk for Schizophrenia: Many Common Variants vs Few Genes of Large Effect

(Nature volume 460, August 2009)

- "Common variants conferring risk of schizophrenia" Stefansson et al, pg 744-47
  - •SGENE-plus sample: 2,663 cases, 13,498 controls from 8 European locations
  - •314,868 markers
- "Common polygenic variation contributes to risk of schizophrenia and bipolar disorder" International Schizophrenia Consortium, pg 748-52
  - •ICS sample: 3,322 European cases, 3,587 controls
  - •739,995 SNPs
- "Common variants on chromosome 6p22.1 are associated with schizophrenia" Shi et al, pg 753-57
  - •MGS sample: 2,681 cases 2,653 controls (European) plus 1,286 cases, 973 controls (African-American); 671,424 SNPs for Europeans; 811,340 for AAs

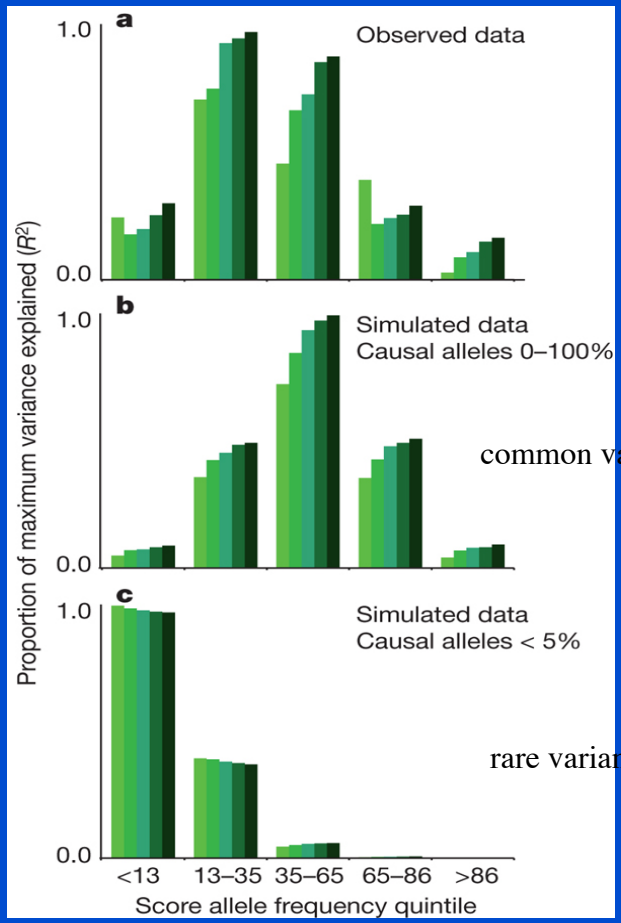
Summary of GWAS Findings

- Even with large samples, none of these studies alone could find a genetic marker significant across the whole genome
- All 3 meta-analyses (each combining data across 3 studies in different ways) point to a region on chromosome 6 (Major Histocompatability Complex)
- One meta analysis points to two new genes- NRGN on 11q24.2 and TCF4 at 18q21.2

Meta-analyses ISC group

- Modeled a risk "score" based on total number of alleles at different significance thresholds
  - Replicated across independent samples
  - Shared across SZ and BP
  - Specific to psychiatric phenotypes
- In simulations, models with a large number of common variants (rather than a few common variants of large effect, or rare variants) fit the observed data significantly better

Analysis stratified by score allele frequency.

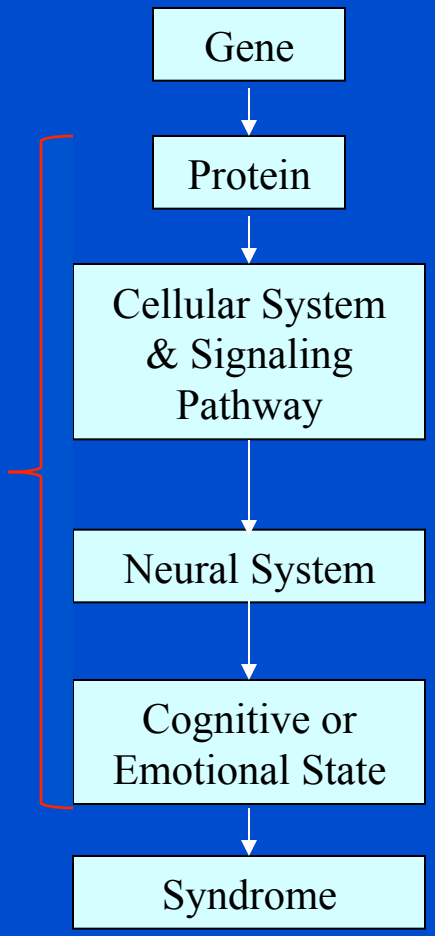


Summary of design issues for case-control GWAS

- Case-Control GWA studies model as a categorical phenotype a set of intrinsically quantitative phenomena
- Syndromal phenotype is the furthest downstream outcome, thus subject to the greatest heterogeneity
  - On one hand, case-control GWAS should thus be best positioned to detect old mutations (SNPs) that have spread the furthest in human populations
    - Supported by replication of genome-wide risk "score" across independent samples in ISC
  - On the other hand, those mutations probably have very small effect sizes on the probability of illness (or would have already been eliminated)
    - Supported by paucity of alleles reaching 10^{-8} significance in samples of +10,000

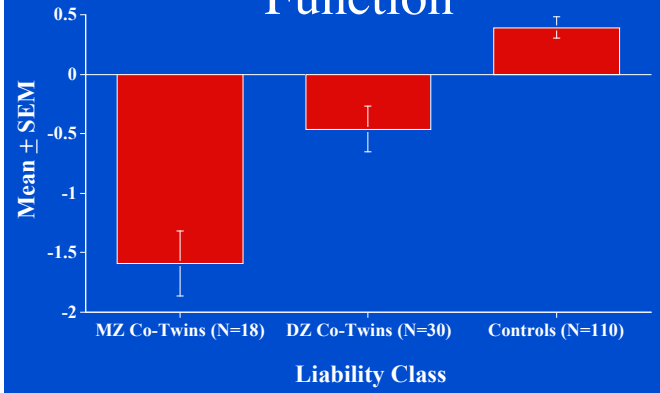
A Strategy for Gene Discovery & Confirmation Based on Endophenotypes

- Identify intermediate phenotypes (e.g., using brain imaging, neurocognitive testing, gene expression profiling) that co-vary in a dose-dependent manner with genetic loading in twin pairs *discordant for illness phenotype* (MZ co-twins>DZ co-twins>normal twins)
  - Use of unaffected twins from discordant pairs disconfounds genetic from phenotypic effects
- Search for genetic polymorphisms (both common and rare) that contribute to quantitative variation in these endophenotypes
- Evaluate transgenic models on panel of homologous endophenotypes in mice



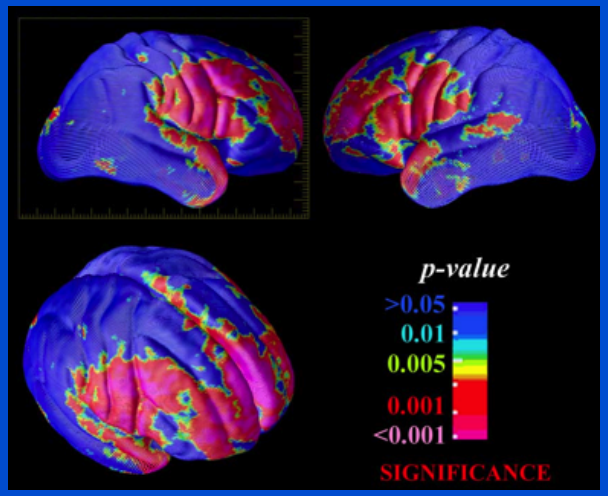
Plasticity-Related Endophenotypes

Short- and Long-Term Memory Function*



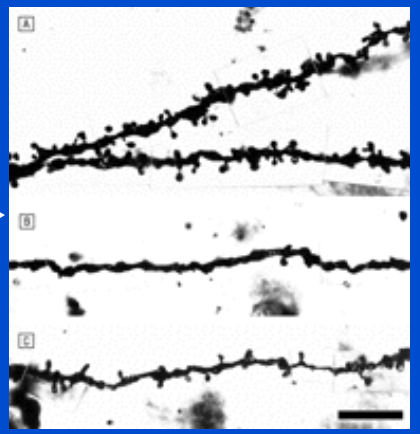
Cannon et al. *Am J Human Genet* 2000;67:369-382.

