

# *Topic 12 – DNA technology*

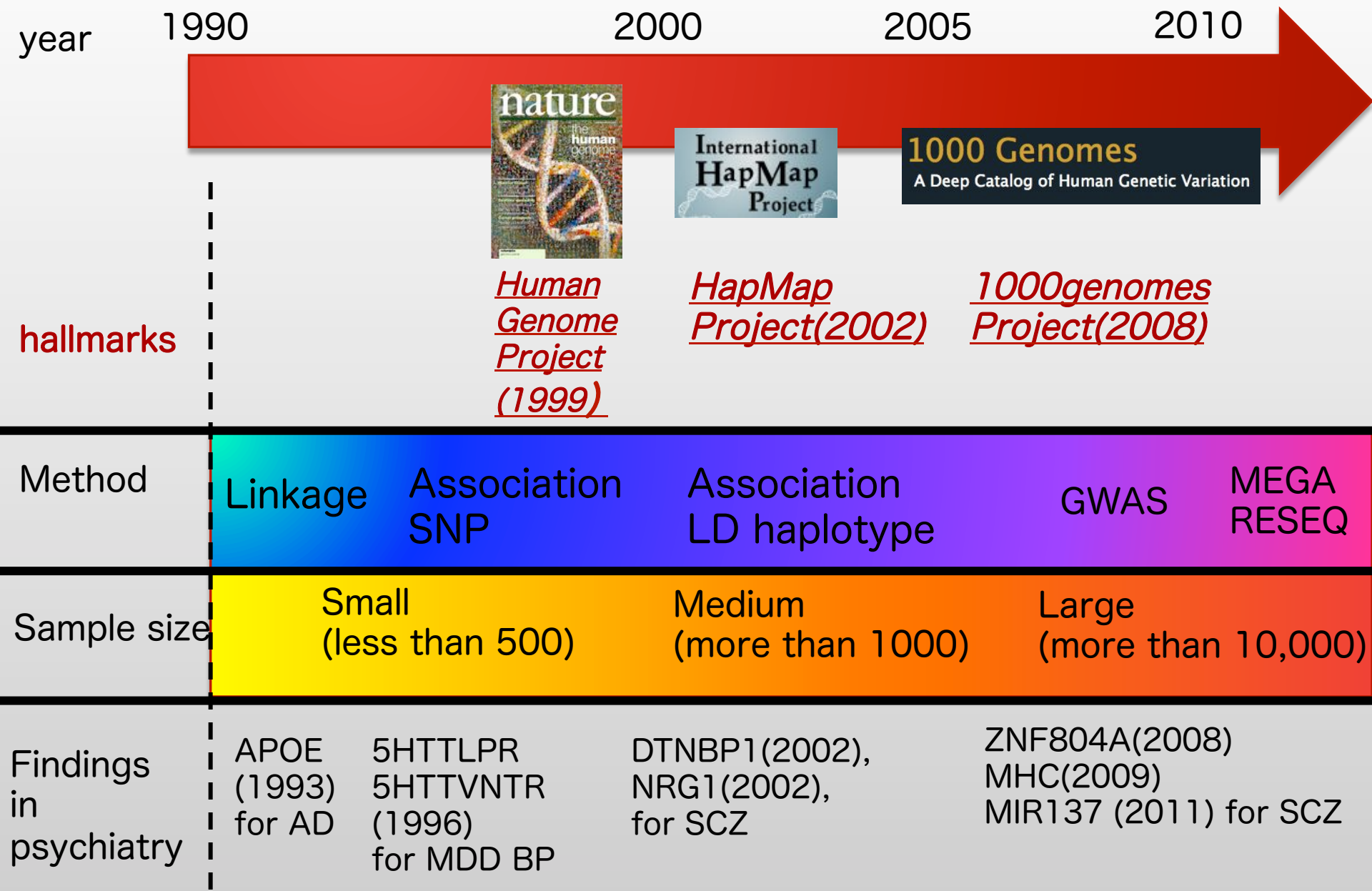
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Copyright materials

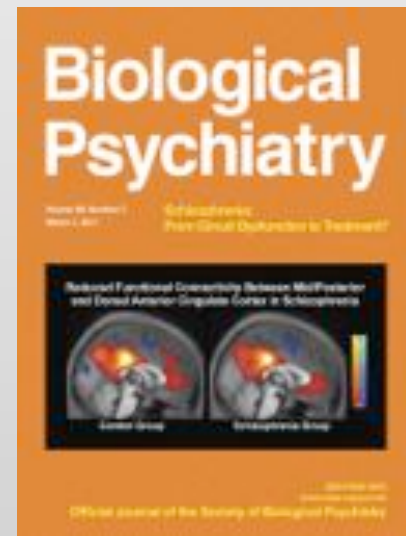
# Overview of genetic study (in psychiatry)



# Today's topics:

- Japanese Schizophrenia GWAS
  - SNP-based analysis
  - Polygenic component analysis
  - Follow-up

# Schizophrenia GWAS



M. Ikeda, B. Aleksic et al, 2011 Biol Psychiatry

# History of Schizophrenia Diagnosis

- Emil Kraepelin (1856-1926): dementia praecox
  - Early onset
  - Progress to dementia
  - Cf. Demetia presenilis (Alzheimer's disease) and manic depressive insanity
- Eugen Bleuler (1857-1939): schizophrenia
  - did not necessarily have an early onset
  - Doesn't (always) progress to dementia



# Schizophrenia now

- Common psychiatric disorder
- Onset is usually in early adulthood
- Characterized by:
  - Positive symptoms (hallucinations)
  - Negative symptoms (lack of motivation, social withdrawal)
  - Cognitive symptoms (decreasing of IQ)
- Usually prolonged medication is required

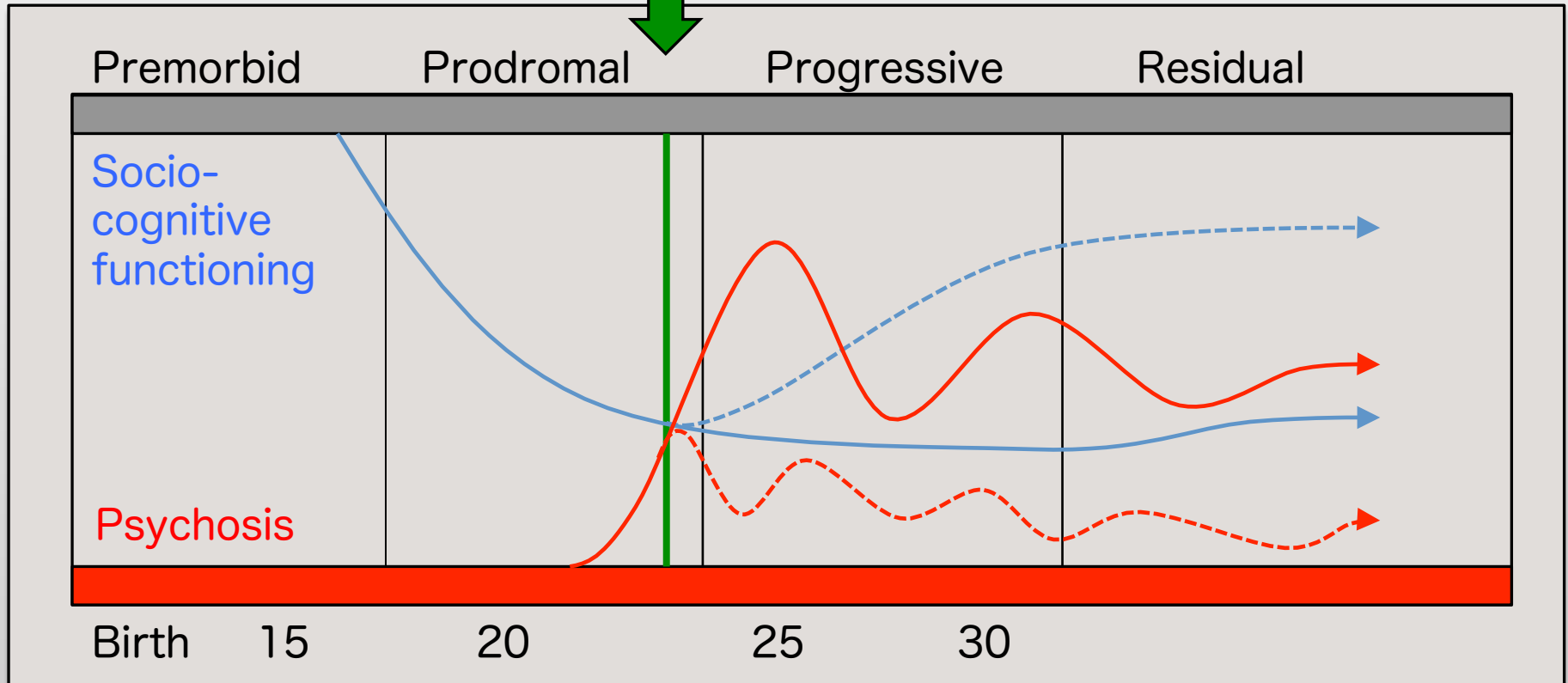
# Clinical course of Schizophrenia

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Treatment

---  
With treatment

—  
Without treatment





# Approximate risks

	Schizophrenia	Schizoaffective	Bipolar disorder
Risk in general population	0.8-1%	0.3%	0.3-1%
Risk to siblings/ first degree rels	10%	2-3%	5-10%
Monozygotic (MZ) twin concordance	45%	40%	40%
Dizygotic (DZ) twin concordance	5-10%	5%	5%
Risk in adoption studies	8%	-	14% (but small sample)

# Heritability: examples

- Schizophrenia 80%+
- Autism 80%+
- Bipolar 60%
  
- Unipolar depression 40-70%
- Alcohol dependence 50-60%
- Anxiety disorders 20-30%

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# Our Platform (affy 5.0)



