Identification by genetics of neurotransmitter and other transporters expressed in the brain as potential novel targets for schizophrenia

13rd November 2014, Drug Transporters symposium, target of avoid?

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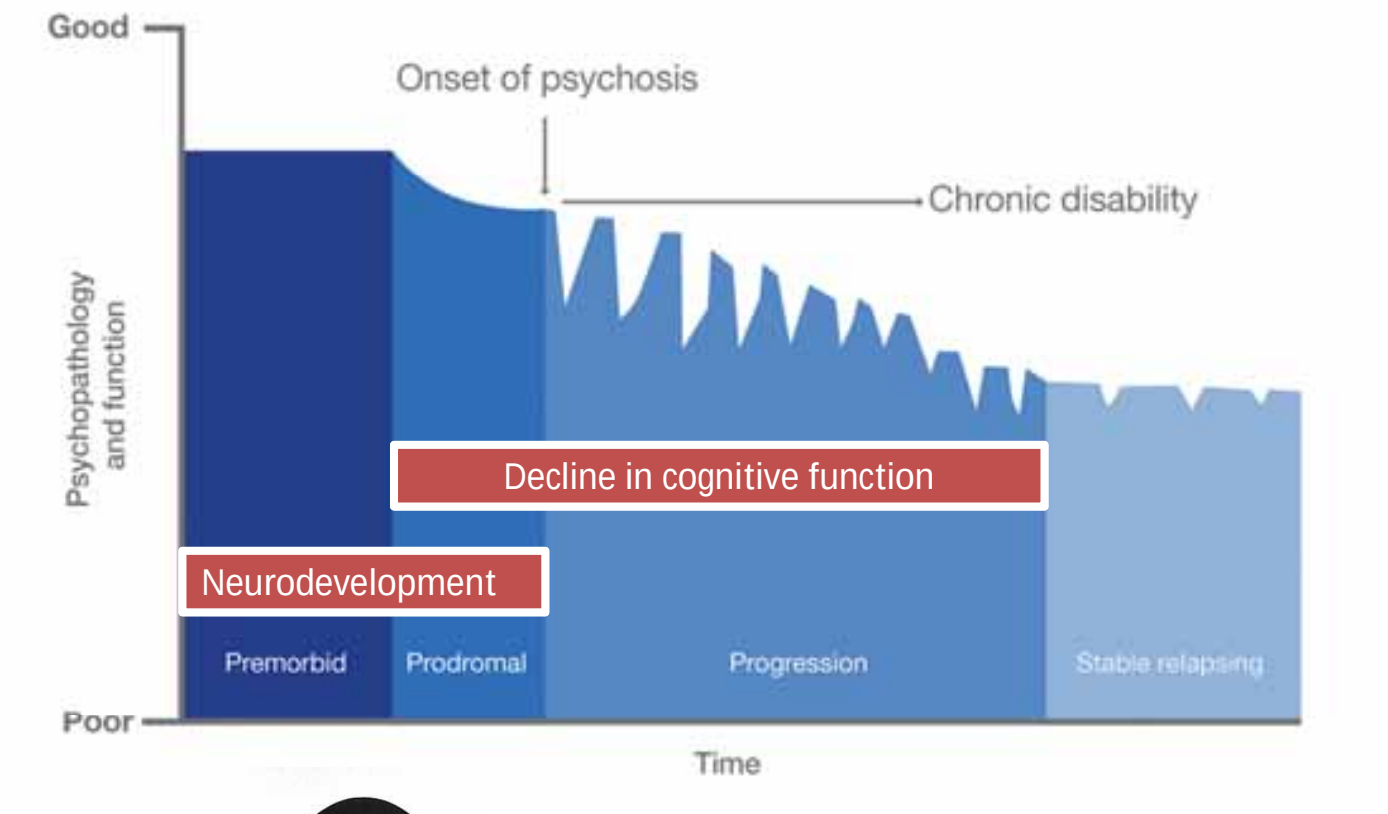
Eli Lilly and Company; King's College London
Schizophrenia Working Group of the
Psychiatric Genomics Consortium

Schizophrenia

Characteristic symptoms

- Two or more of the following:
  - Delusions
  - Hallucinations
  - Disorganized speech (formal thought disorder)
  - Grossly disorganized or catatonic behavior
  - Negative symptoms: Blunted affect, alogia, avolition
- Social or occupational dysfunction
- Significant duration: Continuous signs of the disturbance persist for at least six months.
- Up to 1% of the population

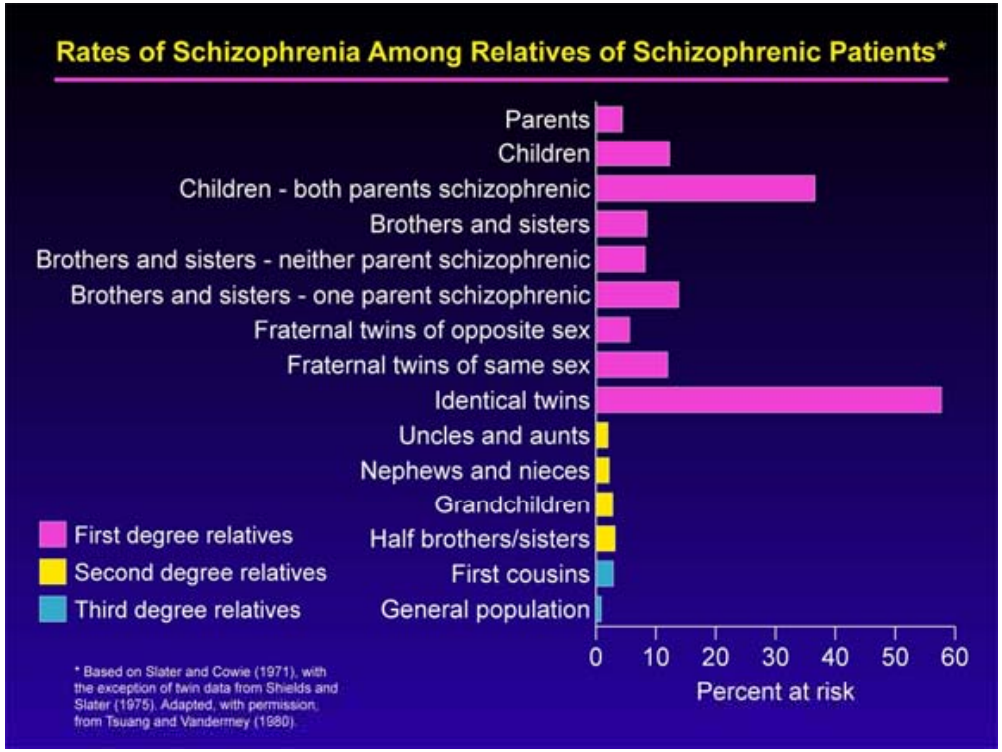


Dementia praecox (a "premature dementia" or "precocious madness") refers to a chronic, deteriorating psychotic disorder characterized by rapid cognitive disintegration, usually beginning in the late teens or early adulthood.

Emil Kraepelin (15 February 1856 – 7 October 1926)