Gray Matter in PFC & MTL*



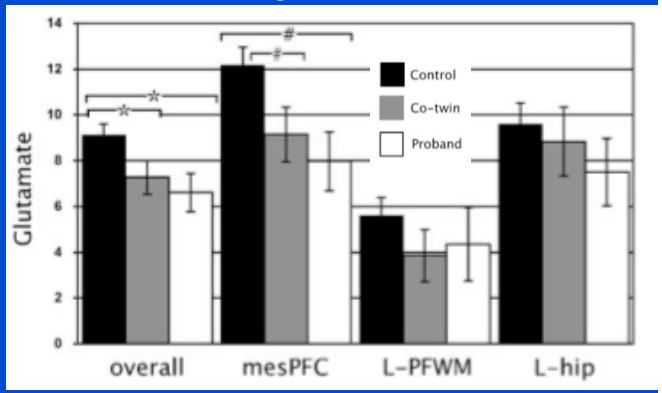
Cannon et al. *PNAS* 2002;99:3228-3233.

Reduced spine density



Glanz & Lewis *Arch Gen Psy* 2000;57:65-73.

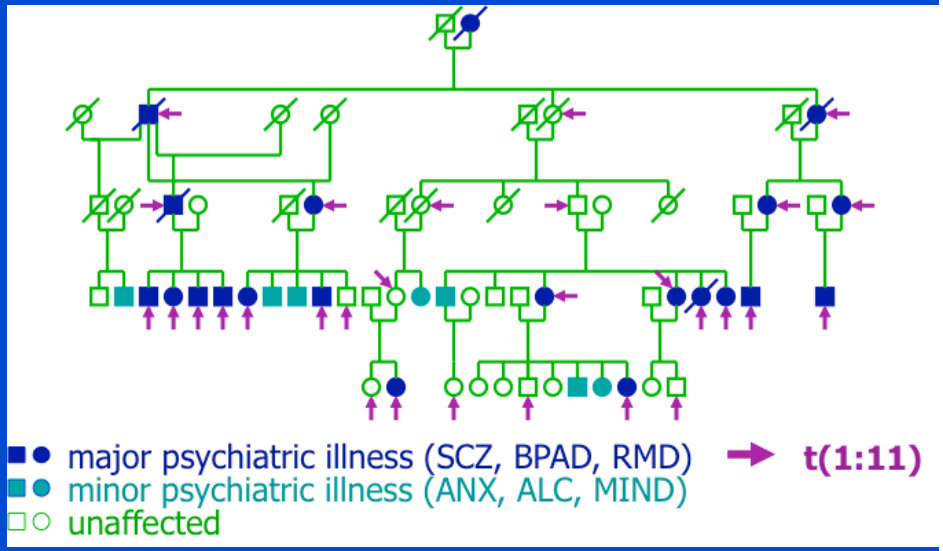
Glutamatergic Transmission

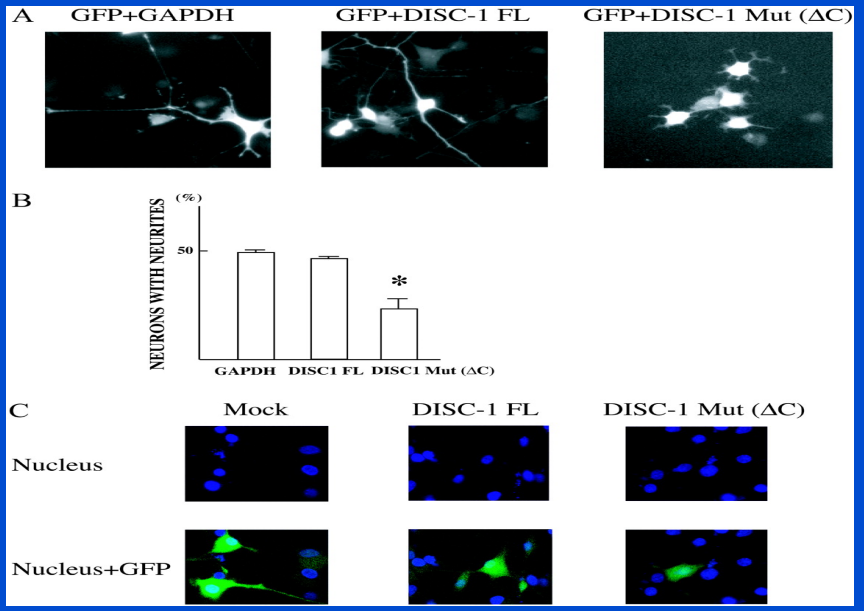


Lutkenhoff et al. *Mol Psychiatry* 2010;15:308-318.

* Validated using MZ and DZ twins discordant for SCZ and healthy controls (disconfounds genetic influences from secondary factors)

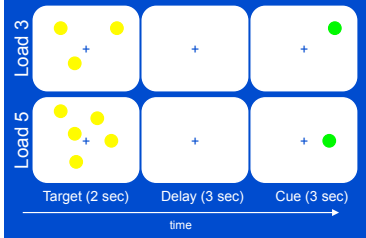
- DISC1 discovered via a balanced translocation
- DISC1 expressed in brain - especially in hippocampus and cortex
- In vitro truncation of DISC1 (inhibiting its interaction with NUDEL) inhibits neurite outgrowth
- DISC1 thus a candidate for explaining disruptions in synaptic plasticity and connectivity in schizophrenia

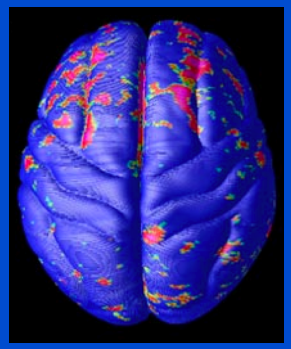




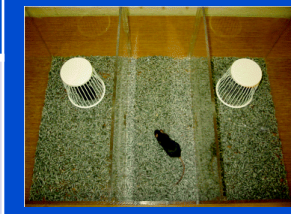
DISC1 Translational Analyses

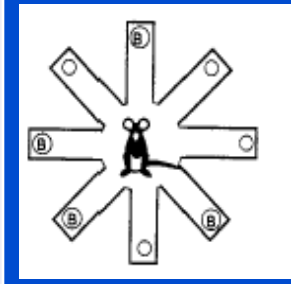
DISC1 Haplotype	Asociality	
	No	Yes
Other	96%	4%
AATG	83%	17%

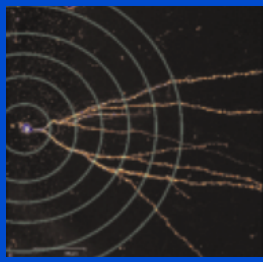




Human (Cannon et al. Arch Gen Psychiatry 2005)	**Mouse** (Li et al. PNAS 2007)
DISC1 HEP2/3 associates with social deficits	Disc1-cc results in reduced sociality
DISC1 HEP2/3 and HEP1 associate with deficits in spatial working memory and long-term memory	Disc1-cc results in reduced spatial working memory
DISC1 HEP2/3 and HEP1 associate with reductions in gray matter in prefrontal cortex and hippocampus	Disc1-cc results in reduced dendritic complexity and basal synaptic transmission of hippocampal neurons