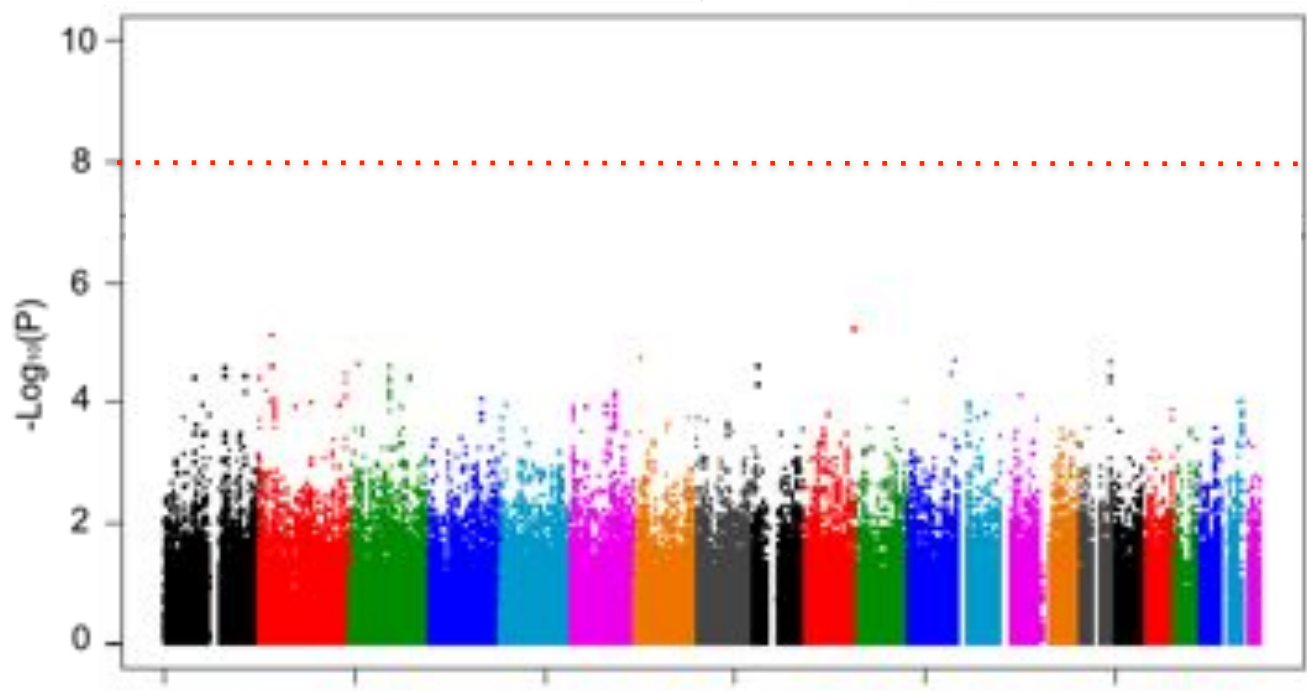
# Samples (SNP-based analysis)

## Meta Analysis

- **GWAS (JPN)**
  - 575 schizophrenia
  - 564 controls
- **Rep\_JPN**
  - Replication1 (main sample for follow-up)
    - 1511 schizophrenia
    - 1517 controls
  - Replication2 (additional con: JPN, public database)
    - 934 controls (Genotyped by Illumina550)
- **Replication3** (WTCCC\_scz: UK)
  - 479 schizophrenia
  - 2938 controls (Genotyped by Affy 500K)

# Manhattan Plot

: GWAS sample (~1,200 sample)



● Chr1	● Chr9	● Chr17
● Chr2	● Chr10	● Chr18
● Chr3	● Chr11	● Chr19
● Chr4	● Chr12	● Chr20
● Chr5	● Chr13	● Chr21
● Chr6	● Chr14	● Chr22
● Chr7	● Chr15	
● Chr8	● Chr16	

***NO SNPs*** with genome-wide significance ( $P < 5 \times 10^{-8}$ )

# Result: CMH analysis

: meta analysis (~8,000 sample)

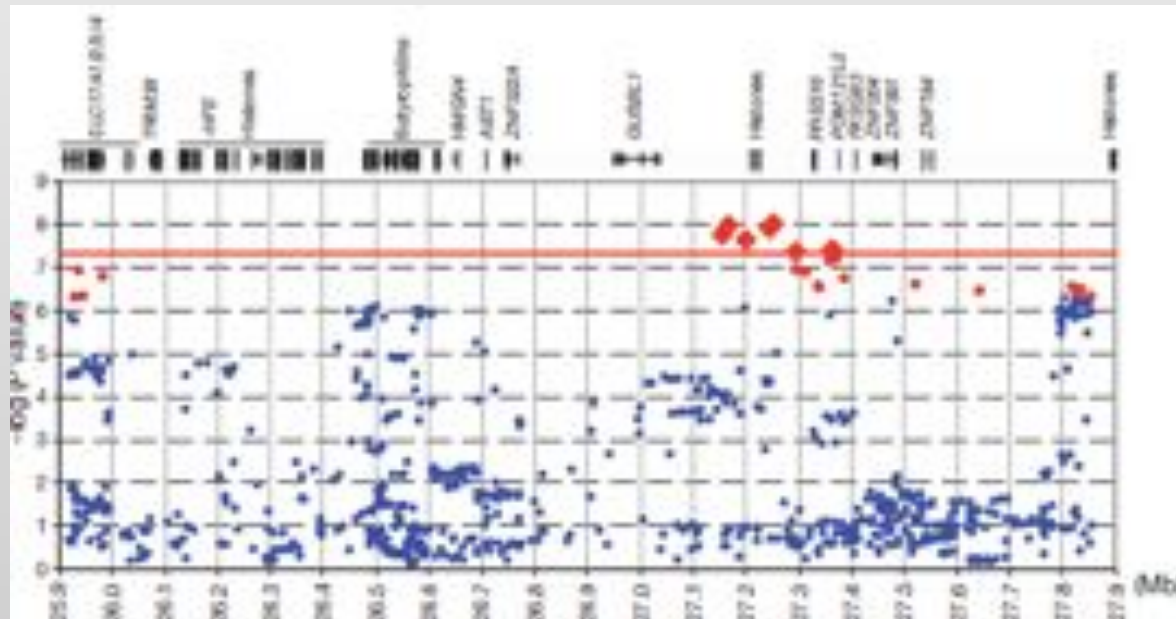
CHR	SNP	closest gene	Meta_all		
			MAF	P <sub>CMH</sub>	OR
2	rs11895771	SULT6B1	0.49	<b>3.7X10<sup>-5</sup></b>	0.84
7	rs1011131	LOC392288	0.070	1.2X10 <sup>-4</sup>	1.30
14	rs1176970	LOC644919	0.15	1.4X10 <sup>-4</sup>	1.22
1	rs4908274	COL11A1	0.28	3.1X10 <sup>-4</sup>	1.20
6	rs2294424	C6orf105	0.41	5.0X10 <sup>-4</sup>	1.15
2	rs13010889		0.15	0.0011	0.85
2	rs17026152		0.26	0.0012	0.85
6	rs2787566	GRIK2	0.039	0.0014	1.34
6	rs2071286	NOTCH4	0.19	0.0014	0.87
8	rs17462248		0.20	0.0017	1.16



Again, **NO SNPs** with genome-wide significance ( $P < 5 \times 10^{-8}$ )

# Promising candidate region reported in already published GWASes focused on schizophrenia

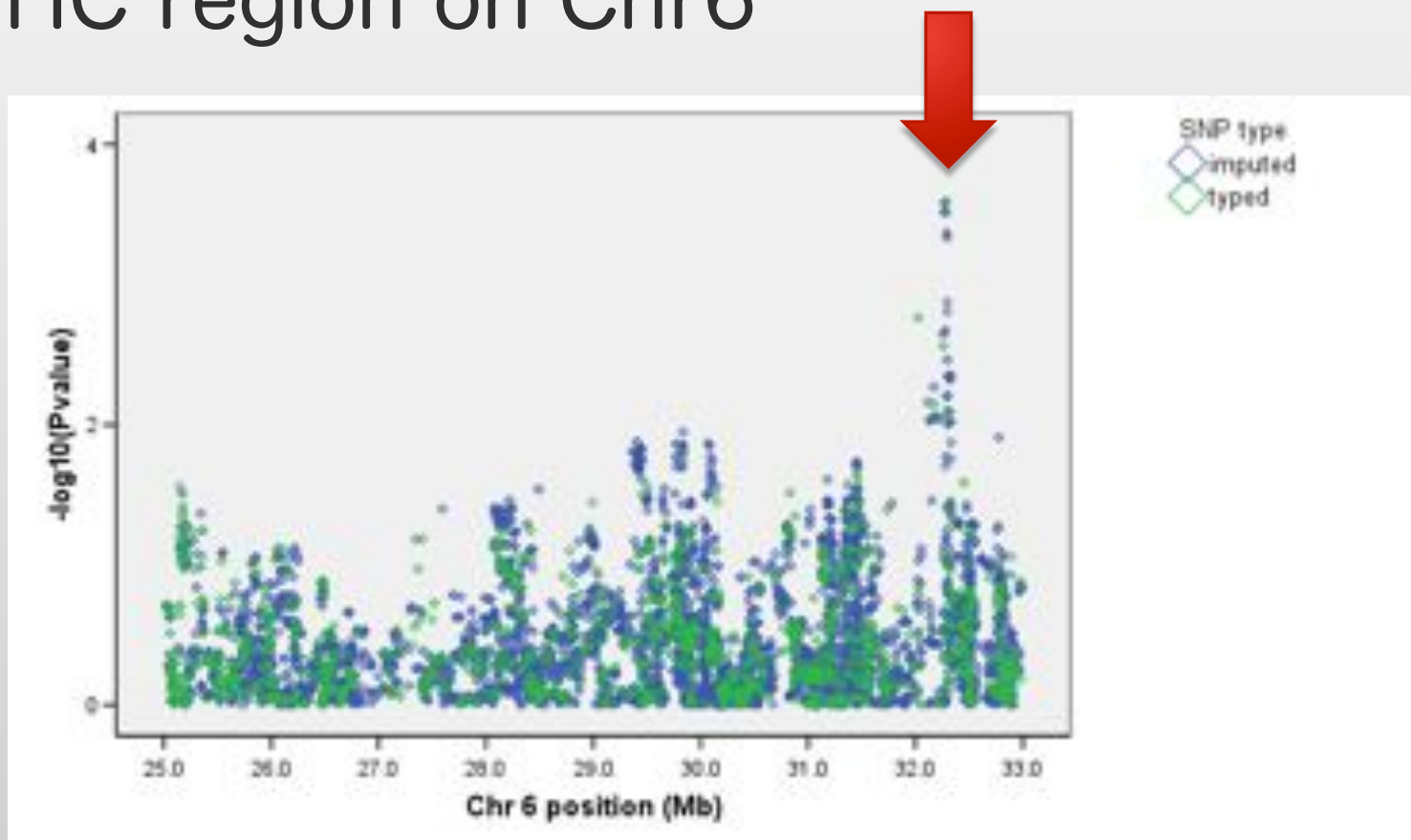
- Several candidate genes with genome-wide significance ( $5 \times 10^{-8}$ )
- MHC region on Chr6 by **SGENE**, **ISC**, **MGS**, **PGC**