Schizophrenia is a complex genetic disorders

strong genetic influence with a role for environmental factors



ADHD: 83-92%

Schizophrenia: 60-80%

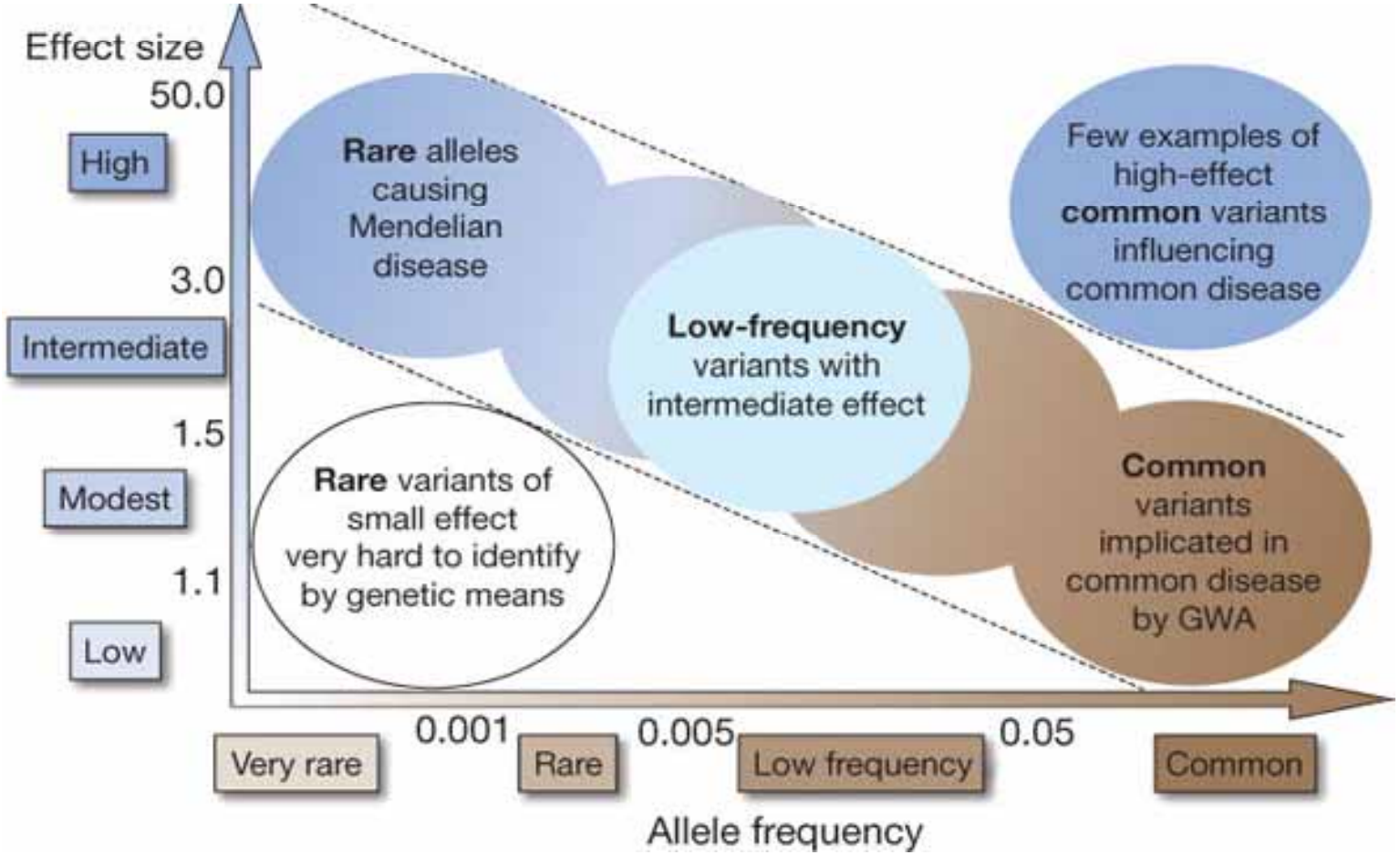
T1 diabetes: 26%

Autism: 70-90%

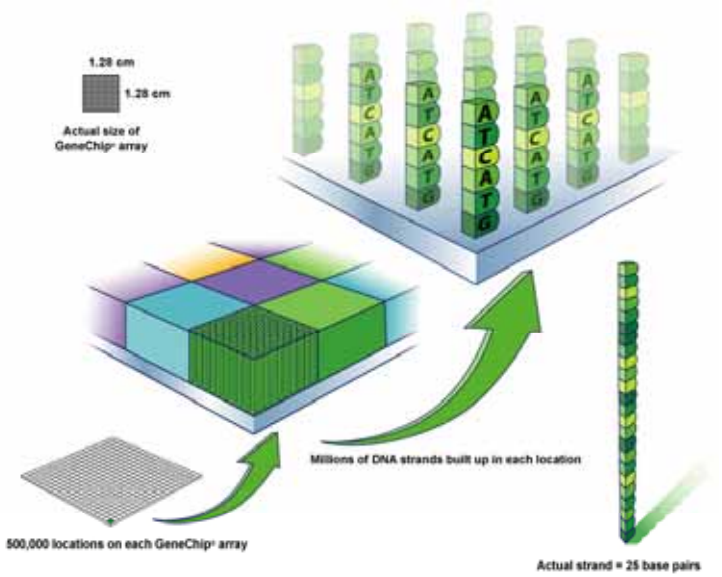
Depression: 30-40%

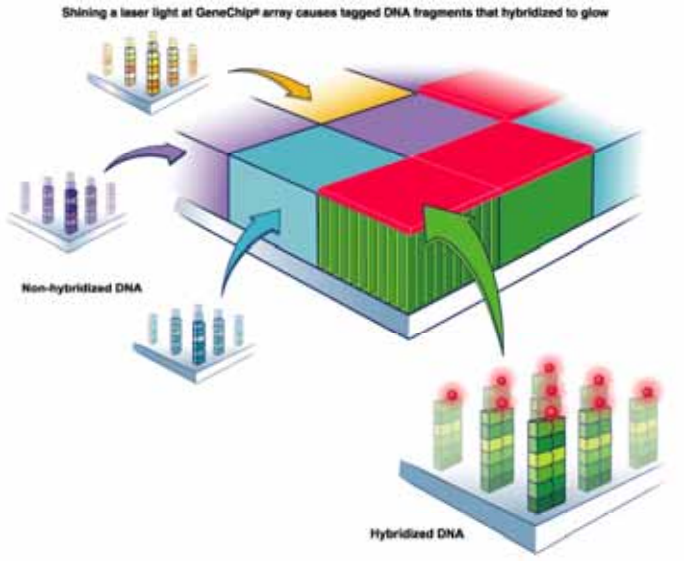
Colorectal cancer: 35%

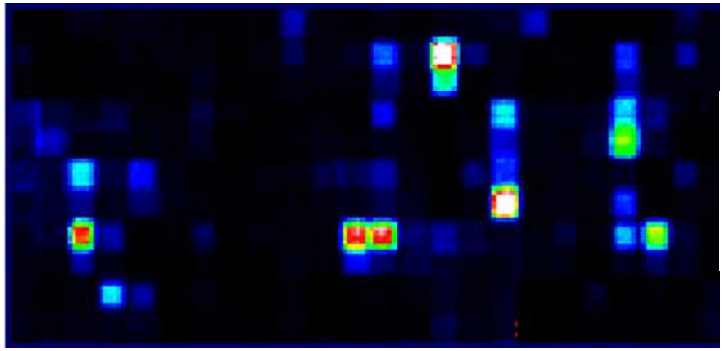
Heart disease: 39-56%


Effect size
50.0
High
3.0
Intermediate
1.5
Modest
1.1
Low
Rare alleles causing Mendelian disease
Few examples of high-effect common variants influencing common disease
Low-frequency variants with intermediate effect
Rare variants of small effect very hard to identify by genetic means
Common variants implicated in common disease by GWA
0.001
0.005
0.05
Very rare
Rare
Low frequency
Common
Allele frequency

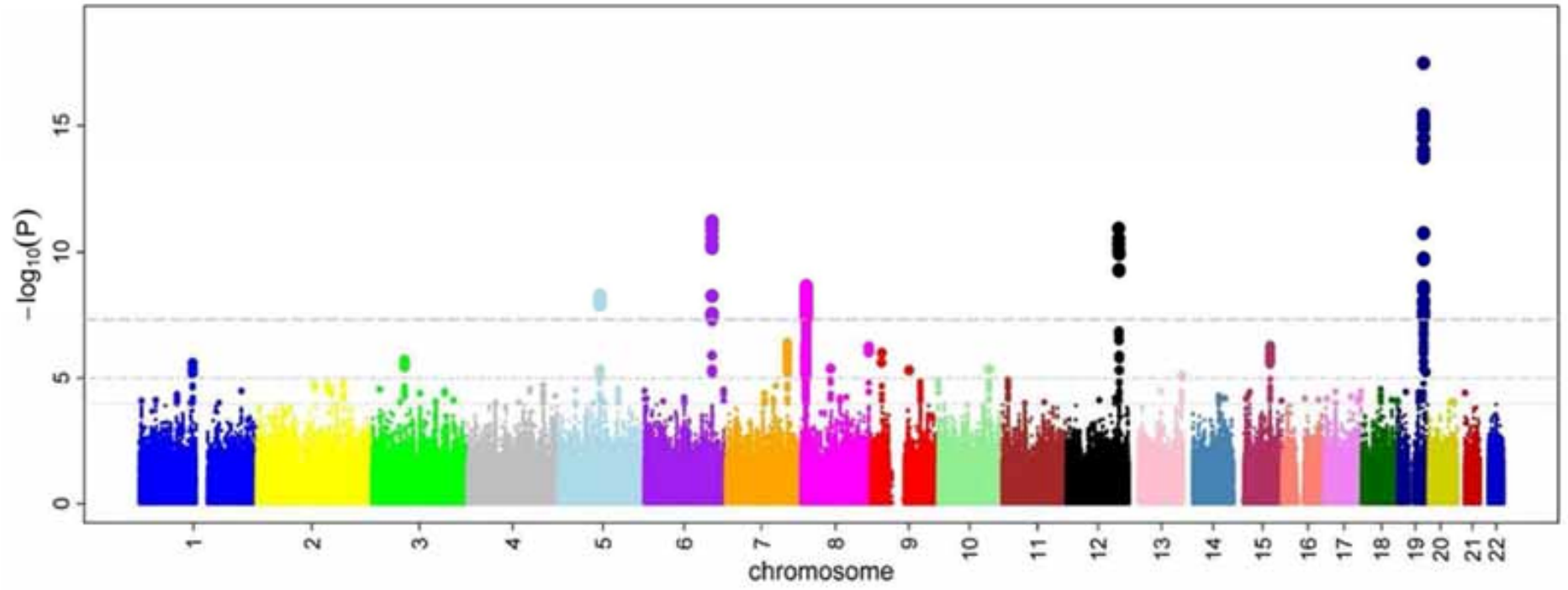
The age of arrays: genome-wide association