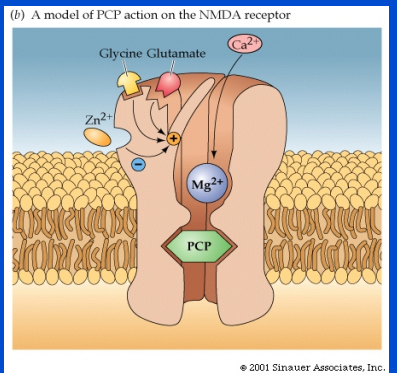


Dysbindin

- Best candidate under a replicated linkage peak at 6p22.3 (Straub 2002)
- A number of dysbindin SNPs and haplotypes associate w/ schizophrenia (though inconsistently across study samples)
- Functional variants remain unknown, though risk may be associated with low expression of mRNA and protein (Bray et al. 2005; Tang et al. 2009)
- Robust associations of dysbindin SNPs with impaired cognition
- Gene encodes dystrobrevin-binding protein: classically thought to be a member of BLOC-1, also known to be involved in glutamate vesicle trafficking to synapse

Reduced NMDAR-mediated synaptic plasticity in schizophrenia

- PCP & ketamine, which block NMDA receptors, induce/exacerbate symptoms of schizophrenia (+, -, cognitive)
- Several studies have observed decreased NMDAR function in schizophrenia, including in drug-free patients



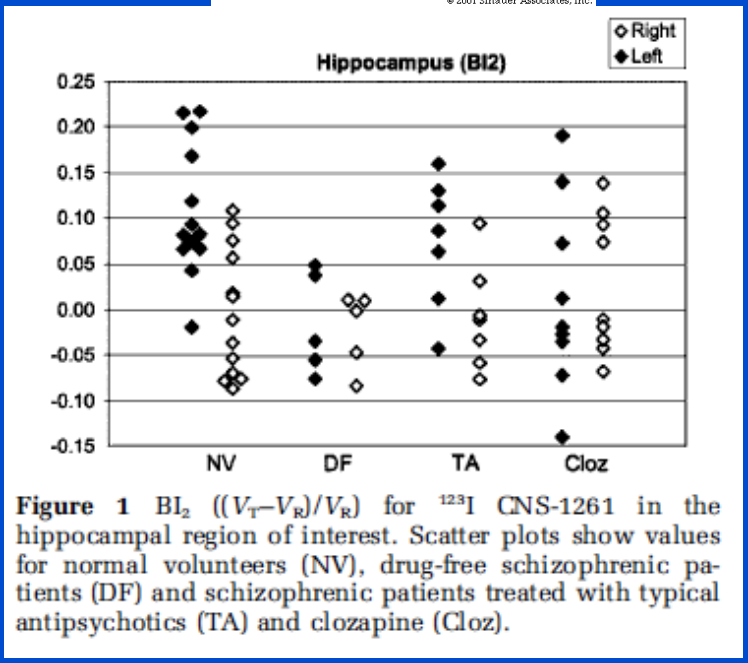
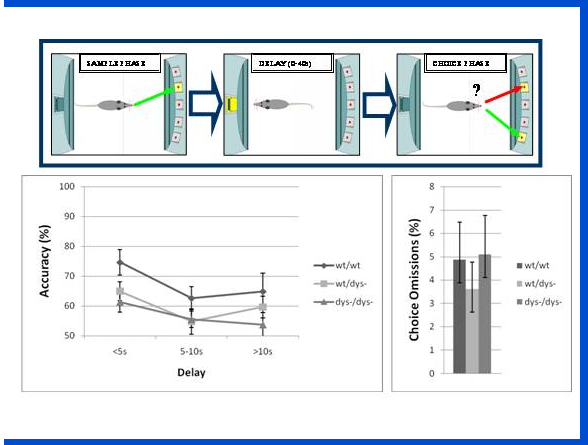


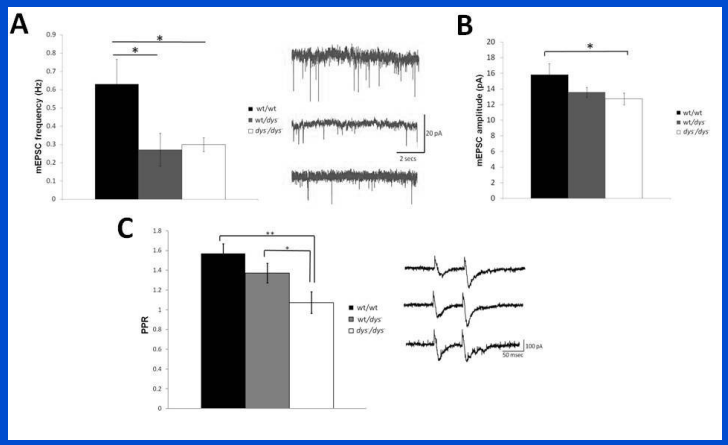
Figure 1 BI_2 $((V_T-V_R)/V_R)$ for ^{123}I CNS-1261 in the hippocampal region of interest. Scatter plots show values for normal volunteers (NV), drug-free schizophrenic patients (DF) and schizophrenic patients treated with typical antipsychotics (TA) and clozapine (Cloz).

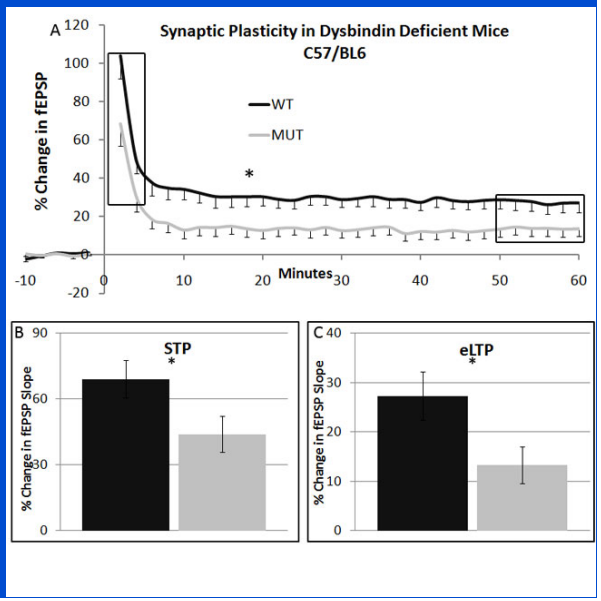
Pilowsky et al., *Mol Psychiatry* 2006;11:118-119

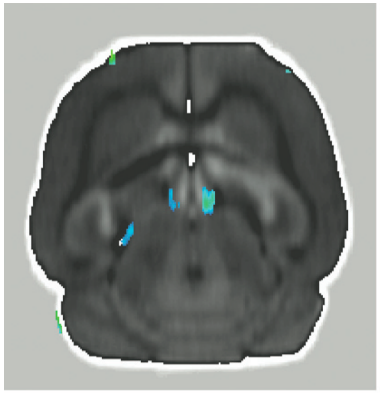
Dysbindin Translational Analyses

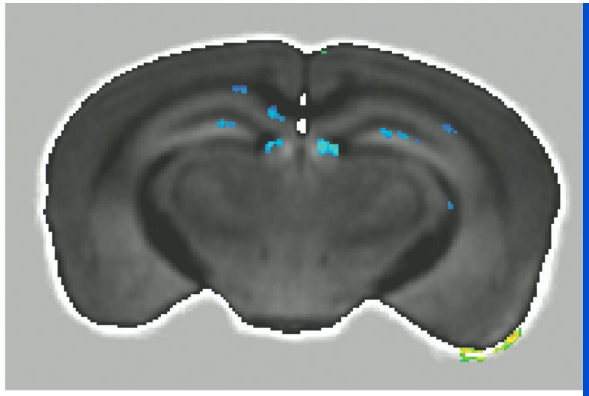
- Null mutant model arose spontaneously on DBA/2J background (sdy mouse); backcrossed to C57Bl
- Mutants show disrupted spatial WM, reduced activity in glutamatergic neurons, reduced expression of NR1 subunit of NMDA receptor, and reduced LTP (rescued with bath application of glycine)
  - Jentsch et al., *Neuropsychopharmacol*. 2009;34:2601-8; Karlsgodt et al. *Biol Psychiatry* 2011;69:28-34; Glen et al., *Neuropsychopharmacol*, in press