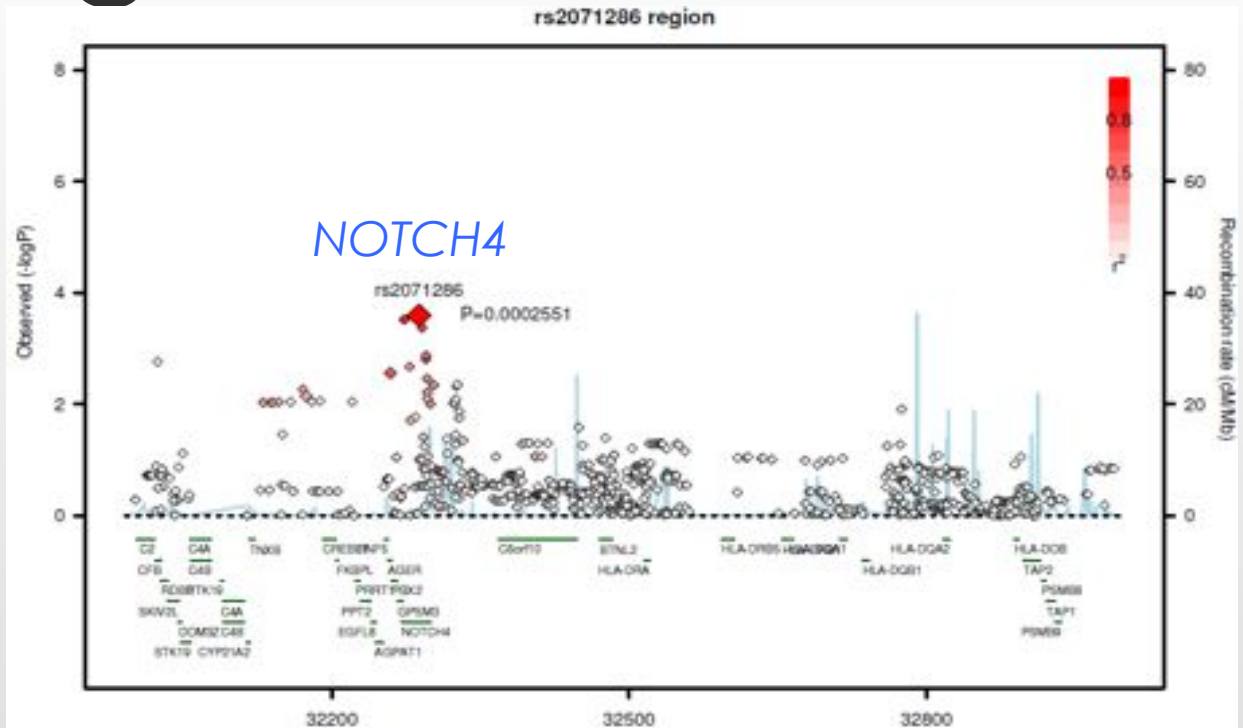


# Japanese GWAS data low magnification on MHC

- MHC region on Chr6

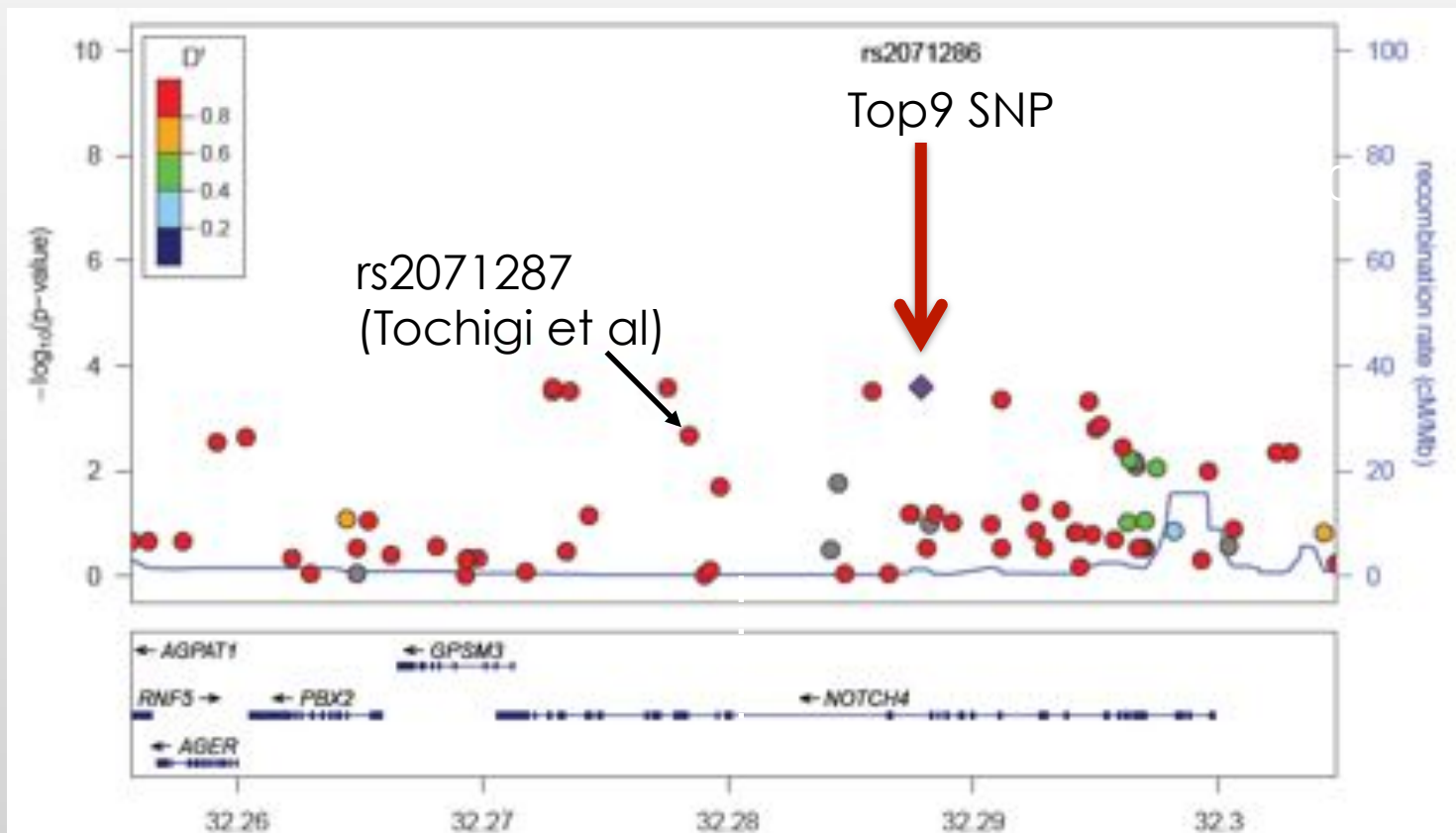


# Zooming in!



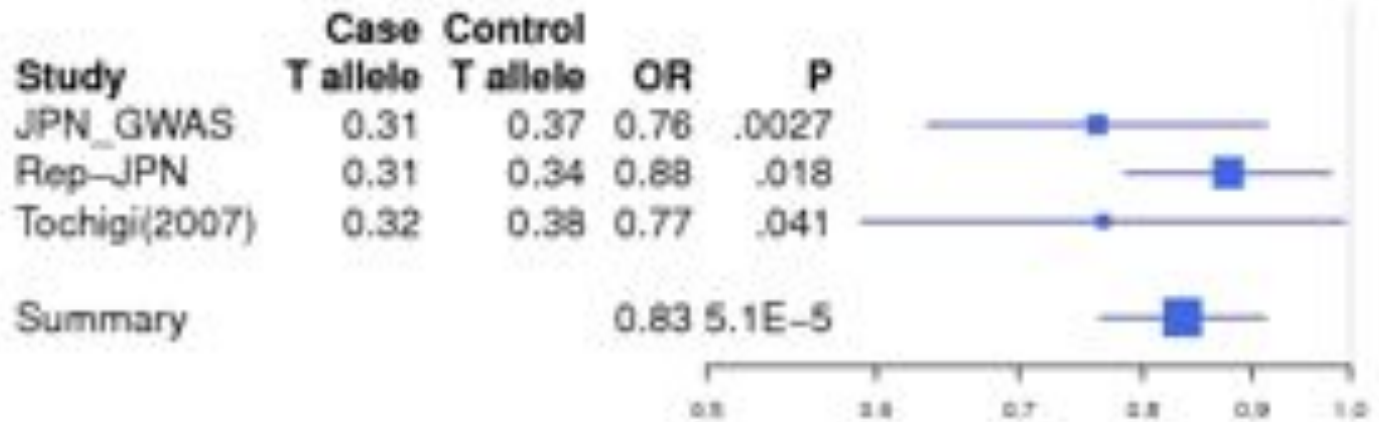
CHR	SNP	closest gene	Meta_all				
			MAF	$P_{CMH}$	OR	L95	U95
2	rs11895771	SULT6B1	0.4892	$3.7 \times 10^{-5}$	0.84	0.7708	0.9117
7	rs1011131	LOC392288	0.07039	$1.2 \times 10^{-4}$	1.30	1.136	1.48
14	rs1176970	LOC644919	0.1524	$1.4 \times 10^{-4}$	1.22	1.10	1.354
1	rs4908274	COL11A1	0.28	$3.1 \times 10^{-4}$	1.20	1.087	1.324
6	rs2294424	C6orf105	0.4128	$5.0 \times 10^{-4}$	1.15	1.063	1.244
2	rs13010889		0.1524	0.0011	0.85	0.7716	0.9375
2	rs17026152		0.2618	0.0012	0.85	0.7673	0.9369
6	rs2787566	GRIK2	0.03883	0.0014	1.34	1.12	1.609
6	rs2071286	NOTCH4	0.1929	0.0014	0.87	0.791	0.9459
8	rs17462248		0.1988	0.0017	1.16	1.056	1.268

# Maximum magnification



# Follow up analysis for *NOTCH4* (rs2071287)

Meta\_JPN + previously published data (JPN)