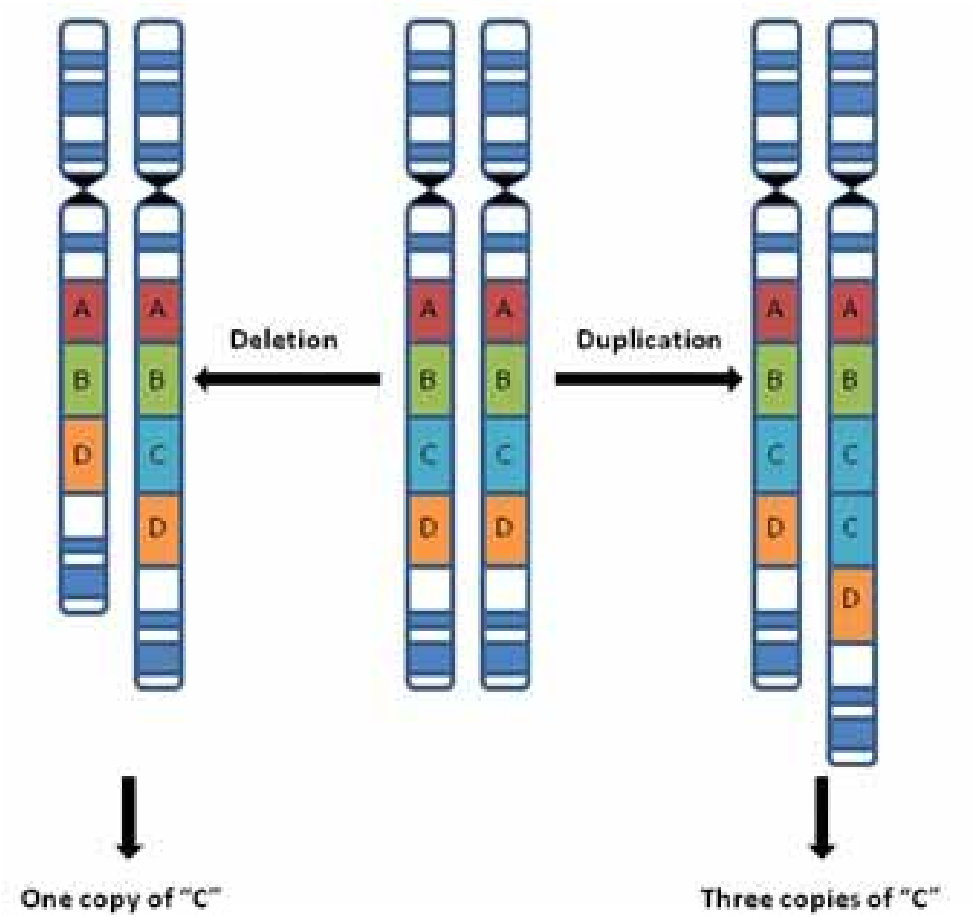




Manhattan plot



Copy number mutations



Large recurrent microdeletions associated with schizophrenia

Hreinn Stefansson[1]*, Dan Rujescu[2]*, Sven Cichon[3,4]*, Olli P. H. Pietiläinen[5], Andres Ingason[1], Stacy Steinberg[1], Ragnheidur Fossdal[1], Engilbert Sigurdsson[6], Thordur Sigmundsson[6], Jacobine E. Buizer-Voskamp[7], Thomas Hansen[8,9], Klaus D. Jakobsen[8,9], Pierandrea Muglia[10], Clyde Francks[10], Paul M. Matthews[11], Arnaldur Gylfason[1], Bjarni V. Halldorsson[1], Daniel Gudbjartsson[1], Thorgeir E. Thorgeirsson[1], Asgeir Sigurdsson[1], Adalbjorg Jonasdottir[1], Aslaug Jonasdottir[1], Asgeir Bjornsson[1], Sigurborg Mattiasdottir[1], Thorarinn Blondal[1], Magnus Haraldsson[6], Brynja B. Magnusdottir[6], Ina Giegling[2], Hans-Jürgen Möller[2], Annette Hartmann[2], Kevin V. Shianna[12], Dongliang Ge[12], Anna C. Need[12], Caroline Crombie[13], Gillian Fraser[13], Nicholas Walker[14], Jouko Lonnqvist[15], Jaana Suvisaari[15], Annamarie Tuulio-Henriksson[15], Tiina Paunio[5,15], Timi Toulopoulou[16], Elvira Bramon[16], Marta Di Forti[16], Robin Murray[16], Mirella Ruggeri[17], Evangelos Vassos[16], Sarah Tosato[17], Muriel Walshe[16], Tao Li[16,18], Catalina Vasilescu[3], Thomas W. Mühleisen[3], August G. Wang[19], Henrik Ullum[20], Srdjan Djurovic[21,22], Ingrid Melle[22], Jes Olesen[23], Lambertus A. Kiemeney[24], Barbara Franke[25], GROUP†, Chiara Sabatti[26], Nelson B. Freimer[27], Jeffrey R. Gulcher[1], Unnur Thorsteinsdottir[1], Augustine Kong[1], Ole A. Andreassen[21,22], Roel A. Ophoff[7,27], Alexander Georgi[28], Marcella Rietschel[28], Thomas Werge[8], Hannes Petursson[6], David B. Goldstein[12], Markus M. Nöthen[3,4], Leena Peltonen[5,29,30], David A. Collier[16,18], David St Clair[13] & Kari Stefansson[1,31]

Reduced fecundity, associated with severe mental disorders[1], places negative selection pressure on risk alleles and may explain, in part, why common variants have not been found that confer risk of disorders such as autism[2], schizophrenia[3] and mental retardation[4]. Thus, rare variants may account for a larger fraction of the overall genetic risk than previously assumed. In contrast to rare single nucleotide mutations, rare copy number variations (CNVs) can be detected using genome-wide single nucleotide polymorphism arrays. This has led to the identification of CNVs associated with mental retardation[4,5] and autism[2]. In a genome-wide search for CNVs associating with schizophrenia, we used a population-based sample to identify *de novo* CNVs by analysing 9,878 transmissions from parents to offspring. The 66 *de novo* CNVs identified were tested for association in a sample of 1,433 schizophrenia cases and 33,250 controls. Three deletions at 1q21.1, 15q11.2 and 15q13.3 showing nominal association with schizophrenia in the first sample (phase I) were followed up in a second sample of 3,285 cases and 7,951 controls (phase II). All three deletions significantly associate with schizophrenia and related psychoses in the combined sample. The identification of these rare, recurrent risk variants, having occurred independently in multiple founders and being subject to negative selection, is important in itself. CNV analysis may also point the way to the identification of additional and more prevalent risk variants in genes and pathways involved in schizophrenia.

The approach we used here was to use a large population-based discovery sample to identify *de novo* CNVs, followed by testing for association in a sample of patients with schizophrenia and psychoses (phase I) and finally replicating the most promising variants from phase I in a second larger sample (phase II). The discovery phase, where

Neurexin 1 alpha deletions in schizophrenia

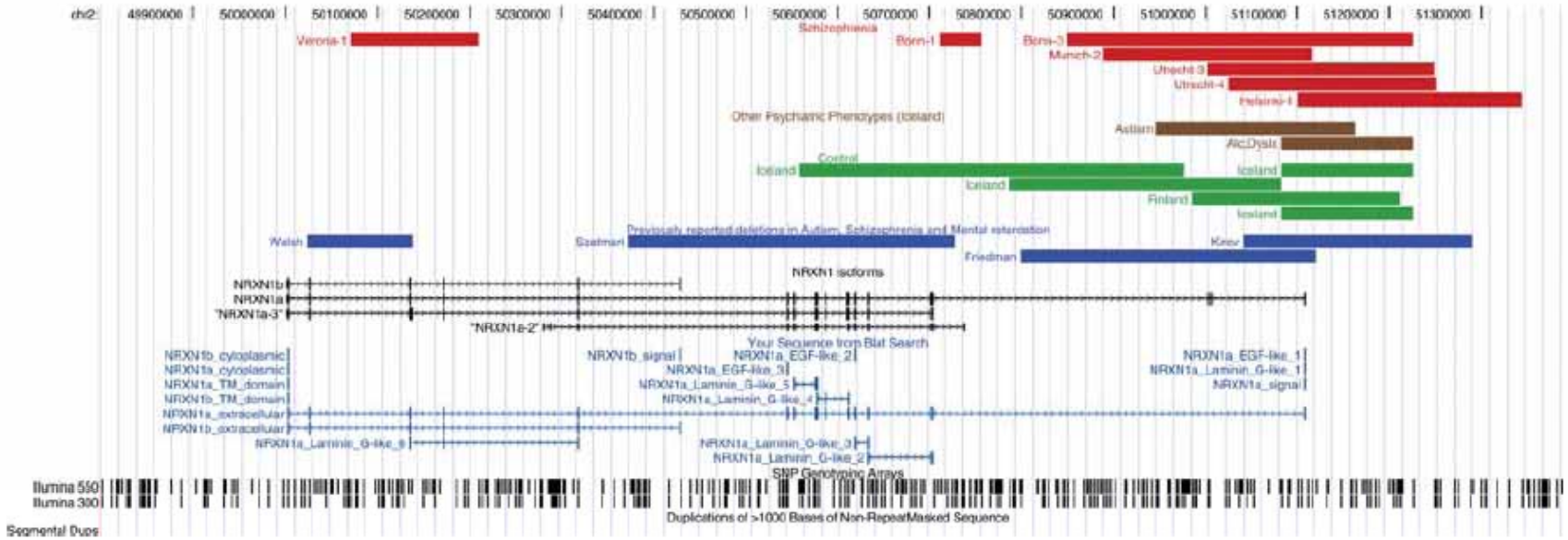
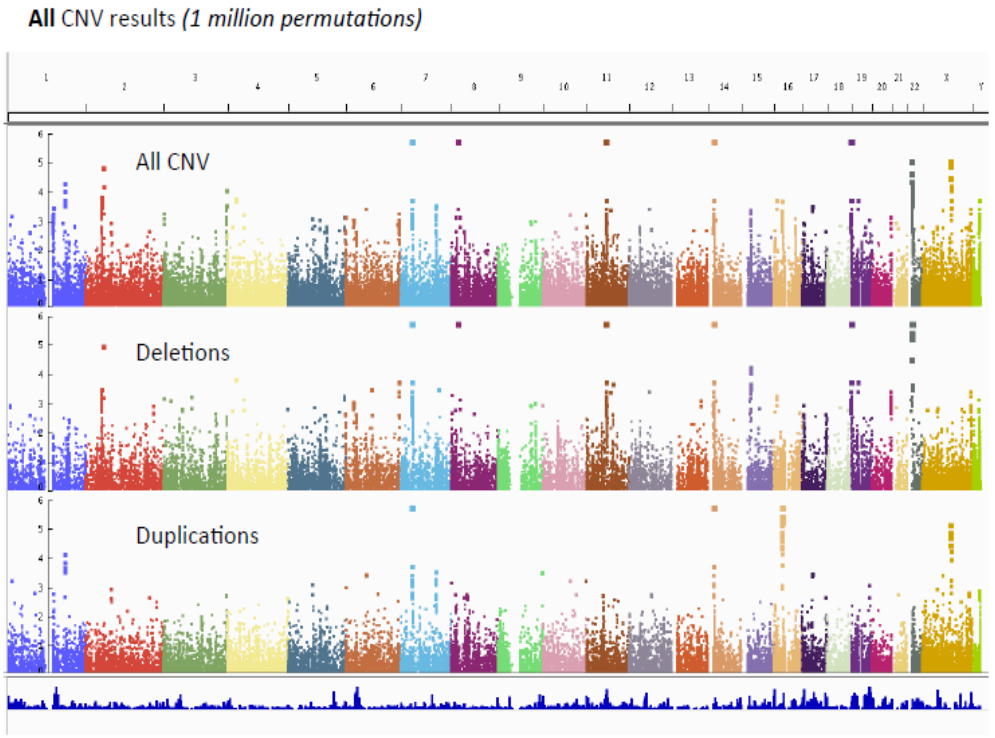
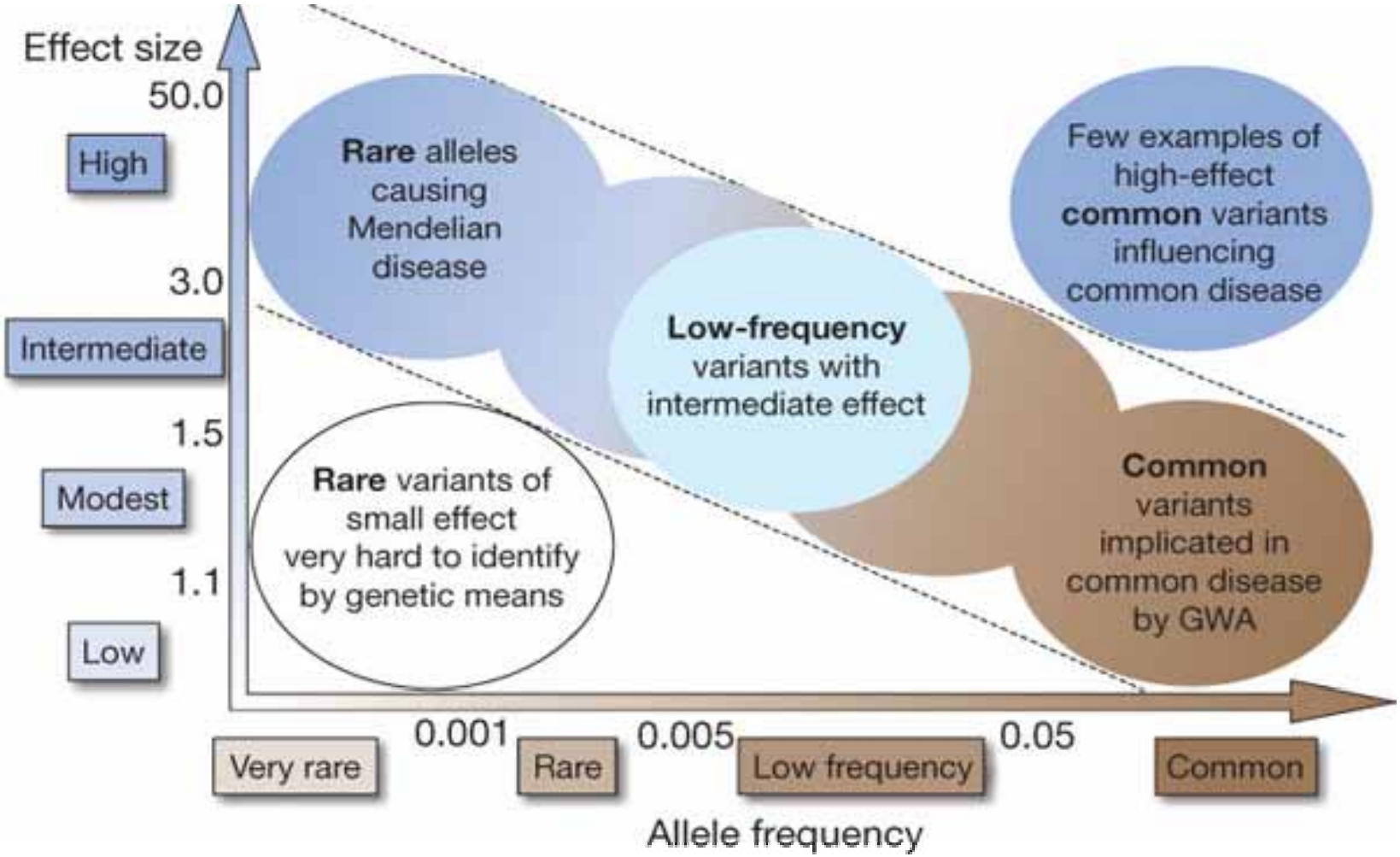