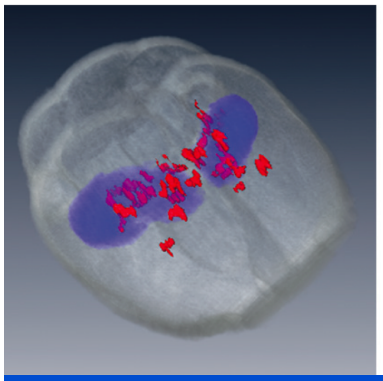


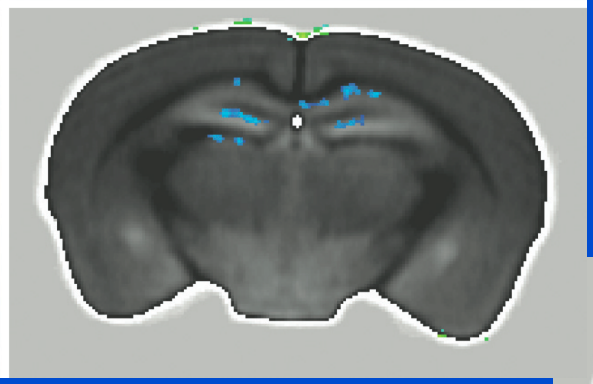






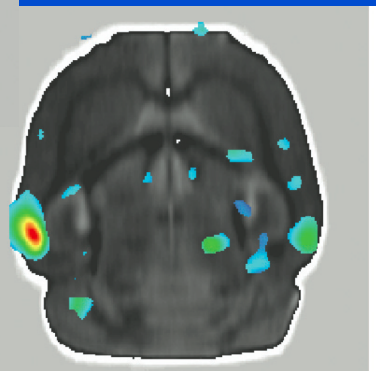


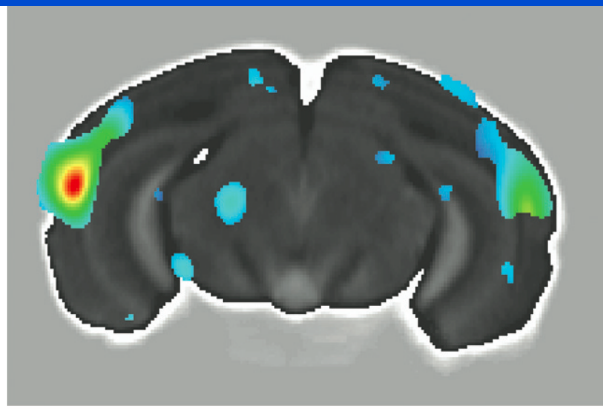




bilateral auditory cortex,
thalamus, and dentate gyrus

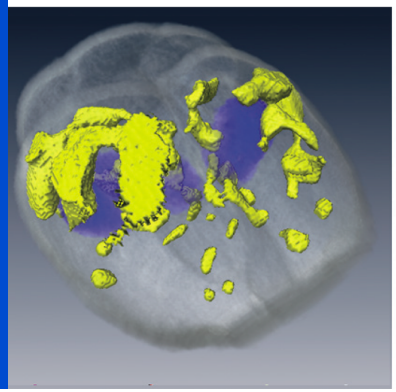
Tensor-Based Morphometry
Dysbindin -/- > Wildtype

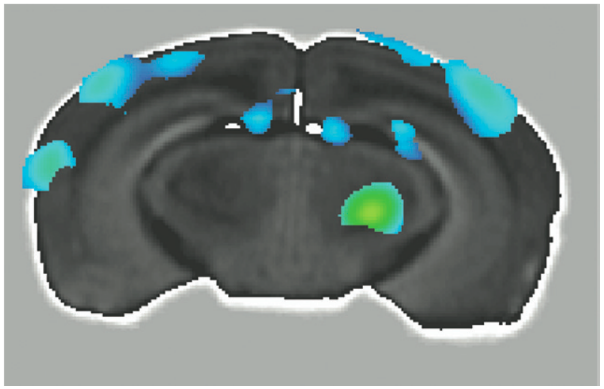




Manganese-Enhanced Contrast
Wildtype > Dysbindin -/-

habenula, dentate, CA1, CA2/3





Lutkenhoff et al. *Under review*

Are DISC1 and Dysbindin susceptibility genes for schizophrenia?

- Linkage
  - Pretty solid statistical evidence of linkage to both regions, replicated across samples and gene pools
  - However, linked regions are relatively large and thus neighboring genes could drive the linkage findings
- Association
  - No SNPs in either gene have reached genome-wide significance in case-control GWA studies
  - Rare CNVs in each gene have been seen in a few cases
  - High likelihood of multiple mutations in both genes in our ancestral past
  - No reason to assume same mutation is relevant to every case
- Biological plausibility
  - SNP associations to validated endophenotypes for schizophrenia
  - Mutant mice show deficits on analogous endophenotypes

Comprehensive Candidate Gene Study

- Examined 3,126 markers from 50 genomic regions based on meta-analysis of all published schizophrenia genetic association studies (www.szgene.org)
  - HuGENet (Human Genome Epidemiology Network) interim criteria based on sample size, heterogeneity across studies, and protection from bias
- Initial series of 135 subjects were scanned & genotyped
  - 26 MZ twin pairs, 34 DZ twin pairs, 15 individuals
  - Status: 96 healthy controls, 26 SZ, 13 BP
  - Mean age = 52 years (SD=10)
- Screened markers for association with global brain volume, then tested regional effects with Tensor Based Morphometry (TBM)

Genomic Regions

Chromosome	Genes (# SNPs)
CHR 1	TSNAX (10), DISC1 (181), PDE4B (196), MTHFR (47), RGS4 (9), GRIK3 (78), PLXNA2 (127), GSTM1 (4), IL10 (9)
CHR 2	GAD1 (14), ZNF804A (65), IL1B (9)
CHR 4	CCKAR (6)
CHR 5	GABRB2 (63), CMYA5 (93)
CHR 6	TNF (15), PRSS16 (44), **PGBD1** (41), NOTCH4 (106), HIST1H2BJ (8), AHI1 (47), RPP21 (22), DTNBP1 (40), C6orf217 (47), MDGA1 (57)
CHR 7	**RELN** (271)
CHR 8	NRG1 (388), PPP3CC (28), SLC18A1 (28)
CHR 10	GWA_10q26.13 (1)
CHR 11	NRGN (4), DRD4 (3), DRD2 (29), GWA_11p14.1 (1), TPH1 (13), OPCML (251)
CHR 12	GRIN2B (205), DAO (5)
CHR 13	DAOA (18), HTR2A (57)
CHR 14	AKT1 (14)
CHR 16	RPGRIP1L (29), HP (1)
CHR 17	SRR (7)
CHR 18	IMPA2 (36), TCF4 (83)
CHR 19	APOE (9)
CHR 22	PRODH (20), ADRBK2 (32), COMT (49)