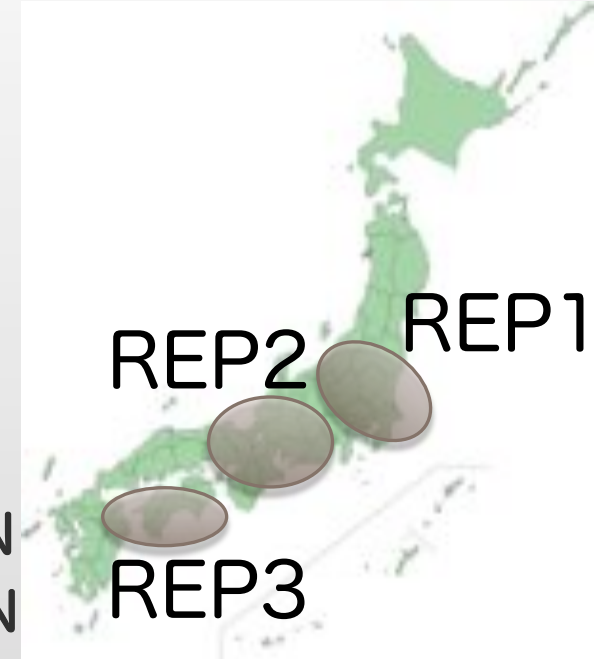


~2,500 SCZ

~2,500 CON

# Further Replication

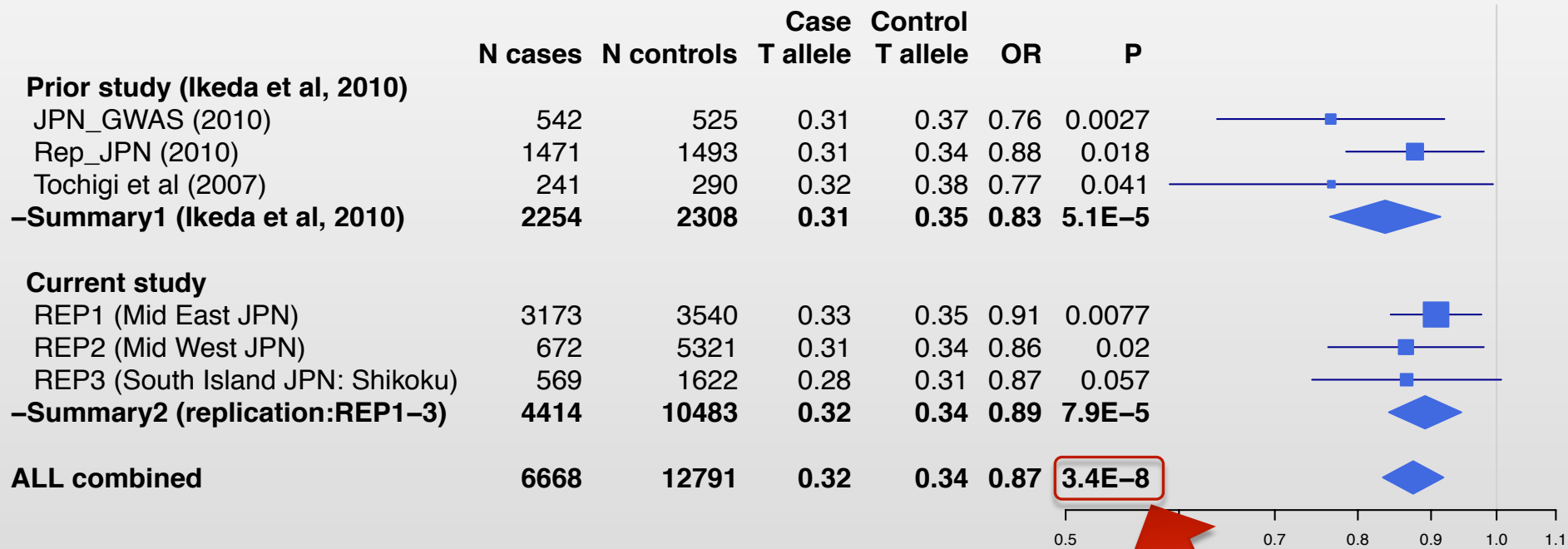
- Samples used in the previous paper (Ikeda et al)
  - JPN\_GWAS: 542 SCZ vs 525 CON
  - REP\_JPN: 1471 SCZ vs 1493 CON
  - Tochigi et al: 241 SCZ vs 290 CON



- New samples
  - REP1: 3150 SCZ vs 3483 CON, Mid-East JPN
  - REP2: 672 SCZ vs 5321 CON, Mid-West JPN
  - REP3: 569 SCZ vs 1622 CON, South Island: Shikoku Island JPN

Extra samples : ~4,400 SCZ  
~10,000 CON

# Meta-analysis



*Total*

~6,700 SCZ

~13,000 CON

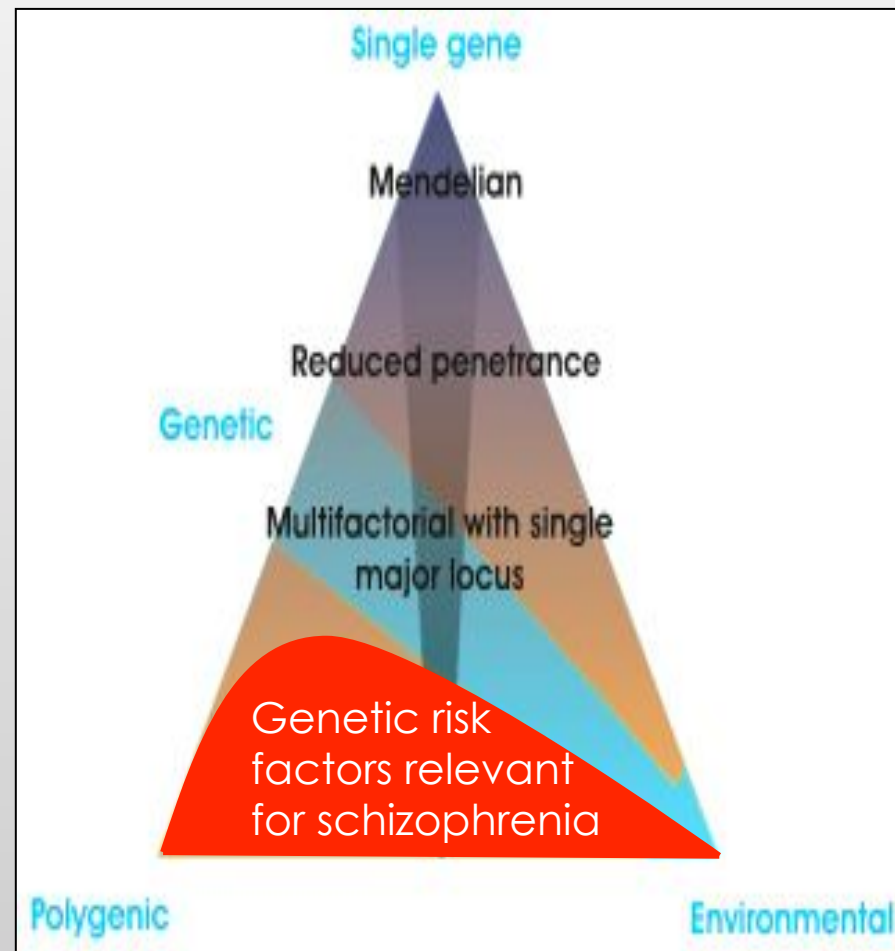
*First genome wide  
significant P-value in  
Japanese schizophrenia  
population based sample*

# Today's topics:

- Japanese Schizophrenia GWAS
  - SNP-based analysis
  - Polygenic component analysis
  - Follow-up

# Next we explored the concept of polygenic Component Analysis (PSA) using our dataset...

- We know that schizophrenia is polygenic
- We know that in case of common SNPs risk effect size is small
- Recent studies showed that in case of schizophrenia common variants do have an important role en masse (cumulative risk effect)



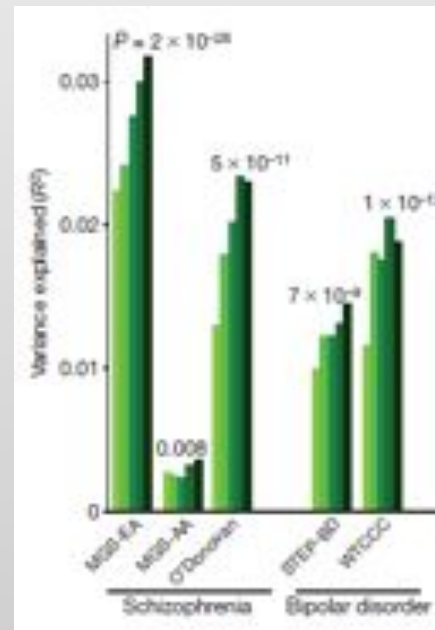


# GWAS in schizophrenia (2)

## Polygenic Component Analysis

- Method used by International SCZ Consortium (ISC)
- Non stringent definition of risk SNPs (*e.g.*  $P < 0.3$ )

$\Sigma xi$



Two GWAS  
sample sets  
are needed  
for polygenic  
component  
analysis !



*Typical genome wide  
association analysis*

*Polygenic component  
analysis*



**Discovery  
sample set**



**Target  
sample set**

Discovery  
sample set

	SNP1	SNP2	SNP3	SNP4	-----
Risk allele	A	T	G	C	
P	.10	.26	.68	.05	

Calculate P value for SNPs/alleles based on  
discovery sample

Discovery  
sample set

$P_T < 0.5$

	SNP1	SNP2	SNP3	SNP4	-----
Risk allele	A	T	G	C	
P	.10	.26	.68	.05	

Assign non stringent statistical threshold ( $P_T$  : P threshold) and define **risk SNPs/alleles (e.g.  $P_T < 0.5$ )** based on discovery sample

Discovery sample set

$P_T < 0.5$

	SNP1	SNP2	SNP3	SNP4	----
Risk allele	A	T	G	C	
P	.10	.26	.68	.05	

Check the genotypes in TARGET sample

Target sample set

	SNP1	SNP2	SNP3	SNP4
case1	AA	TT		CT
case2	AT	TT		CT
con1	AT	TC		TT
con2	TT	TC		TT



Discovery sample set

$P_T < 0.5$

	SNP1	SNP2	SNP3	SNP4	-----
Risk allele	A	T	G	C	
P	.10	.26	.68	.05	

Count number of risk alleles in cases and controls

(TARGET sample)

Target sample set

	SNP1	SNP2	SNP3	SNP4
case1	AA	TT		CT
case2	AT	TT		CT
con1	AT	TC		TT
con2	TT	TC		TT

**Polygenic score**

SNP1	SNP2	SNP4	
2	2	1	= 5
1	2	1	= 4
1	1	0	= 2
0	1	0	= 1

Discovery  
sample set

$P_T < 0.5$

	SNP1	SNP2	SNP3	SNP4	-----
Risk allele	A	T	G	C	
P	.10	.26	.68	.05	

Calculate mean  
polygenic score

(TARGET sample)

Target sample

	SNP1	SNP2	SNP3	SNP4
case1	AA	TT		CT
case2	AT	TT		CT
con1	AT	TC		TT
con2	TT	TC		TT

**Polygenic score**

SNP1	SNP2	SNP4	Mean
2 + 2 + 1 = 5			4.5
1 + 2 + 1 = 4			
1 + 1 + 0 = 2			1.5
0 + 1 + 0 = 1			

Discovery  
sample set

$P_T < 0.5$

	SNP1	SNP2	SNP3	SNP4	-----
Risk allele	A	T	G	C	
P	.10	.26	.68	.05	

Compare  
mean values

TARGET sample

Target sample

	SNP1	SNP2	SNP3	SNP4
case1	AA	TT		CT
case2	AT	TT		CT
con1	AT	TC		TT
con2	TT	TC		TT

**Polygenic score**

SNP1	SNP2	SNP4	Mean
2 + 2 + 1 = 5			4.5
1 + 2 + 1 = 4			
1 + 1 + 0 = 2			1.5
0 + 1 + 0 = 1			



# Samples used for Polygenic Score Analysis (PSA)

- Datasets

- JPN

UK

- WTCCC Schizophrenia

479 SCZ vs 2938 CON  
(O'Donovan et al ,2008)

- WTCCC Bipolar

1868 BP vs 2938 CON  
(WTCCC, 2007)

- Statistical analysis

- Logistic Regression

- P value

- Nagelkerke Pseudo  $R^2$  as measure of explained variability

- The more variability explained, the better the model

# Polygenic component analysis (PCA)

## -Central hypothesis-

- Can PCA predict
  - status (schizophrenia or healthy) within Japanese GWAS sample
  - status within UK sample based on Japanese SNPs and vice versa