Figure 2. UCSC browser output showing the positions of exon-disrupting CNVs discovered in the study relative to the 2p16.3 CNVs from the Kirov *et al.* (2008), Walsh *et al.* (2008), Friedman *et al.* (2008) and Szatzmar *et al.* (2008) studies and known (schizophrenia, red lines; other psychiatric diagnoses, brown lines; controls, green lines; previously described CNVs, blue lines). The four putative Neurexin isoforms are shown below the deletions, along with protein domains aligned to genomic sequence.

Copy number variants in schizophrenia

- **22q11.2 deletion** (~45 genes) 0.3%; OR~25
- **3q29 deletion** (25 genes) 0.08% OR ~ 25
- **16p11.2 proximal duplication** (~28 genes) 0.31% OR~16
- **16p11.2 distal deletion** (9 genes) 0.11% OR~11
- **15q13.3 interstitial deletion** (CHRNA7) 0.19% OR~9
- **7qter duplication (VIPR2)** 0.13% OR~3-7

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Very rare
Rare
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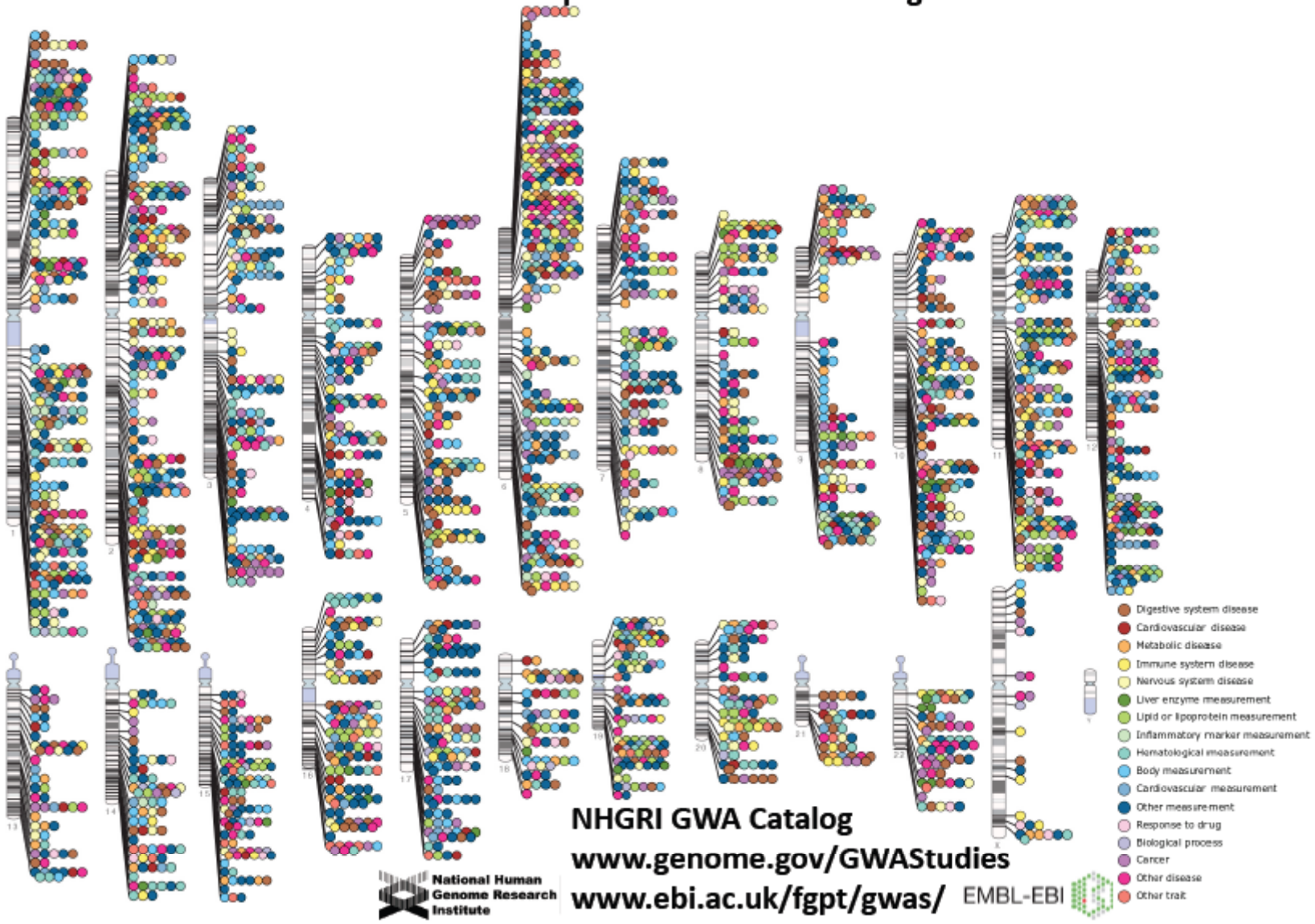
Genome wide association: GWAS

- Basic Idea: examination a significant proportion of COMMON genetic variation across the human genome, in order to to identify genetic associations
- Uses a large number (300,000-2 million) SNP markers spread throughout the genome
- Look for pair-wise associations between the genotypes at each locus and disease status
- **A numbers game: all disorders have succeeded in finding multiple loci once samples size is large enough (>10,000 cases)**

Published Genome-Wide Associations through 12/2012

Published GWA at $p \leq 5X10^{-8}$ for 17 trait categories



NHGRI GWA Catalog
www.genome.gov/GWAStudies
www.ebi.ac.uk/fgpt/gwas/

National Human Genome Research Institute

EMBL-EBI

nature Vol 460 | 6 August 2009 | doi:10.1038/nature08186

LETTERS

Common variants conferring risk of schizophrenia

A list of authors and their affiliations appears at the end of the paper

Schizophrenia is a complex disorder, caused by both genetic and environmental factors and their interactions. Research on pathogenesis has traditionally focused on neurotransmitter systems in the brain, particularly those involving dopamine. Schizophrenia has been considered a separate disease for over a century, but in the absence of clear biological markers, diagnosis has historically been based on signs and symptoms. A fundamental message emerging from genome-wide association studies of copy number variations (CNVs) associated with the disease is that its genetic basis does not necessarily conform to classical nosological disease boundaries. Certain CNVs confer not only high relative risk of schizophrenia but also of other psychiatric disorders[1–3]. The structural variations associated with schizophrenia can involve several genes and the phenotypic syndromes, or the 'genomic disorders', have not yet been characterized[4]. Single nucleotide polymorphism (SNP)-based genome-wide association studies with the potential to implicate individual genes in complex diseases may reveal underlying biological pathways. Here we combined SNP data from several large genome-wide scans and followed up the most significant association signals. We found significant association with several markers spanning the major histocompatibility complex (MHC) region on chromosome 6p21.3-22.1, a marker located upstream of the neurogranin gene (*NRGN*) on 11q24.2 and a marker in intron four of transcription factor 4 (*TCF4*) on 18q21.2. Our findings implicating the MHC region are consistent with an immune component to schizophrenia risk, whereas the association with *NRGN* and *TCF4* points to perturbation of pathways involved in brain development, memory and cognition.