Genotyping Quality Control

- 19 SNPs excluded for failing Hardy-Weinberg Equilibrium (HWE) test ($p<0.001$)
- 568 SNPs excluded for minor allele frequencies (MAF) < 0.01
- 7 SNPs excluded for frequency of missing data > 0.05
- No SNPs excluded for zygosity checks, genotype frequency and category checks
- 6 individuals excluded by population stratification test (EIGENSTRAT)
- 2 individual excluded by inbreeding test (too much homozygosity)
- Final Data:
- 2345 total markers in 50 genes
- 127 individuals

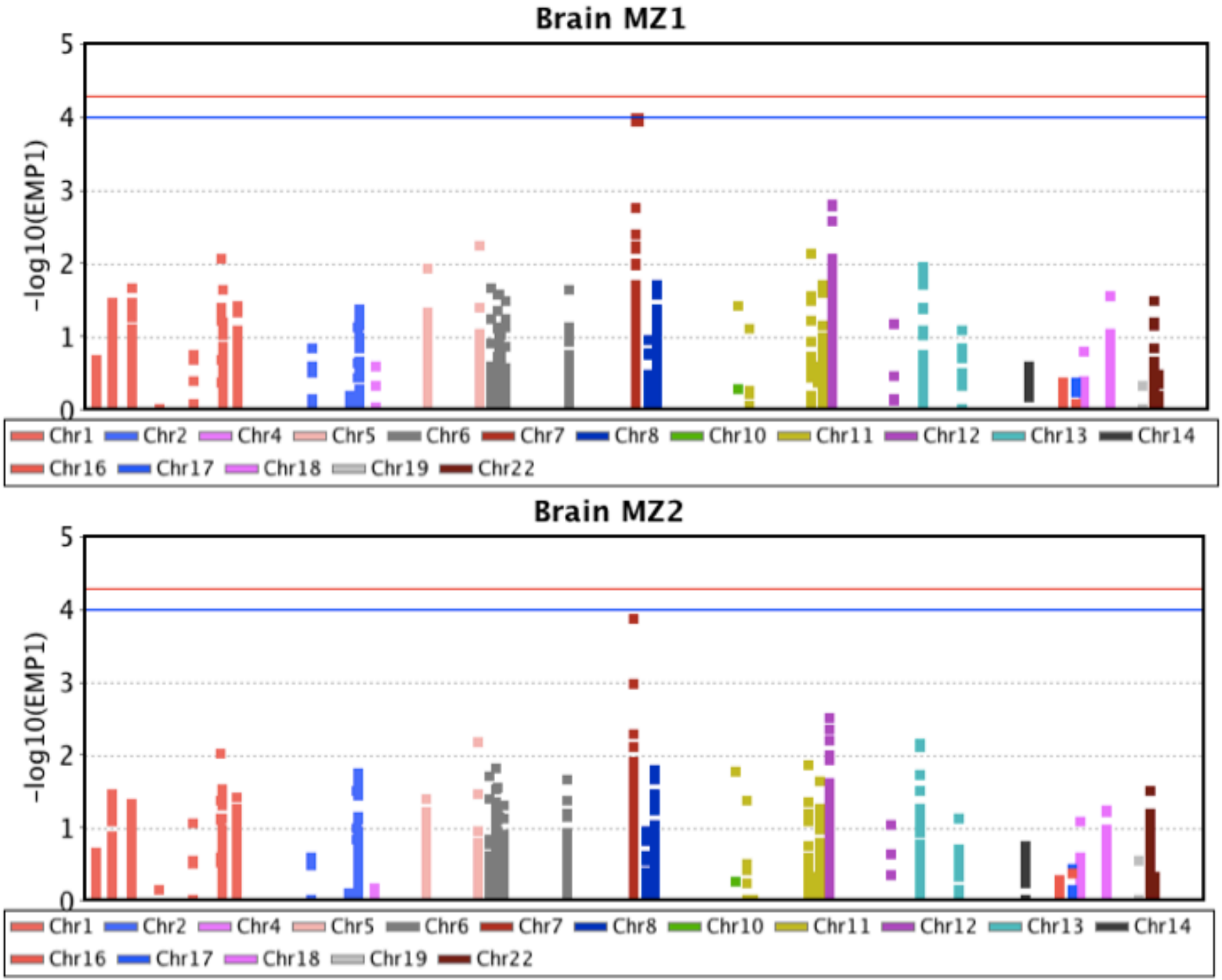
Association Analysis Model

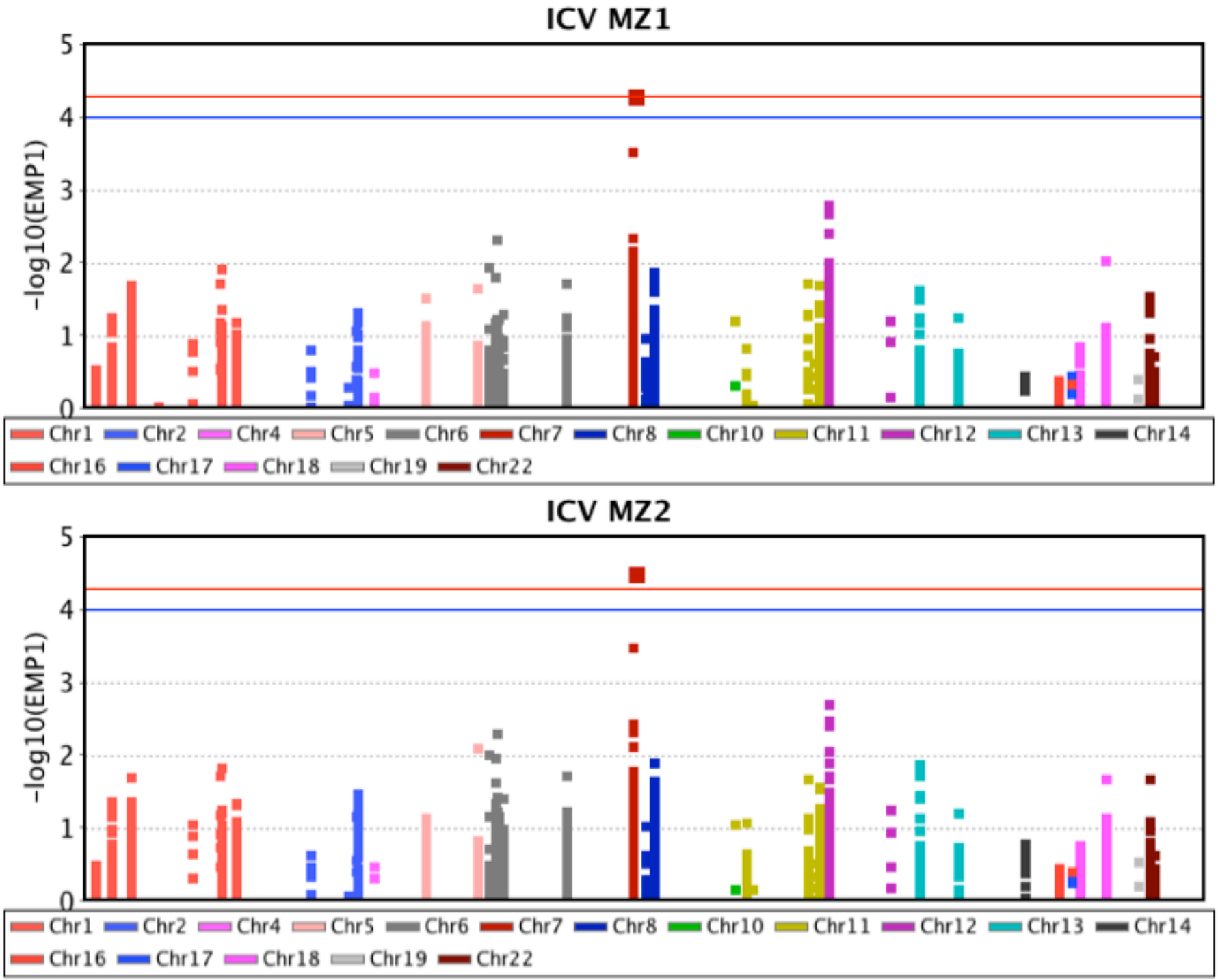
- **PLINK: QFAM** (Shaun Purcell et al, 2007 AJHG)
  - Family-based association tests for quantitative traits
  - Based on QTDT package (Fulker et al,1999, AJHG and Abecasis et al, 2000, AJHG)
  - Accounts for twinship, but not completely for MZ twins:
    - 1 co-twin excluded from each MZ twin pair for analysis
    - Then analysis re-run with other co-twin from each MZ twin pair excluded
  - 2 step permutation:
    1. QFAM total (between and within components) with adaptive permutation on all quality controlled marker)
    2. QFAM total with max(T) 100,000,000 permutations on significant markers from 1st step