# Discovery/Target pair

Within JPN samples: random Division

Case



Control



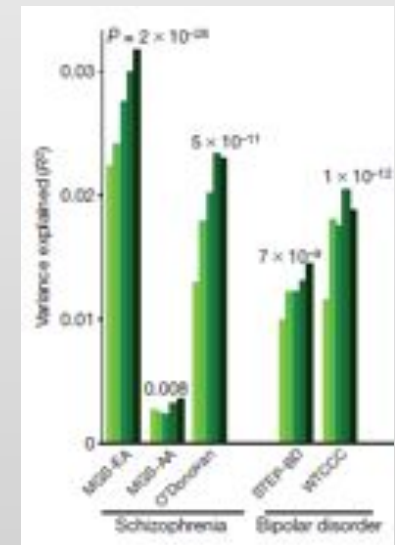
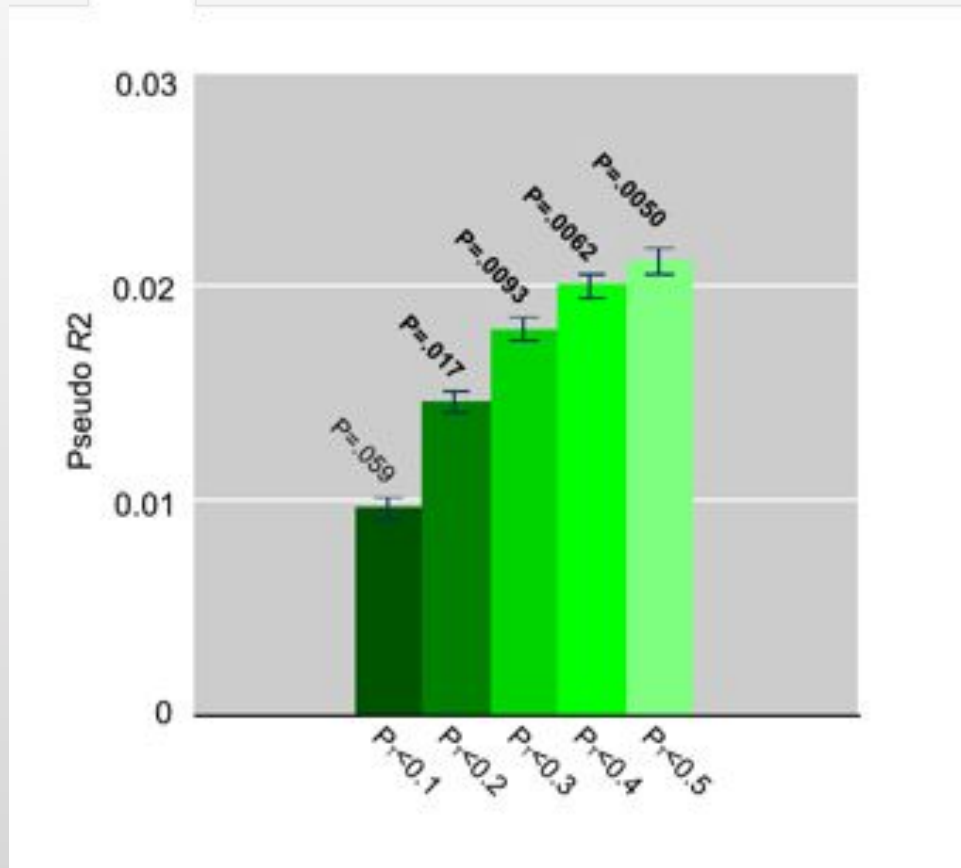
Only one GWAS dataset in JPN sample ...

Discovery set  
For 'risk' alleles

Target set  
For polygenic scores

# Within JPN samples

1<sup>st</sup>/2<sup>nd</sup>: discovery/target pair



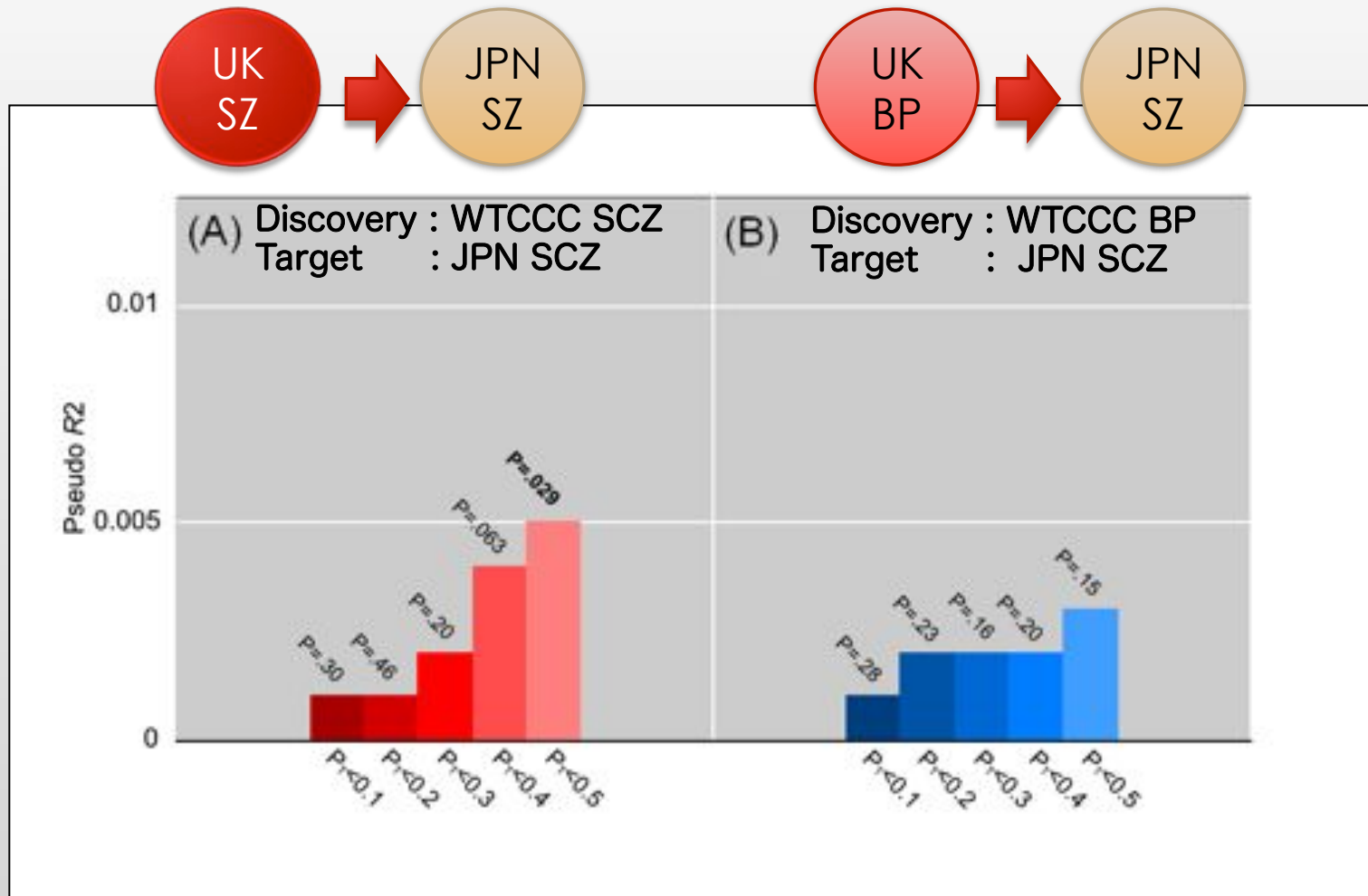
Significant enrichment (risk alleles)

$R^2 \sim 2\%$  ( $P_T < 0.5$ )  $\rightarrow$  en masse increase the risk

#  $R^2$  in ISC 3%

# PCA Results

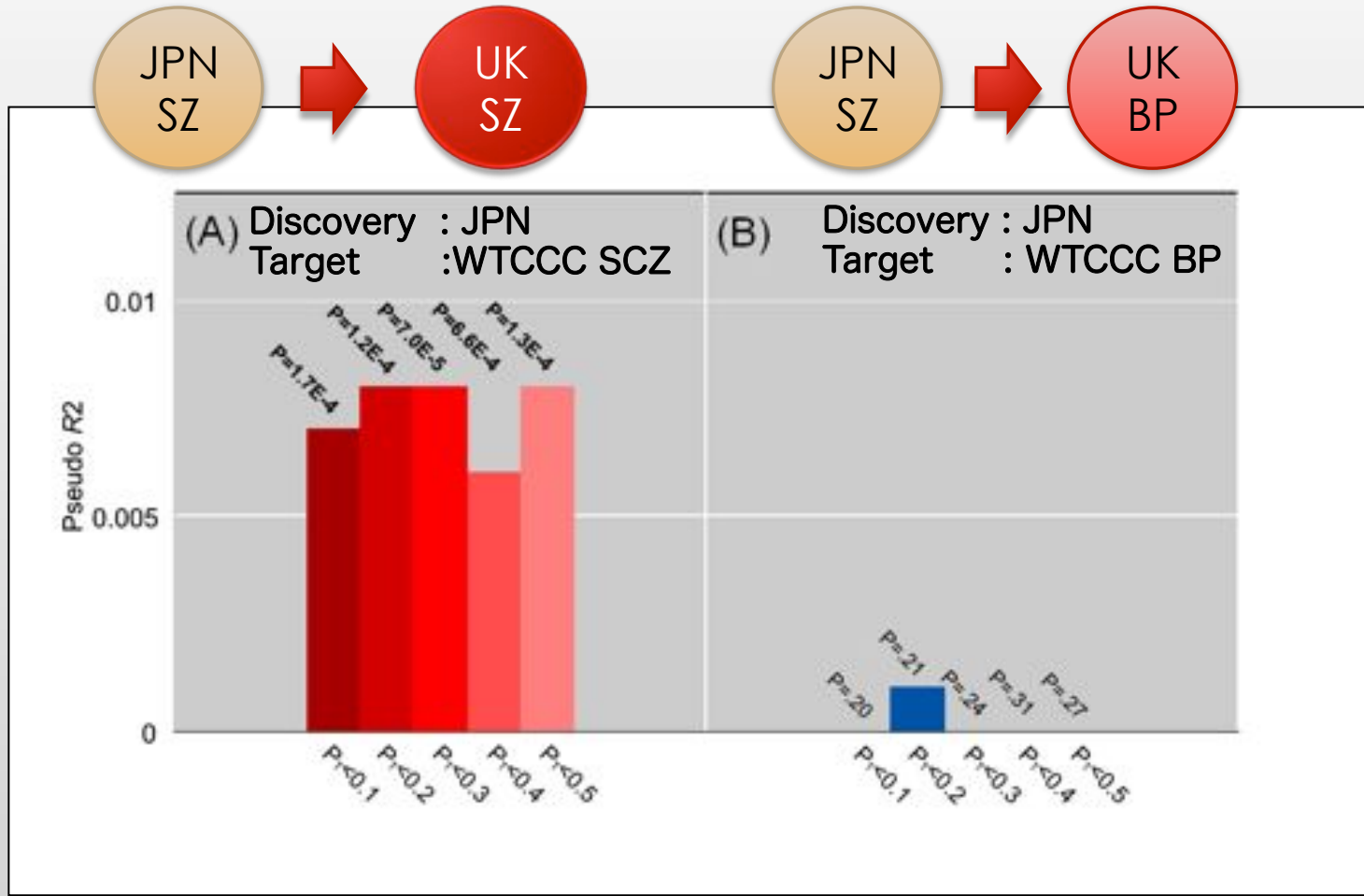
*(UK schizophrenia and bipolar SNPs vs. Japanese schizophrenia SNPs)*