3,634 controls from the Netherlands; set 2, 3,330 cases and 6,892 controls from Denmark (Aarhus), Denmark (Copenhagen), Germany (Bonn), Germany (Munich), Hungary, the Netherlands, Norway, Russia and Sweden; set 3, 287 cases and 3,987 controls from Finland; set 4, 667 cases and 1,042 controls from Spain (Santiago) and Spain (Valencia)) (Supplementary Table 3).

Three markers, all in the extended MHC region on the short arm of chromosome 6, showed genome-wide significance in the combination of SGENE-plus and the follow-up samples described above (Table 1). In addition, four other markers—two in the MHC region, one at 11q24.2 and one at 18q21.2—showed genome-wide significance when results from the International Schizophrenia Consortium and the Molecular Genetics of Schizophrenia study were included (Table 1).

In the MHC region on chromosome 6p21.3-22.1, the five genome-wide significant markers (P ranging from 1.1×10^{-9} to 1.4×10^{-12} in all samples combined) have risk alleles with average control frequencies between 78% and 92% (Table 1). Combined odds ratios (ORs) for the markers range from 1.15 to 1.24 (Table 1) with no significant heterogeneity between the study groups ($P > 0.25$, Supplementary Table 4). For all of the markers, the multiplicative model for risk provides an adequate fit ($P > 0.62$).

Despite spanning about five megabases (Mb), the five chromosome 6p markers cover only about 1.4 centimorgans (cM) and substantial linkage disequilibrium exists between them (Supplementary Table 5). The association of rs6932590 (the most significant marker), however, cannot account for all of the association of the four remaining markers (Supplementary Table 6).

Human Molecular Genetics, 2011, Vol. 20, No. 20 4076–4081
doi:10.1093/hmg/ddr325
Advance Access published on July 26, 2011

Common variants at *VRK2* and *TCF4* conferring risk of schizophrenia

Stacy Steinberg[1], Simone de Jong[2], Irish Schizophrenia Genomics Consortium[†], Ole A. Andreassen[3], Thomas Werge[4], Anders D. Børglum[5,6], Ole Mors[5], Preben B. Mortensen[7], Omar Gustafsson[1,3], Javier Costas[8], Olli P. H. Pietiläinen[10,11], Ditte Demontis[6], Sergi Papiol[12,13], Johanna Huttenlocher[1,51], Manuel Mattheisen[15], René Breuer[16], Evangelos Vassos[17,18], Ina Giegling[19], Gillian Fraser[20], Nicholas Walker[21], Annamari Tuulio-Henriksson[22], Jaana Suvisaari[22], Jouko Lönnqvist[22], Tiina Paunio[23], Ingrid Agartz[3], Ingrid Melle[3], Srdjan Djurovic[3], Eric Strengman[2], GROUP[†], Gesche Jürgens[24], Birte Glenthøj[25], Lars Terenius[26], David M. Hougaard[27], Torben Ørntoft[28], Carsten Wiuf[29], Michael Didriksen[30], Mads V. Hollegaard[27], Merete Nordentoft[31], Ruud van Winkel[32,33], Gunter Kenis[33], Lilia Abramova[34], Vasily Kaleda[34], Manuel Arrojo[9], Julio Sanjuán[35], Celso Arango[36], Swetlana Sperling[13], Moritz Rossner[12,14], Michele Ribolsi[37], Valentina Magni[37], Alberto Siracusano[37], Claus Christiansen[38], Lambertus A. Kiemeney[39], Jan Veldink[40], Leonard van den Berg[40], Andres Ingason[1], Pierandrea Muglia[41], Robin Murray[17], Markus M. Nöthen[42], Engilbert Sigurdsson[43,44], Hannes Petursson[43,44], Unnur Thorsteinsdottir[1,44], Augustine Kong[1], I. Alex Rubino[37], Marc De Hert[32], János M. Réthelyi[45], István Bitter[45], Erik G. Jönsson[26], Vera Golimbet[34], Angel Carracedo[46], Hannelore Ehrenreich[12,13], Nick Craddock[47], Michael J. Owen[47], Michael C. O'Donovan[47], Wellcome Trust Case Control Consortium 2[†], Mirella Ruggeri[48], Sarah Tosato[48], Leena Peltonen[10,11,49], Roel A. Ophoff[2,52], David A. Collier[18], David St Clair[20], Marcella Rietschel[16], Sven Cichon[15,42,50], Hreinn Stefansson[1], Dan Rujescu[19] and Kari Stefansson[1,44,*]

Common sequence variants have recently joined rare structural polymorphisms as genetic factors with strong evidence for association with schizophrenia. Here we extend our previous genome-wide association study and meta-analysis (totalling 7 946 cases and 19 036 controls) by examining an expanded set of variants using an enlarged follow-up sample (up to 10 260 cases and 23 500 controls). In addition to previously reported alleles in the major histocompatibility complex region, near *neurogranin* (*NRGN*) and in an intron of *transcription factor 4* (*TCF4*), we find two novel variants showing genome-wide significant association: rs2312147[C], upstream of *vaccinia-related kinase 2* (*VRK2*) [odds ratio (OR) = 1.09, $P = 1.9 \times 10^{-8}$] and rs4309482[A], between *coiled-coiled domain containing 68* (*CCDC68*) and *TCF4*, about 400 kb from the previously described risk allele, but not accounted for by its association (OR = 1.09, $P = 7.8 \times 10^{-6}$).

Psychiatric Genomics Consortium

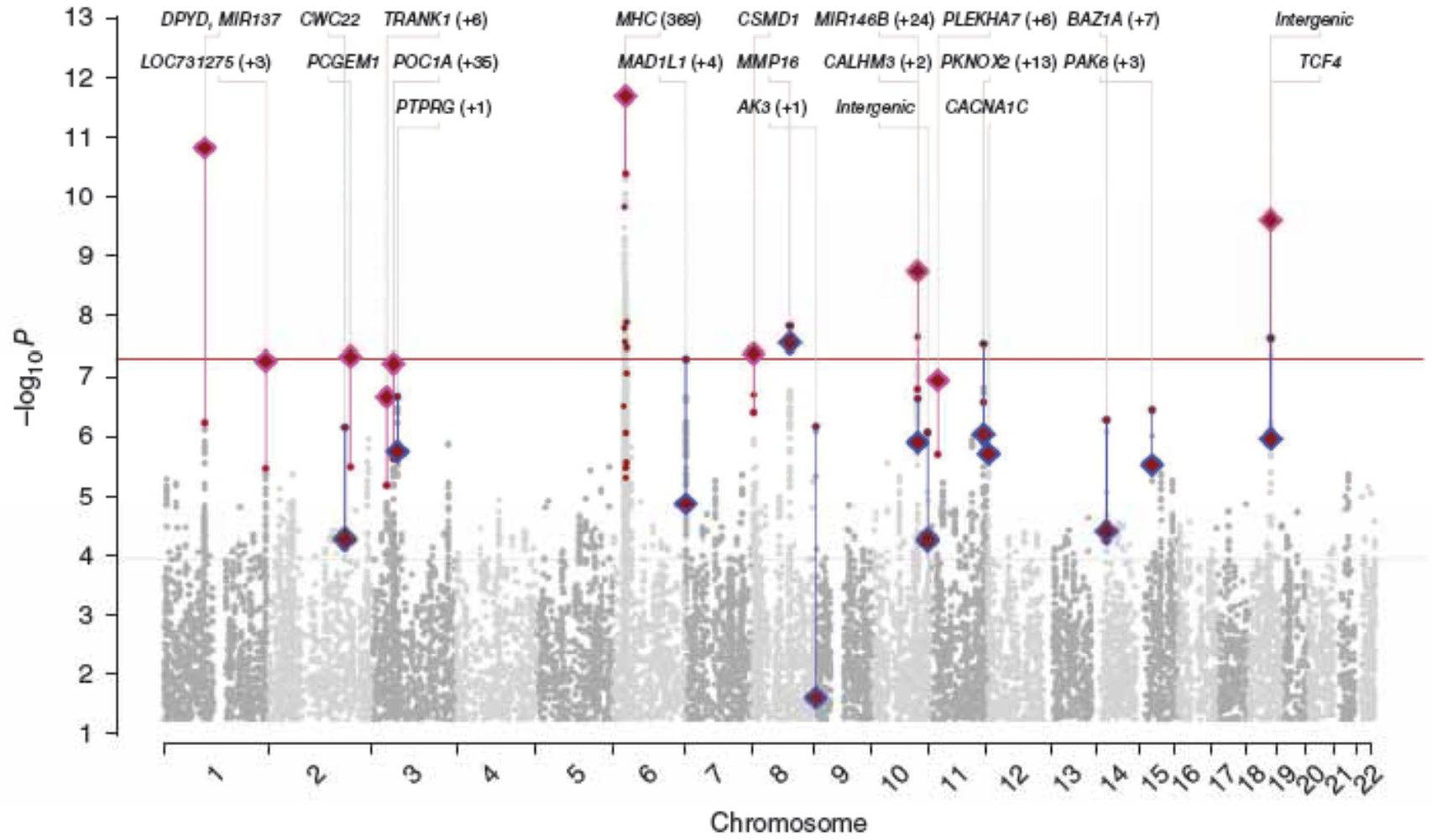
Genome-wide association study identifies five new schizophrenia loci

The Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium[1]

We examined the role of common genetic variation in schizophrenia in a genome-wide association study of substantial size: a stage 1 discovery sample of 21,856 individuals of European ancestry and a stage 2 replication sample of 29,839 independent subjects. The combined stage 1 and 2 analysis yielded genome-wide significant associations with schizophrenia for seven loci, five of which are new (1p21.3, 2q32.3, 8p23.2, 8q21.3 and 10q24.32-q24.33) and two of which have been previously implicated (6p21.32-p22.1 and 18q21.2). The strongest new finding ($P = 1.6 \times 10^{-11}$) was with rs1625579 within an intron of a putative primary transcript for *MIR137* (microRNA 137), a known regulator of neuronal development. Four other schizophrenia loci achieving genome-wide significance contain predicted targets of *MIR137*, suggesting *MIR137*-mediated dysregulation as a previously unknown etiologic mechanism in schizophrenia. In a joint analysis with a bipolar disorder sample (16,374 affected individuals and 14,044 controls), three loci reached genome-wide significance: *CACNA1C* (rs4765905, $P = 7.0 \times 10^{-9}$), *ANK3* (rs10994359, $P = 2.5 \times 10^{-8}$) and the *ITIH3-ITIH4* region (rs2239547, $P = 7.8 \times 10^{-9}$).