Multiple Testing Correction

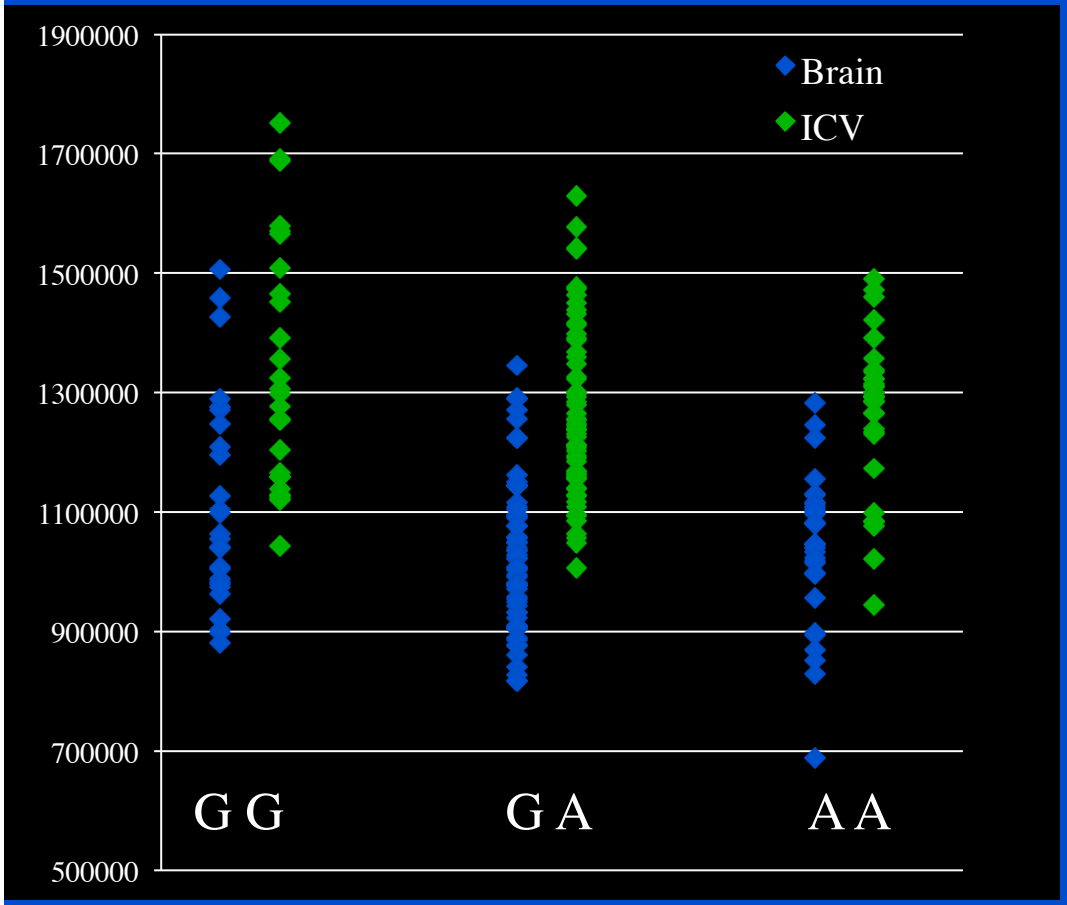
- Used an adjusted Bonferroni correction that models the underlying linkage disequilibrium (LD) structure among the markers to determine the number of effectively independent tests (Nicodemus, Liu et al. 2005)
  - 2,345 total markers used in analysis
  - 941 SNPs in independent LD blocks

- Corrected p value for 941 tests:
  - $p = 0.05 / 941 = 5.31 \times 10^{-5}$
- Therefore, p-values less than 5.31×10^{-5} are statistically significant (p-values less then 10^{-4} considered suggestive)
- P-values were empirically derived using permutation


Brain MZ1
-log10(EMP1)
0
1
2
3
4
5
Chr1 Chr2 Chr4 Chr5 Chr6 Chr7 Chr8 Chr10 Chr11 Chr12 Chr13 Chr14
Chr16 Chr17 Chr18 Chr19 Chr22
Brain MZ2
-log10(EMP1)
0
1
2
3
4
5
Chr1 Chr2 Chr4 Chr5 Chr6 Chr7 Chr8 Chr10 Chr11 Chr12 Chr13 Chr14
Chr16 Chr17 Chr18 Chr19 Chr22


ICV MZ1
−log10(EMP1)
0
1
2
3
4
5
Chr1 Chr2 Chr4 Chr5 Chr6 Chr7 Chr8 Chr10 Chr11 Chr12 Chr13 Chr14
Chr16 Chr17 Chr18 Chr19 Chr22
ICV MZ2
−log10(EMP1)
0
1
2
3
4
5
Chr1 Chr2 Chr4 Chr5 Chr6 Chr7 Chr8 Chr10 Chr11 Chr12 Chr13 Chr14
Chr16 Chr17 Chr18 Chr19 Chr22