Low pseudoR<sup>2</sup> ~ 0.5% ( $P_T < 0.5$ )

# PCA Results

(Japanese schizophrenia SNPs vs. UK schizophrenia and bipolar SNPs)



Low pseudoR<sup>2</sup>~0.7% (P<sub>T</sub><0.5)

# Summary: GWAS

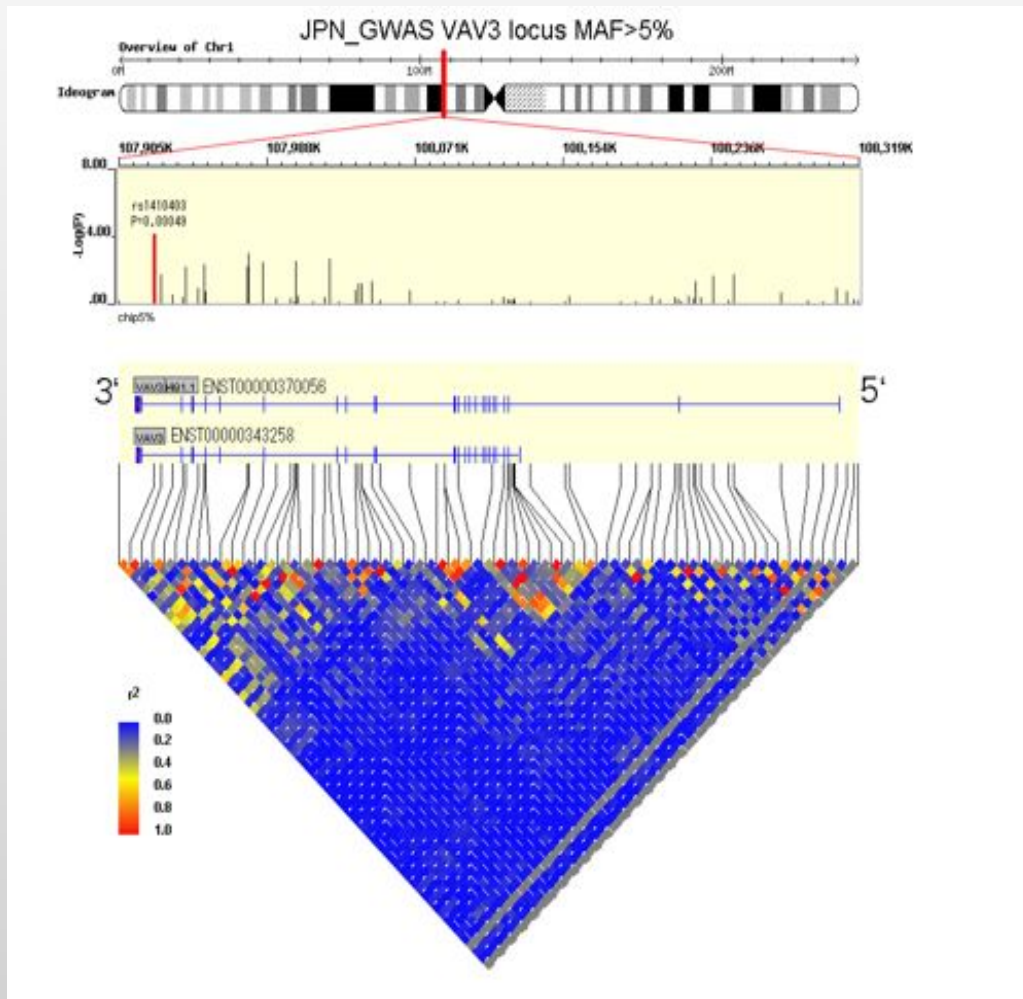
- Effect size of risk SNPs (common) is very small
- It is important to chase sub GWAS P-threshold in underpowered sample sets
- We replicated the observation of a polygenic component to schizophrenia within the Japanese population ( $p = .005$ )
- Our trans Japan-UK analysis of schizophrenia also revealed a significant correlation (best  $p = 7.0 \times 10^{-5}$ ) in the polygenic component across populations
- These results indicate a shared polygenic risk of schizophrenia between Japanese and Caucasian samples, although we did not detect unequivocal evidence for a novel susceptibility gene for schizophrenia

# Today's topics:

- Japanese Schizophrenia GWAS
  - SNP-based analysis
  - Polygenic component analysis
  - Follow-up



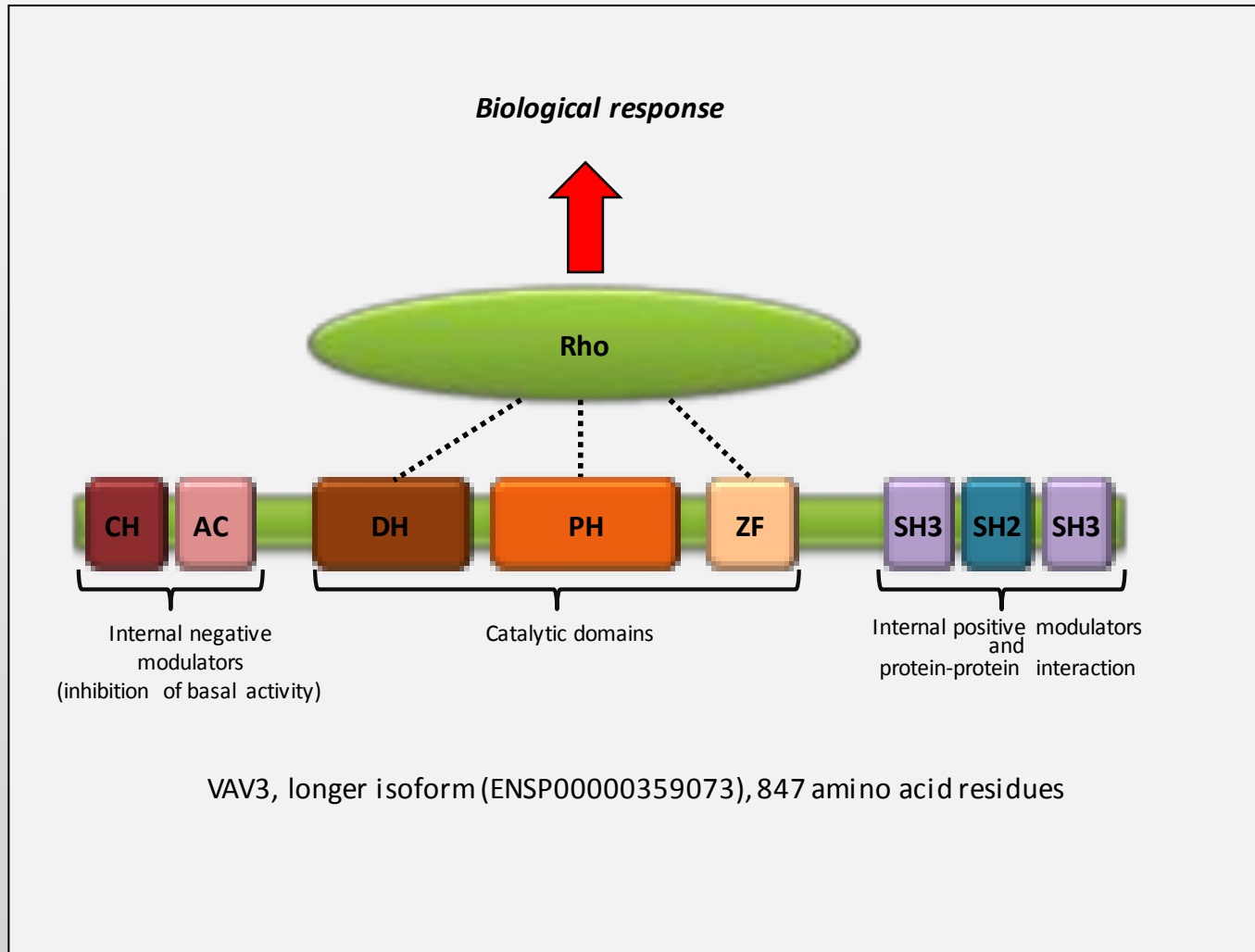
# Association signal in JGWAS



*Gene centered data:*

- Location: 1p13.3
- Size: 400 kbps
- 2 isoforms has been reported

# VAV3 structure



# VAV3 and Schizophrenia

- Related to the axon guidance (process identified as disturbed in schizophrenic patients)
- Identified by linkage study in Japanese population

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Schizophrenia

Increased risk for schizophrenia

Gene X  
(hypothetical schizophrenia susceptibility gene)