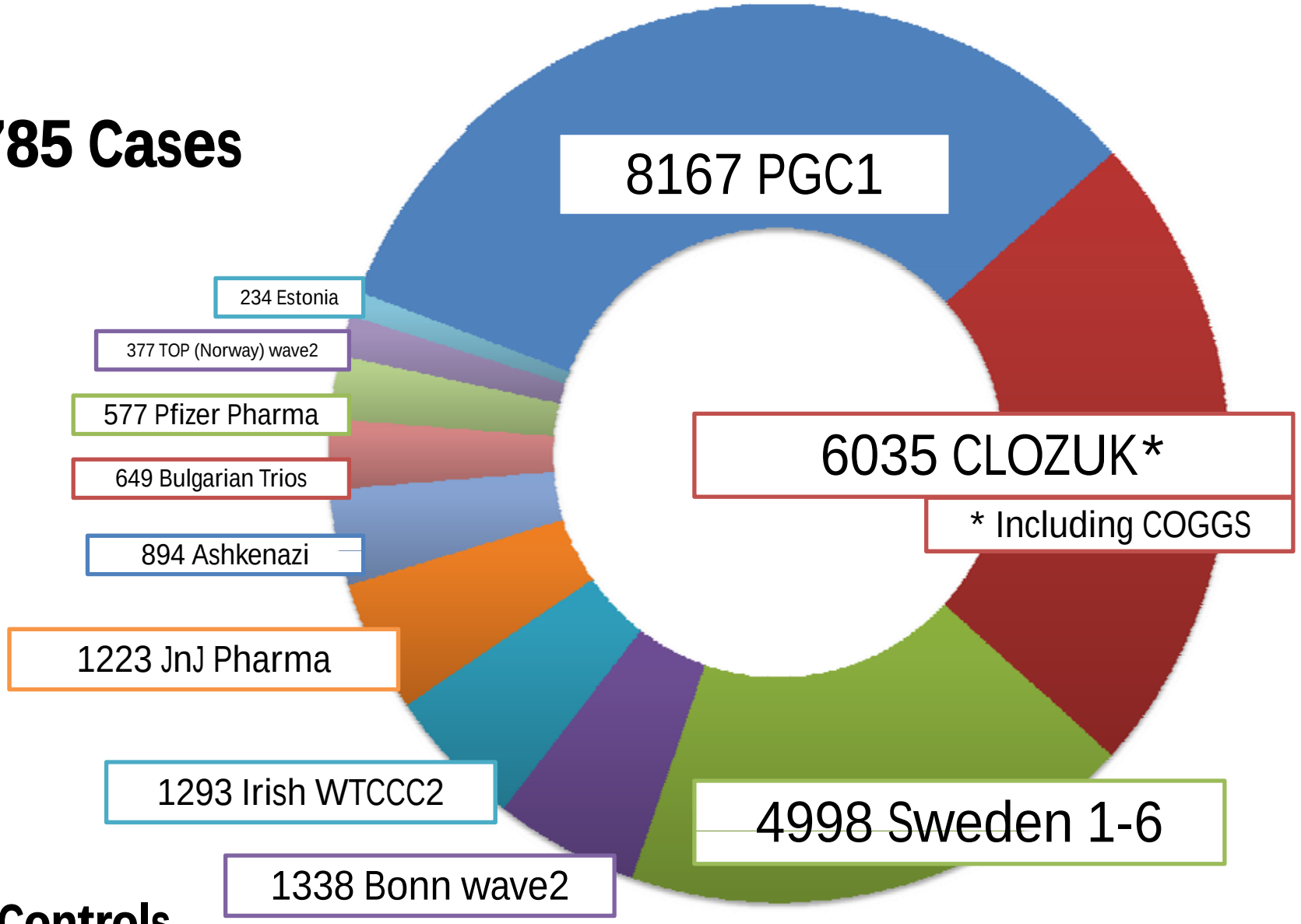
showed greater inflation in the test statistics than we saw for all markers (AIMs $\lambda = 2.26$ compared to all markers $\lambda = 1.56$). After inclusion of principal components, the distributions of the test statistics did not differ between AIMs ($\lambda = 1.18$) and all markers ($\lambda = 1.23$), a result inconsistent with population stratification explaining the residual deviation seen in **Supplementary Figure 1**. Moreover, the results of a meta-analysis using summary results generated using study specific principal components (**Supplementary Note**) were highly correlated with those from the mega-analysis (Pearson correlation = 0.94, with a similar $\lambda = 1.20$; **Supplementary Fig. 2**). Of the ten SNPs in **Table 2**, four increased and six decreased in significance, suggesting that the most extreme values did not result from systematic inflation artifacts. Therefore, our primary analysis used unadjusted *P* values (nevertheless, see **Table 2** for stage 1 *P* values adjusted for λ (ref. 6).

In stage 1 (**Table 2**, **Supplementary Table 4** and **Supplementary Figs. 3** and **4**), 136 associations reached genome-wide significance ($P < 5 \times 10^{-8}$)[7]. The majority of these associations ($N = 129$) mapped to 5.5 Mb in the extended major histocompatibility complex (MHC, 6p21.32-p22.1), a region of high linkage disequilibrium (LD) previously implicated in schizophrenia in a subset of the samples used here[4,8,9]. The other stage 1 regions included new regions (10q24.33 and 8q21.3) and previously reported regions (18q21.2 at *TCF4* (encod-


DPYD, MIR137
LOC731275 (+3)
CWC22
PCGEM1
TRANK1 (+6)
POC1A (+35)
PTPRG (+1)
MHC (369)
MAD1L1 (+4)
CSMD1
MMP16
AK3 (+1)
MIR146B (+24)
CALHM3 (+2)
Intergenic
PLEKHA7 (+6)
PKNOX2 (+13)
CACNA1C
BAZ1A (+7)
PAK6 (+3)
Intergenic
TCF4
−log10P
Chromosome