RELN: rs262366

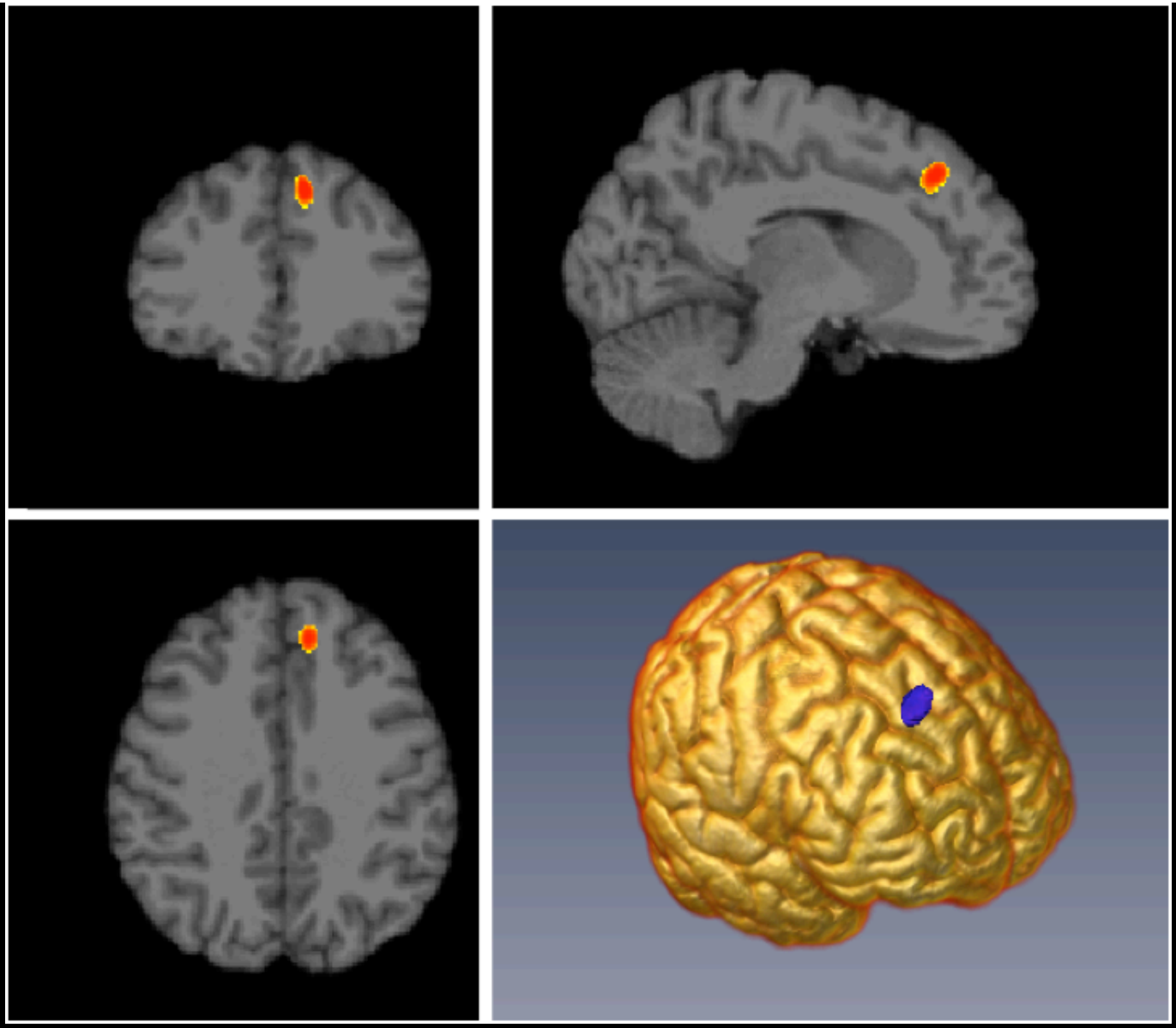


Major allele = A
Minor allele = G
MAF = 0.4945

RELN: Reelin
Chromosome 7
rs262366 located in intron

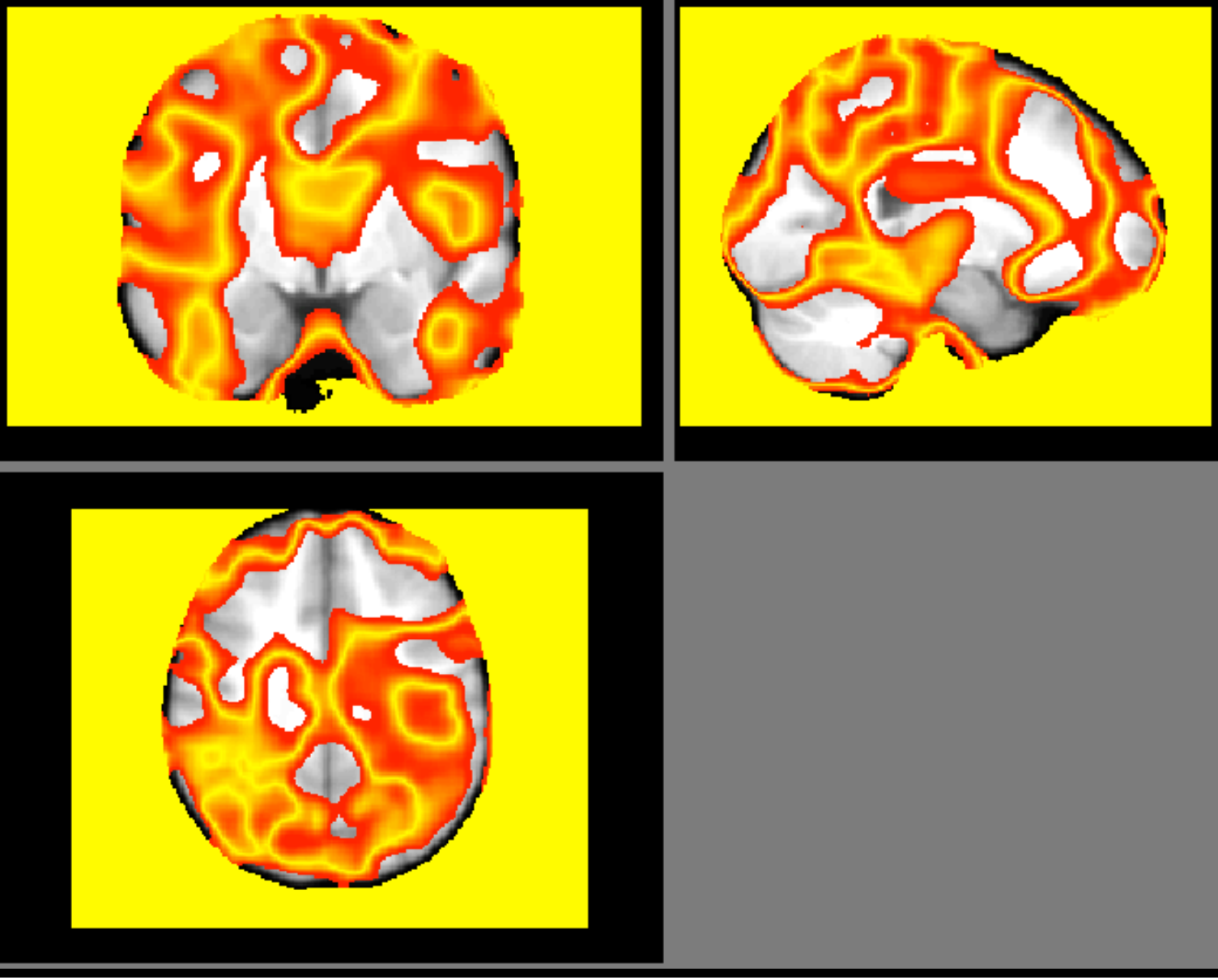
rs262366	Brain	ICV
MZ 1	9.25E-05	4.19E-05
MZ 2	1.17E-04	2.70E-05