Sequencing

GWAS

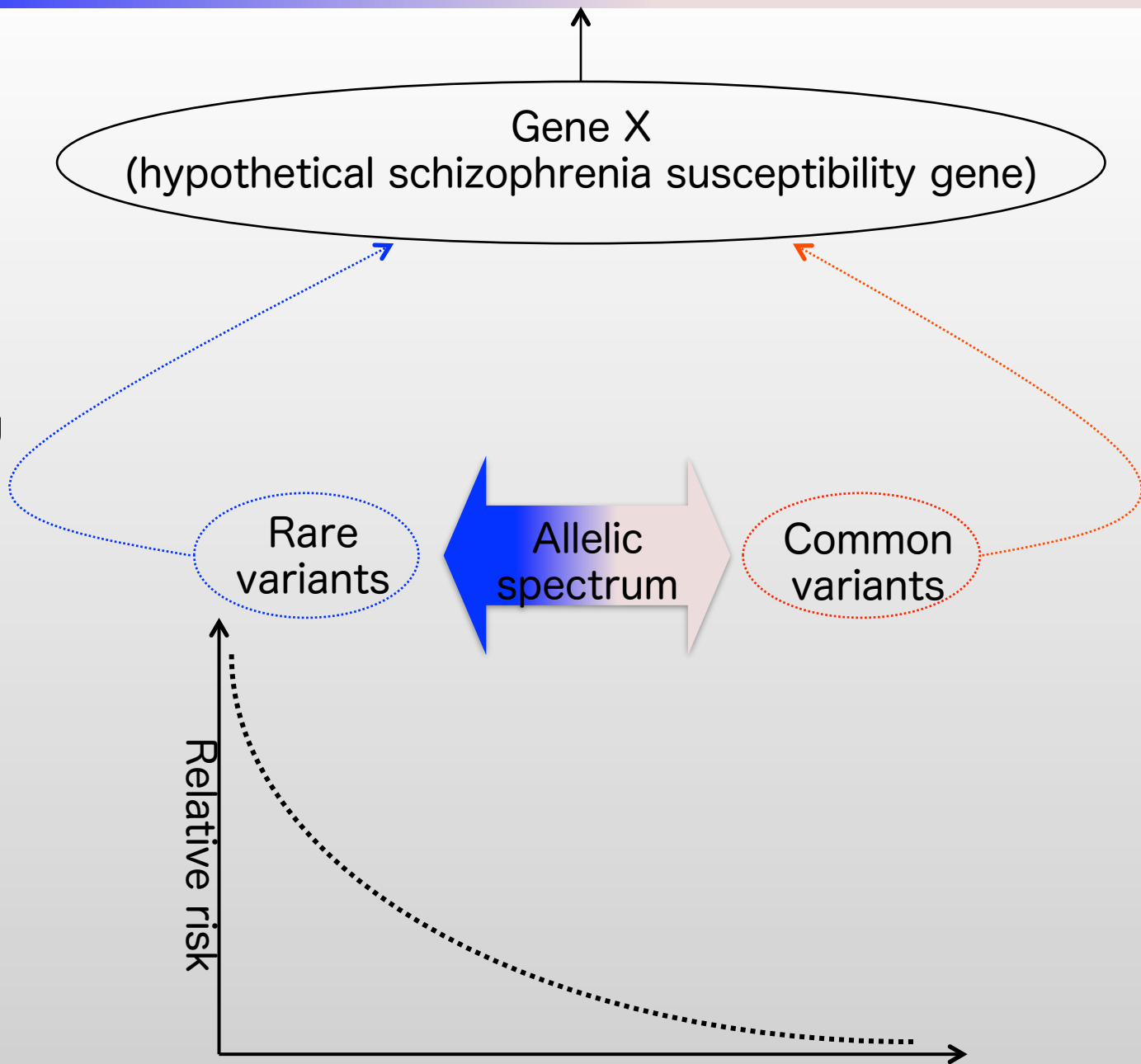
Rare  
variants

Allelic  
spectrum

Common  
variants

Relative risk

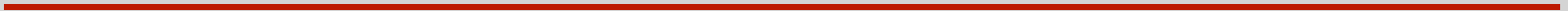
Population frequency



# Our study of *VAV3*

- Mutation screening and association analysis of associated rare variants
- Check the effect of associated common SNP on brain morphology (MRI)

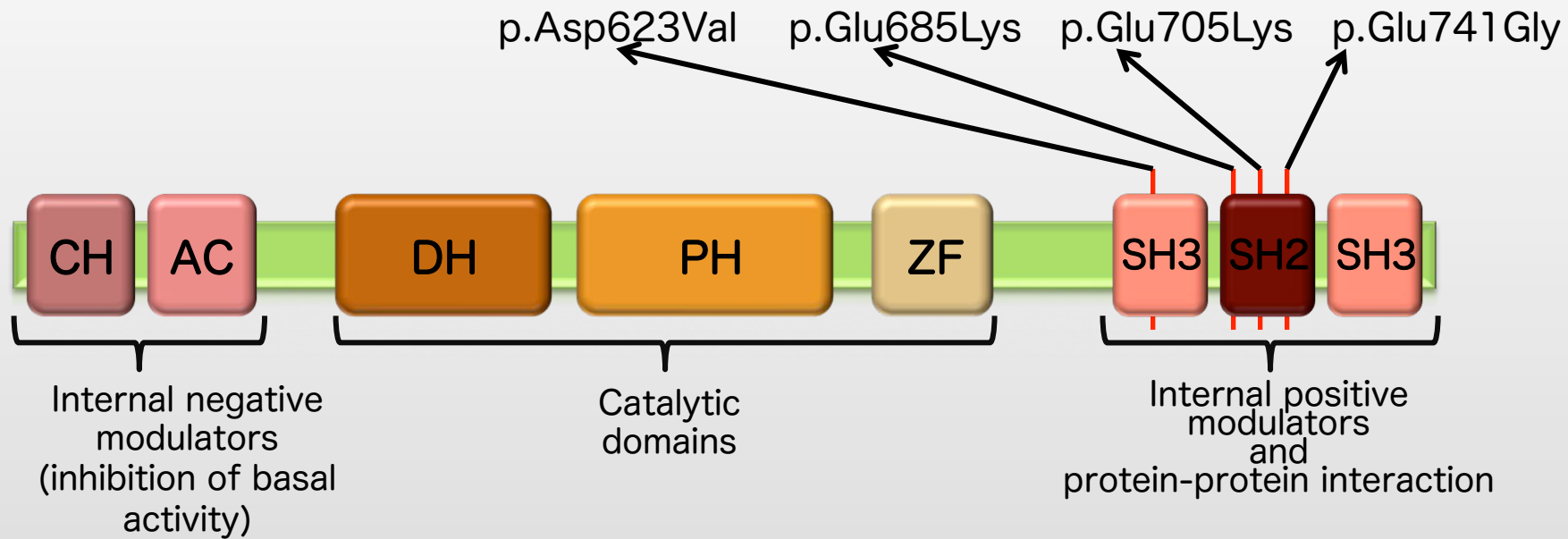
# FOCUS ON THE RARE VARIANTS



# Mutation screening -strategy-

- Custom array resequencing method
- Focusing on exons only
  - Novel rare missense/nonsense variants
- Screening only cases (N=321)
- Follow up candidates in large case-control sample

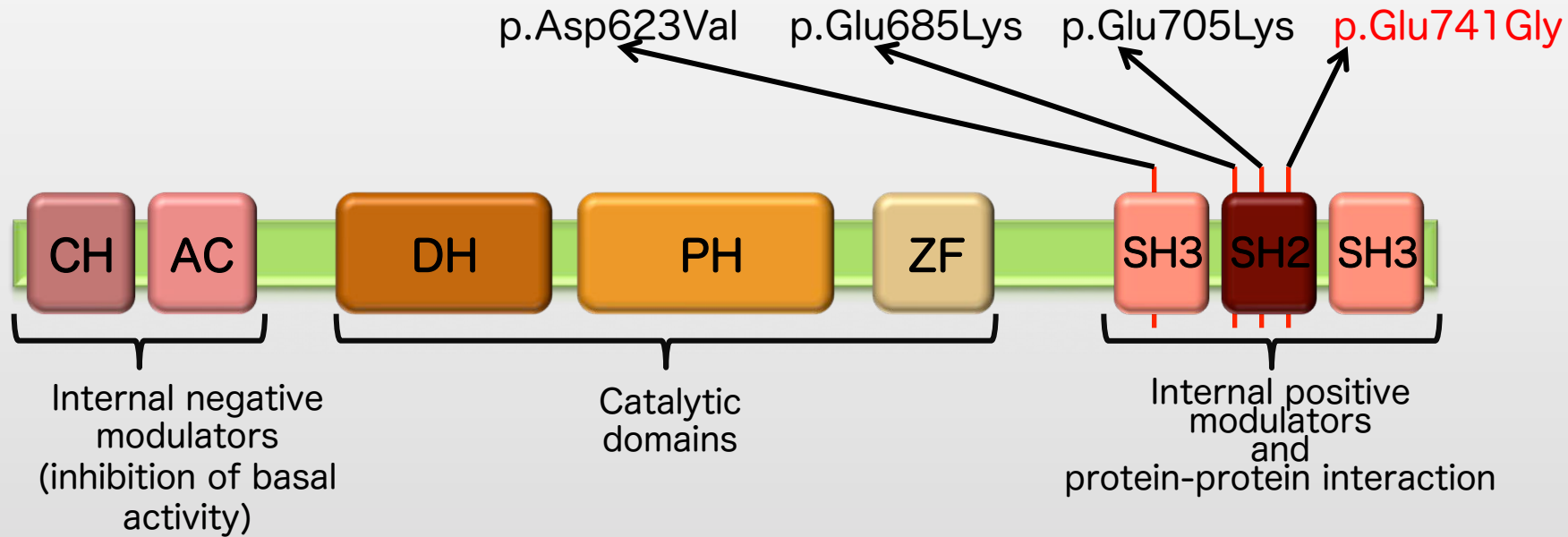
# Discovered variants



Species	Protein	p.Asp623Val	p.Glu685Lys	p.Glu705Lys	p.Glu741Gly	Pairwise Alignment Scores vs H.sapiens		
						Protein identity %	DNA Identity %	Substitution Rate
Homo sapiens	NP_006104.4	L Q A G <b>D</b> T V	L Q A E T <b>E</b> L	K E S G <b>E</b> Y A	L M <b>E</b> L V E Y	n/a	n/a	n/a
Canis lupus familiaris	XP_537047.2	I Q A G <b>D</b> T V	L Q A E T <b>E</b> L	K E S G <b>E</b> Y A	L M <b>E</b> L V E Y	97.1	92.9	0.075
Bos taurus	XP_615898.4	I Q A G <b>D</b> T V	L Q A E T <b>E</b> L	R E S G <b>E</b> Y A	L M <b>E</b> L V E Y	95.7	92.8	0.076
Mus musculus	NP_065251.2	I Q A G <b>D</b> T V	L Q A E T <b>E</b> L	K E S G <b>E</b> Y A	L M <b>E</b> L V E Y	95.4	90.5	0.101
Rattus norvegicus	XP_227600.4	I Q A G <b>D</b> T V	L Q A E T <b>E</b> L	K E S G <b>E</b> Y A	L M <b>E</b> L V E Y	94.8	89.6	0.112
Gallus gallus	NP_996745.1	I Q I G <b>D</b> T I	L Q A E S <b>E</b> L	K E S G <b>E</b> Y A	L M <b>E</b> L V D Y	86.3	81.3	0.215
Danio rerio	XP_687553.3	A Q I G <b>D</b> V I	H H A E S <b>E</b> L	R E S R <b>E</b> Y A	V L G L V E Y	71.2	68.2	0.414
Caenorhabditis elegans	NP_001041223.1	F A K G <b>D</b> R I	A K A E S T L	K N R K Q T A	T V <b>E</b> L V Q Y	35.3	46.0	0.953