2013: PGC Schizophrenia, second wave

25,785 Cases



28,441 Controls

ARTICLE

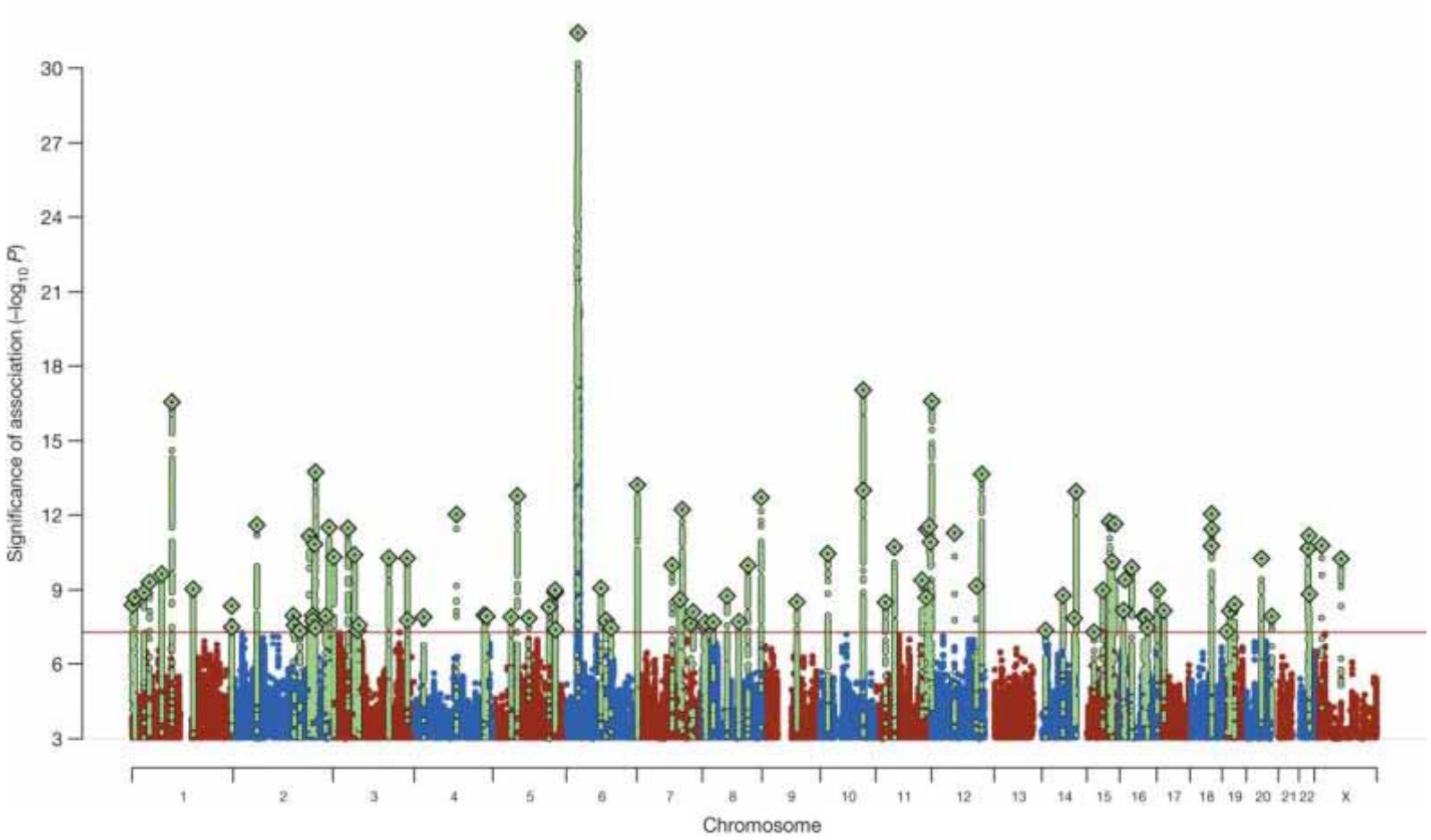
doi:10.1038/nature13595

Biological insights from 108 schizophrenia-associated genetic loci

Schizophrenia Working Group of the Psychiatric Genomics Consortium*

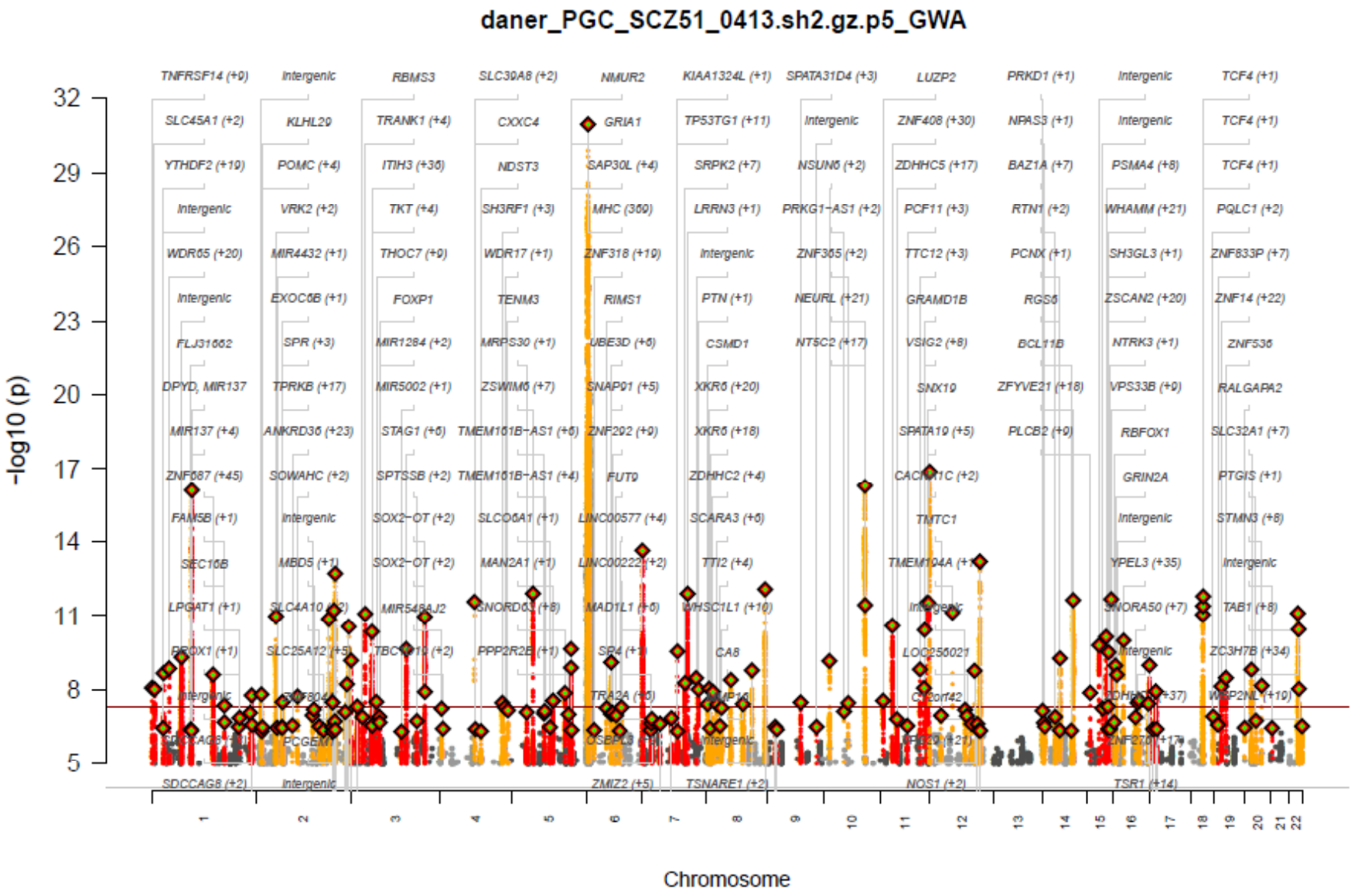
Schizophrenia is a highly heritable disorder. Genetic risk is conferred by a large number of alleles, including common alleles of small effect that might be detected by genome-wide association studies. Here we report a multi-stage schizophrenia genome-wide association study of up to 36,989 cases and 113,075 controls. We identify 128 independent associations spanning 108 conservatively defined loci that meet genome-wide significance, 83 of which have not been previously reported. Associations were enriched among genes expressed in brain, providing biological plausibility for the findings. Many findings have the potential to provide entirely new insights into aetiology, but associations at *DRD2* and several genes involved in glutamatergic neurotransmission highlight molecules of known and potential therapeutic relevance to schizophrenia, and are consistent with leading pathophysiological hypotheses. Independent of genes expressed in brain, associations were enriched among genes expressed in tissues that have important roles in immunity, providing support for the speculated link between the immune system and schizophrenia.

Manhattan plot showing schizophrenia associations.



nature

S Ripke *et al. Nature* **000**, 1-7 (2014) doi:10.1038/nature13595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-log10 (p)
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Chromosome
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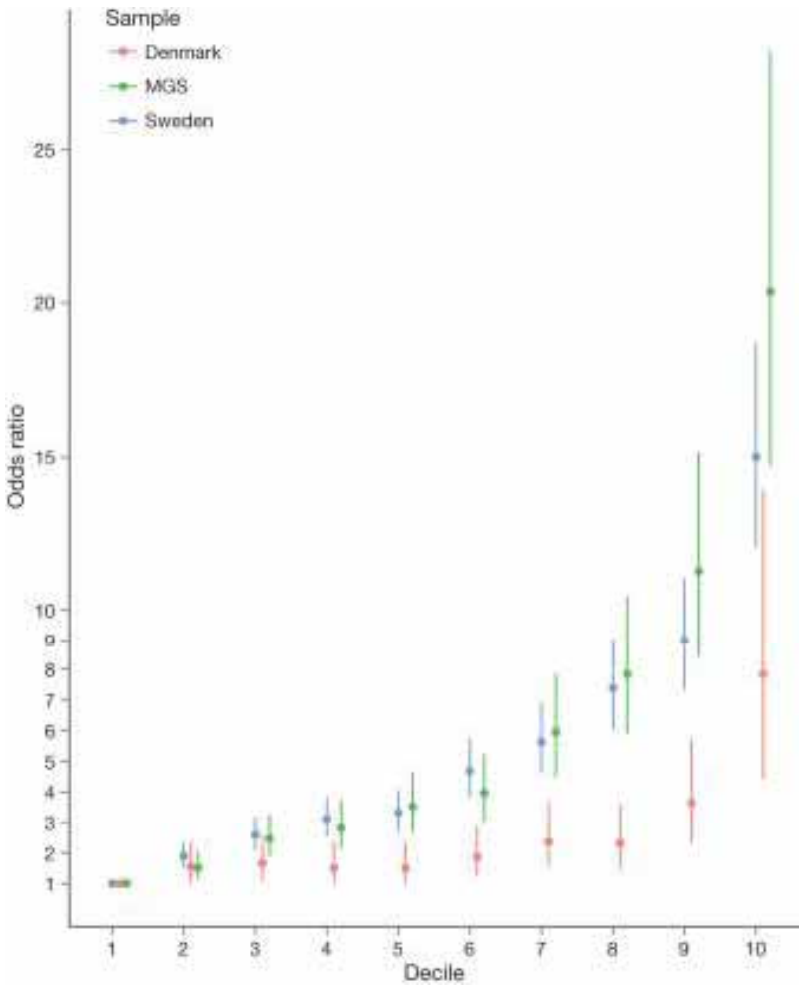
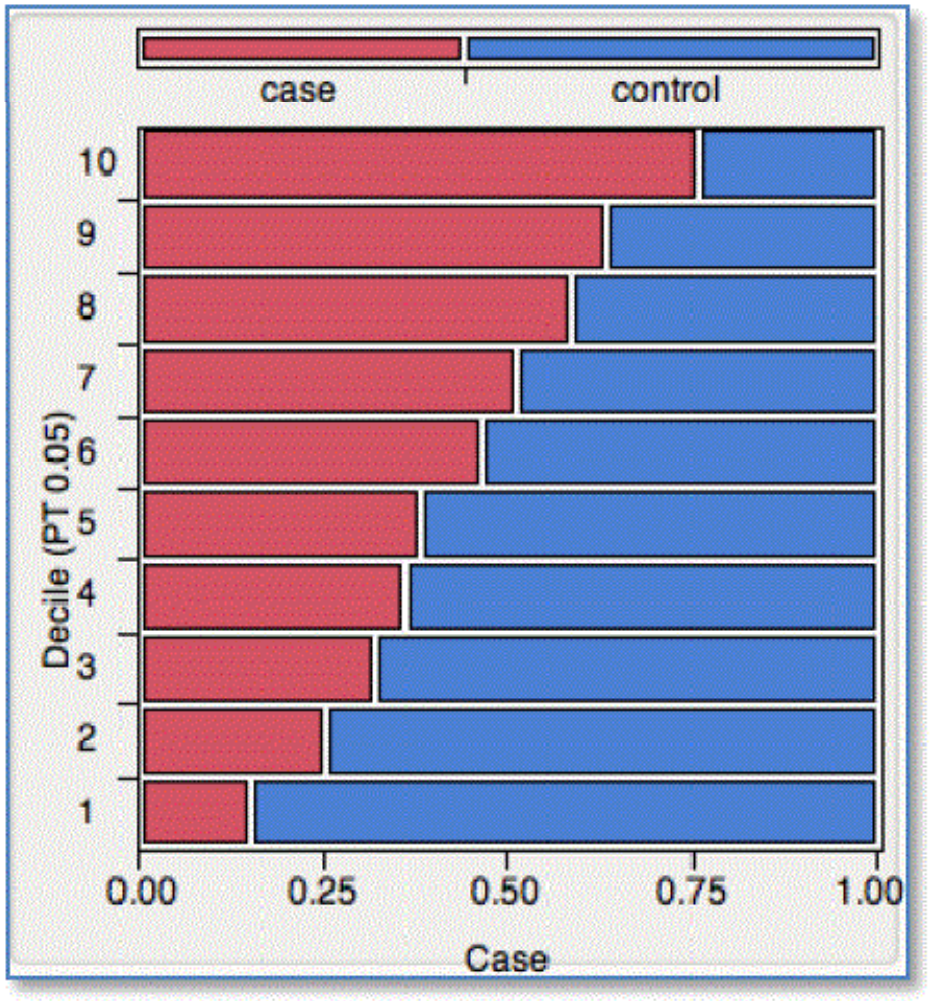
Odds ratio by risk score profile.