rs262366	BP	SZ	Control
AA	2	10	32
GA	20	11	60
GG	10	6	22

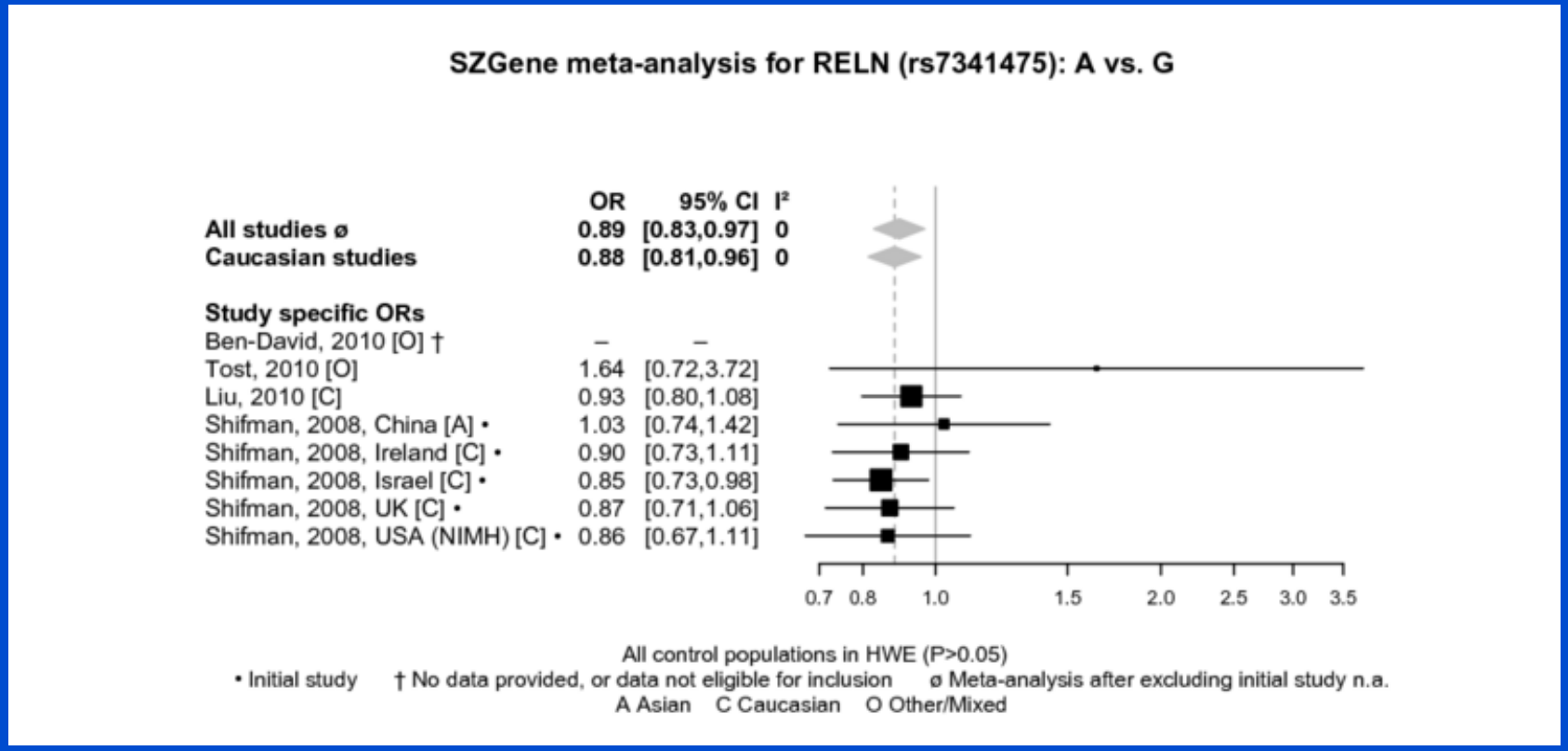


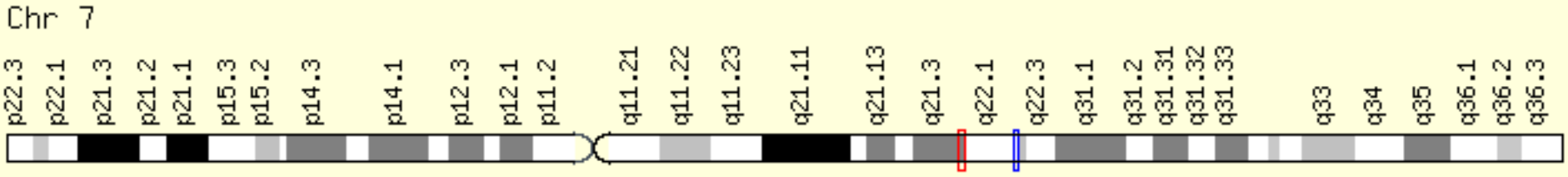
Orthographic views of EMMAX modeling for effect of RELN polymorphism rs262366 on TBM maps; significantly associating voxels in red and yellow located in the superior frontal gyrus and anterior cingulate gyrus (515 voxels, FDR corrected; $p < 1.24 \times 10^{-05}$) . Three-dimensional volume rendering of representative brain with rs262366 association result displayed in blue (lower right).

rs262366 (RELN): No threshold



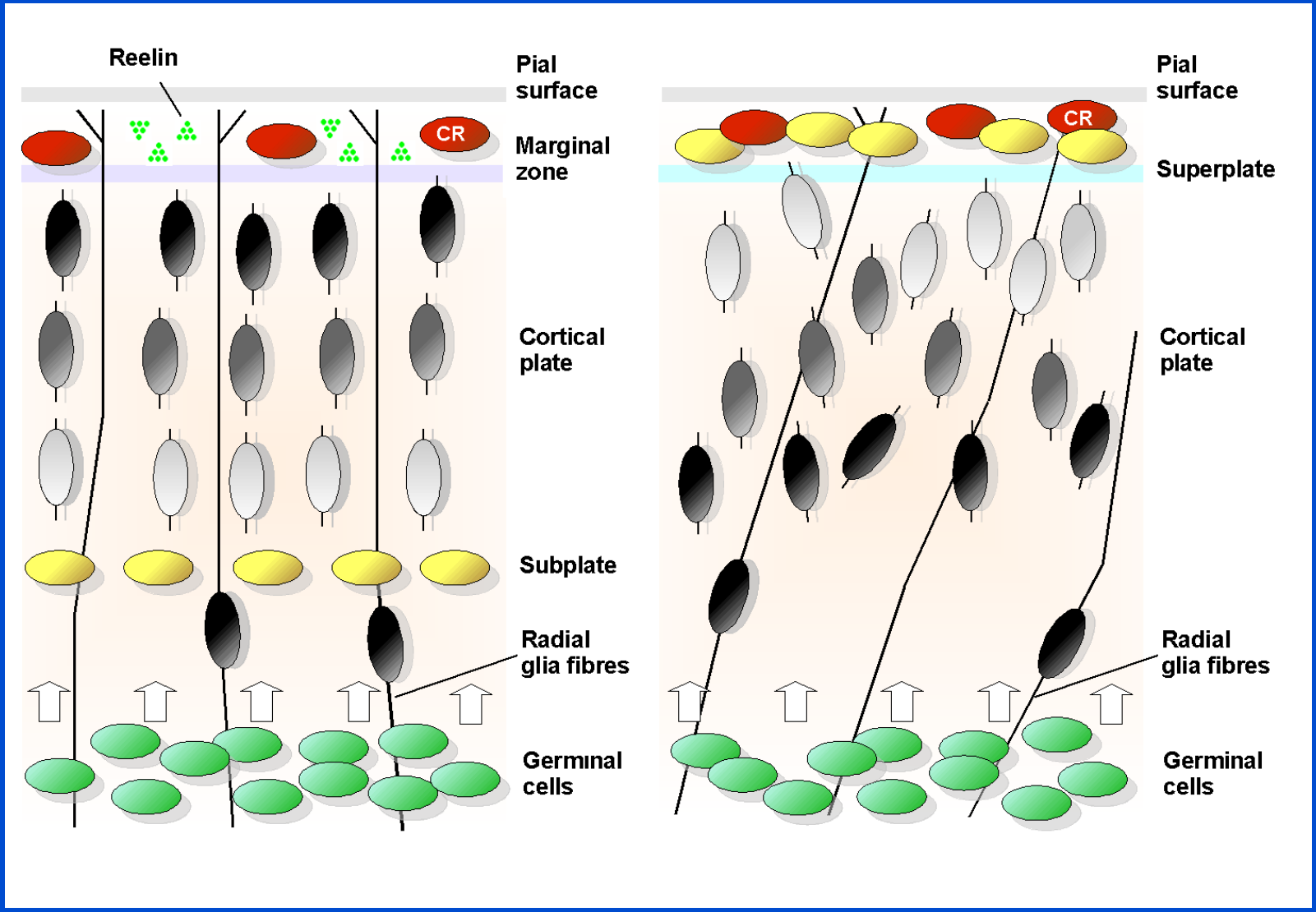
Reelin & Schizophrenia





Reelin Linkage Disequilibrium

Reelin & Neural Development



Wildtype

Reelin Mutation

Cece2309. Wikimedia Commons. 2007.

Conclusions

- Imaging phenotypes can be informative for genetic studies of complex illness
  - Quantitative, multidimensional, and translational nature represent significant advantages
- In schizophrenia, some utility of this approach in relation to some candidate genes, including DISC1, dysbindin, reelin
- Power and sample size the biggest rate limiting step both at the genetic (SNP) and phenotypic (voxel) levels for both candidate gene and GWA

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