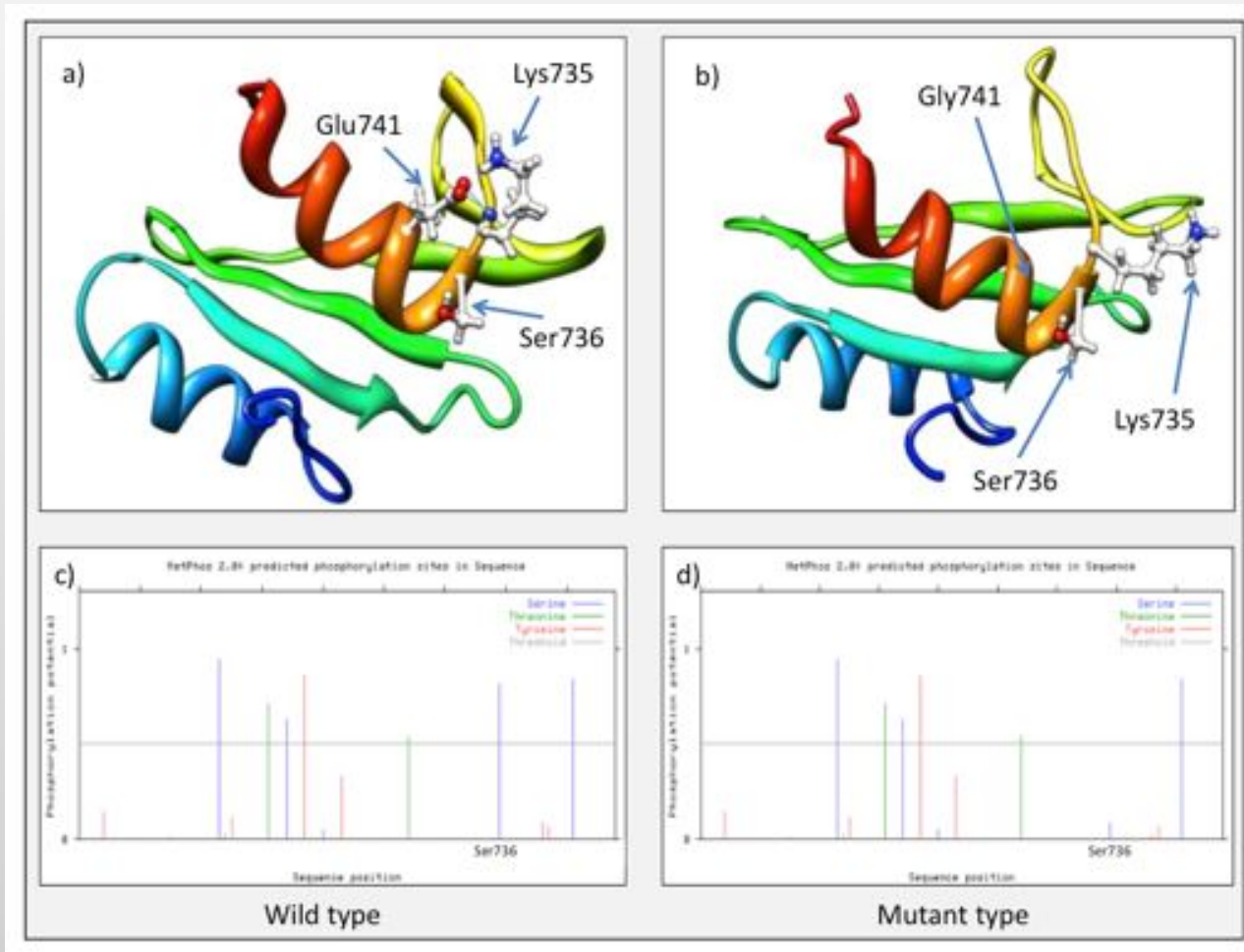


# Association analysis

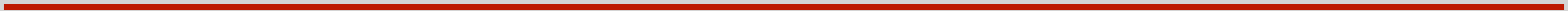


Chr	Variant	Physical position <sup>1</sup>	Protein domain	M <sup>2</sup>	JMut (minor allele count)	m <sup>2</sup>	JPN_GWAS (MAF) N=1100				Rep_JPN (MAF) N=3000				Meta analysis N=4200	
							Cases <sup>3</sup>	Control <sup>3</sup>	P <sub>allele</sub>	OR <sup>4</sup>	Cases <sup>3</sup>	Control <sup>3</sup>	P <sub>allele</sub>	OR <sup>4</sup>	P <sub>CMH</sub> <sup>5</sup>	OR <sup>4</sup>
1	p.Asp623Val	107,986,810	N-SH3	A	2	T	0.0006964	0.0008993	0.8561	0.7742	0.0003344	0	0.3171	NA	0.6649	1.662
1	p.Glu685Lys	107,947,271	SH2	G	1	A	0.0006974	0.001821	0.4151	0.3824	0.0003336	0	0.3168	NA	0.8415	0.8246
1	p.Glu705Lys	107,947,211	SH2	G	3	A	0.0007022	0.0009074	0.8557	0.7737	0.0006658	0.0003311	0.5605	2.011	0.7354	1.355
1	p.Glu741Gly	107,940,485	SH2	A	7	G	0.004972	0.01087	<b>0.09038</b>	0.4547	0.0074480	0.0117400	<b>0.0897</b>	0.6314	<b>0.02065</b>	0.5821

# In-silico modeling of SH2 domain (VAV3)



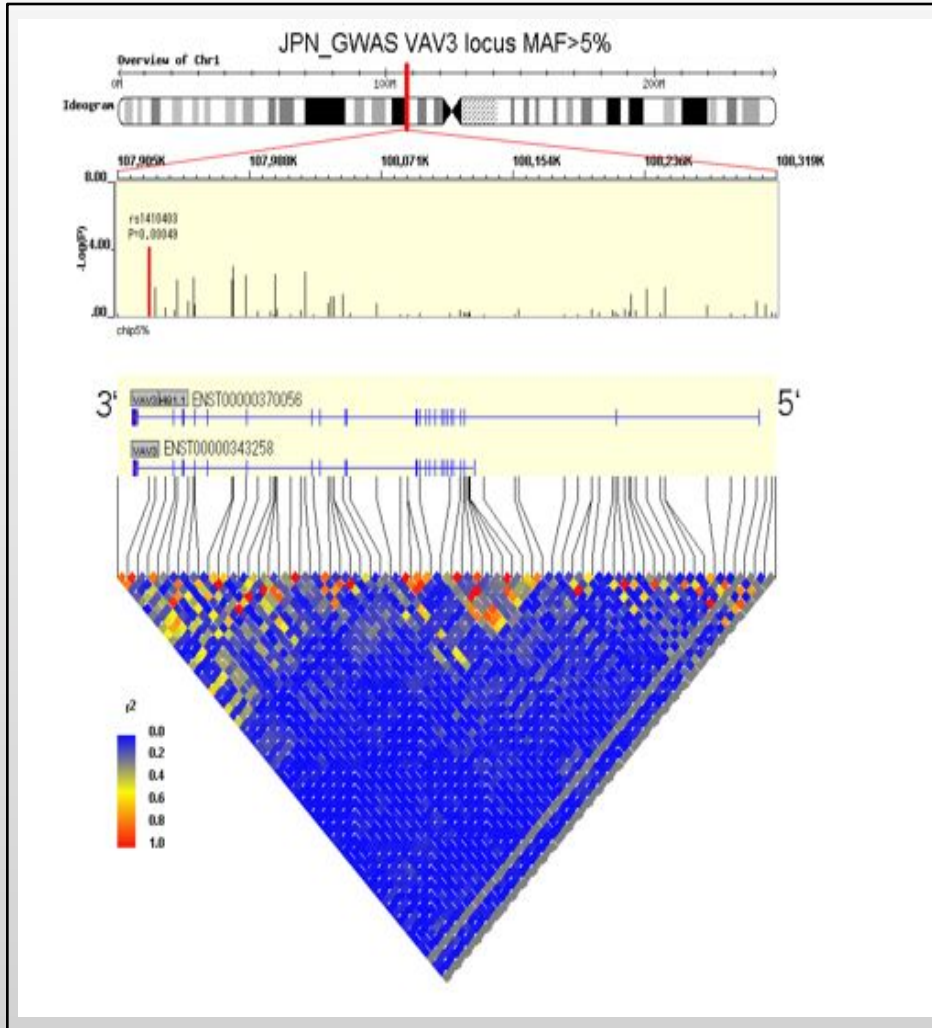
# COMMON VARIANTS



# Voxel based morphometry

- We followed up rs1410403 (SNP associated with schizophrenia in JGWAS)
- $P_{\text{CMH}}=9.3 \times 10^{-4}$ , odds ratio=0.86
- case control sample was comprised of 100 patients with schizophrenia ( $38.3 \pm 13.0$ ) and 264 healthy controls ( $36.7 \pm 11.9$ )
- All magnetic resonance imaging was performed on a 1.5T GE Sigma EXCITE system

# Association signal in JGWAS



*Gene centered data:*

- Location: 1p13.3
- Size: 400 kbps
- 2 isoforms has been reported

# Effect on the brain morphology

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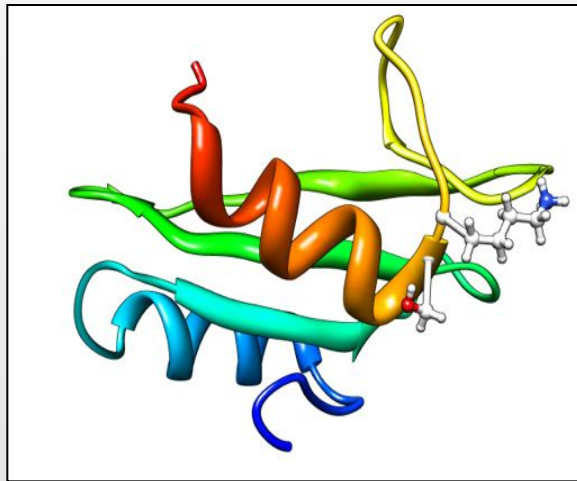
- Rs1410403 (A/G) was followed up
- Minor allele frequency (G) in *CONTROLS* of this SNP was 37%
- Minor allele frequency (G) in *CASES* of this SNP was 32%
- OR=0.86

# Summary of findings

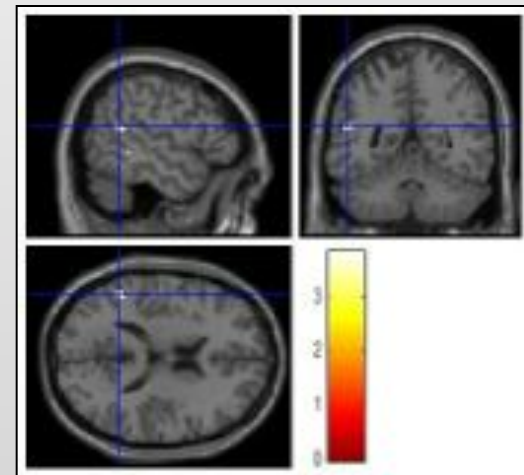
- p.Glu741Gly might be responsible for genetic susceptibility to schizophrenia-biological consequences are unknown (OR=0.52)
- Rs1410403 might influence volume of gray matter in schizophrenic patients (OR=0.86)

# VAV3 effects of associated variants

High  
Odds ratio  
Low



- Change in protein structure
- Biological consequences unknown



- Protein structure unchanged
- Variation in gray matter volume

Low

Frequency

High



# Challenges and future directions

- Consideration of the effect of environmental factors such as maternal infection or drug use
- Consideration of epigenetic mechanism
- Use of high-throughput whole genome sequencing
  - Has potential to detect virtually all SNPs/SNVs
  - Will provide comprehensive information of individual at DNA-single base pair level
  - Very costly

# Take Home Messages

- Strong genetic basis of SZ proven from age-old family studies to the ultra modern GWAS
- Specific genes and loci are not definitely established (i.e. lack of consistent replication)
- Problem arising from multiple factors
  - Lack of operationalized phenotypes
  - Presence of large number of risk variants with relatively small effect size
  - Cost, manpower and expertise inadequacy

*Thank you for your  
attention!  
Any questions or  
comments?*