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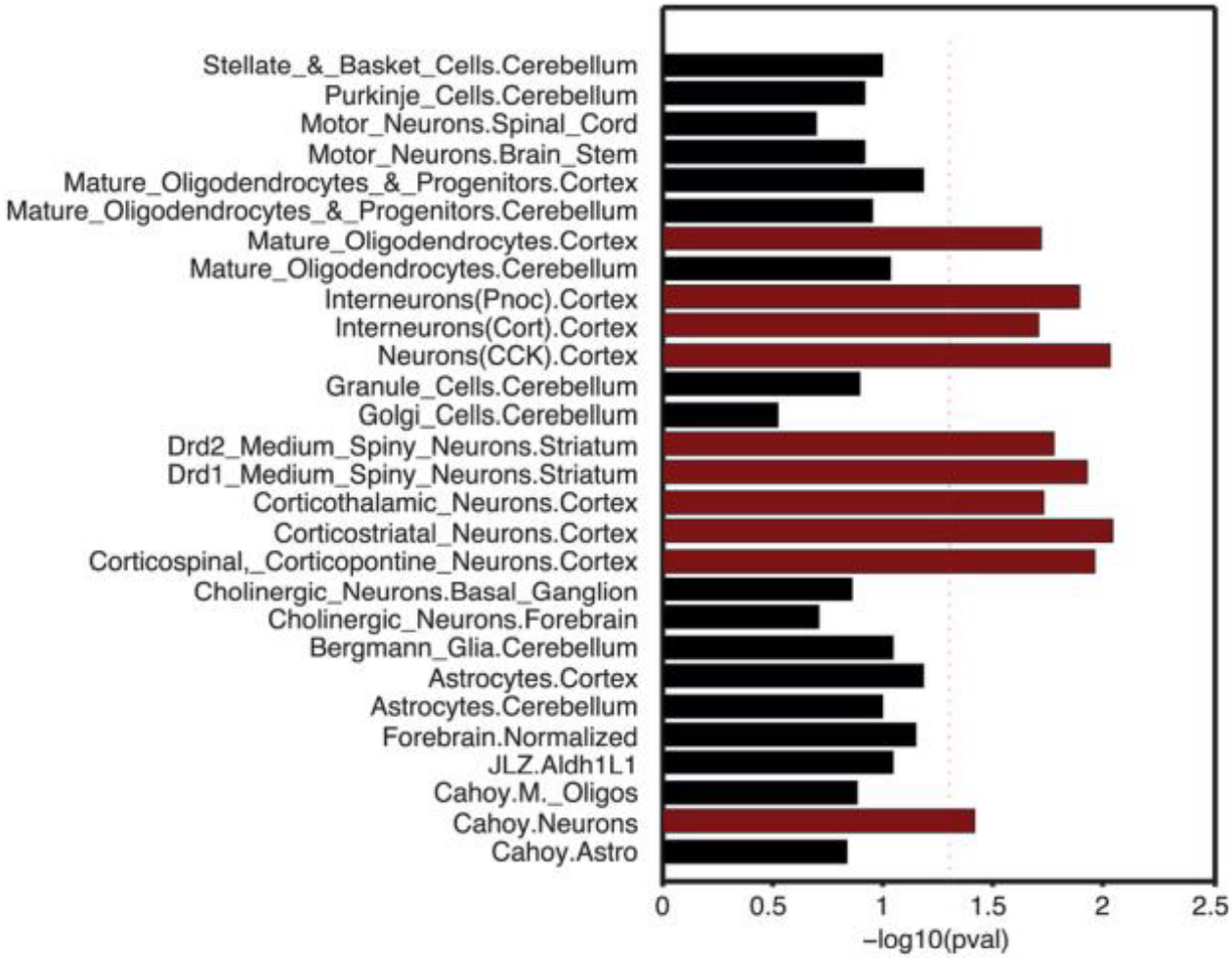
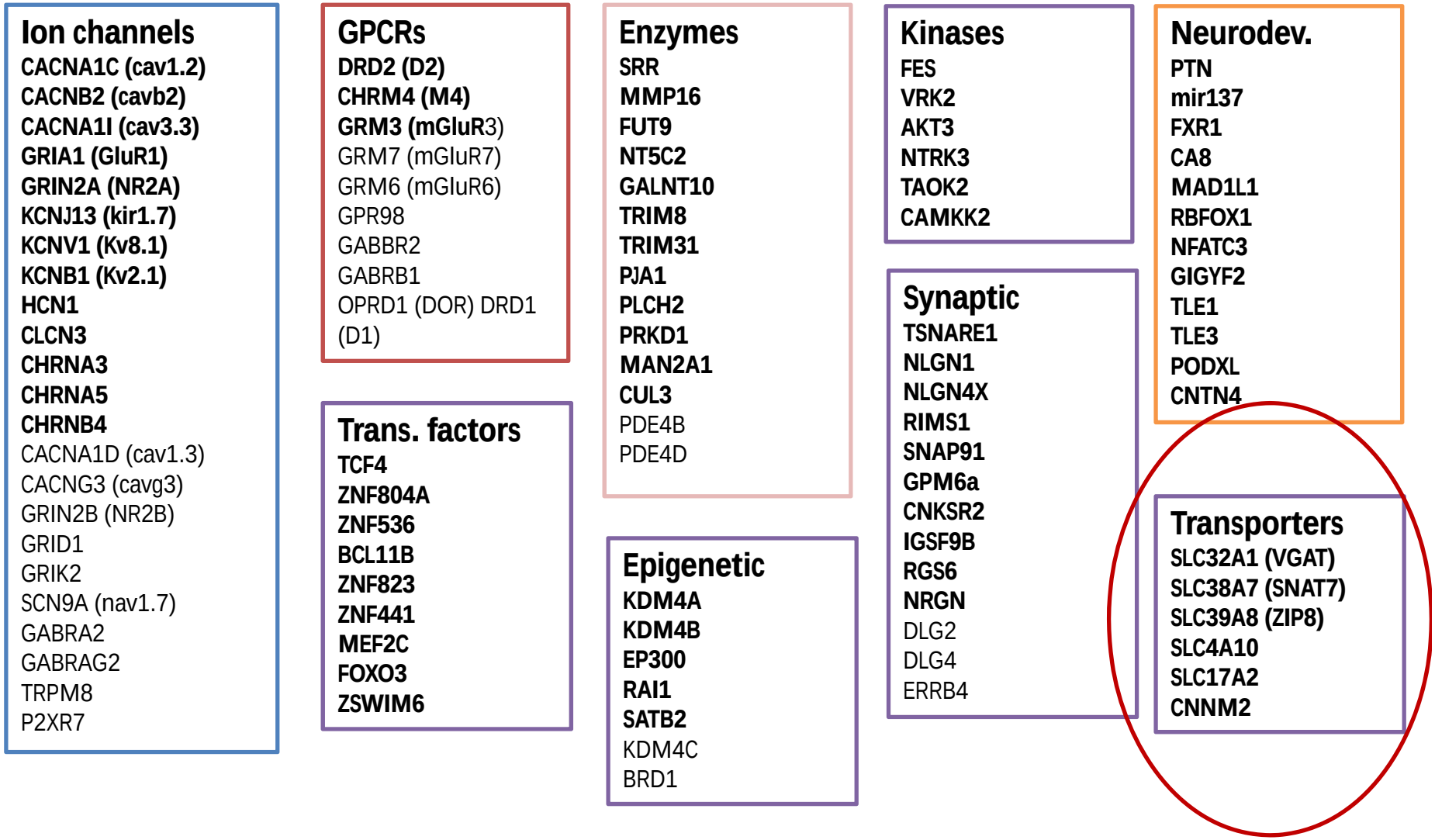
Enrichment of associations in tissues and cells



Individual targets by class: unranked



Can GWAS identify drug targets?

Has GWAS identified targets for drugs that have been shown to be efficacious in phase III trials or are in preclinical trials?

DISEASE MECHANISMS

Genetics of osteoporosis from genome-wide association studies: advances and challenges

J. Brent Richards[1,2], Hou-Feng Zheng[1] and Tim D. Spector[2]

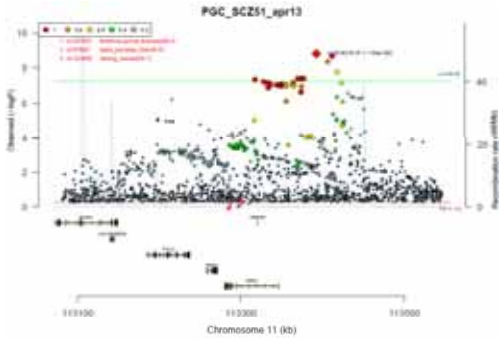
Abstract | Osteoporosis is among the most common and costly diseases and is increasing in prevalence owing to the ageing of our global population. Clinically defined largely through bone mineral density, osteoporosis and osteoporotic fractures have reasonably high heritabilities, prompting much effort to identify the genetic determinants of this disease. Genome-wide association studies have recently provided rapid insights into the allelic architecture of this condition, identifying 62 genome-wide-significant loci. Here, we review how these new loci provide an opportunity to explore how the genetics of osteoporosis can elucidate its pathophysiology, provide drug targets and allow for prediction of future fracture risk.

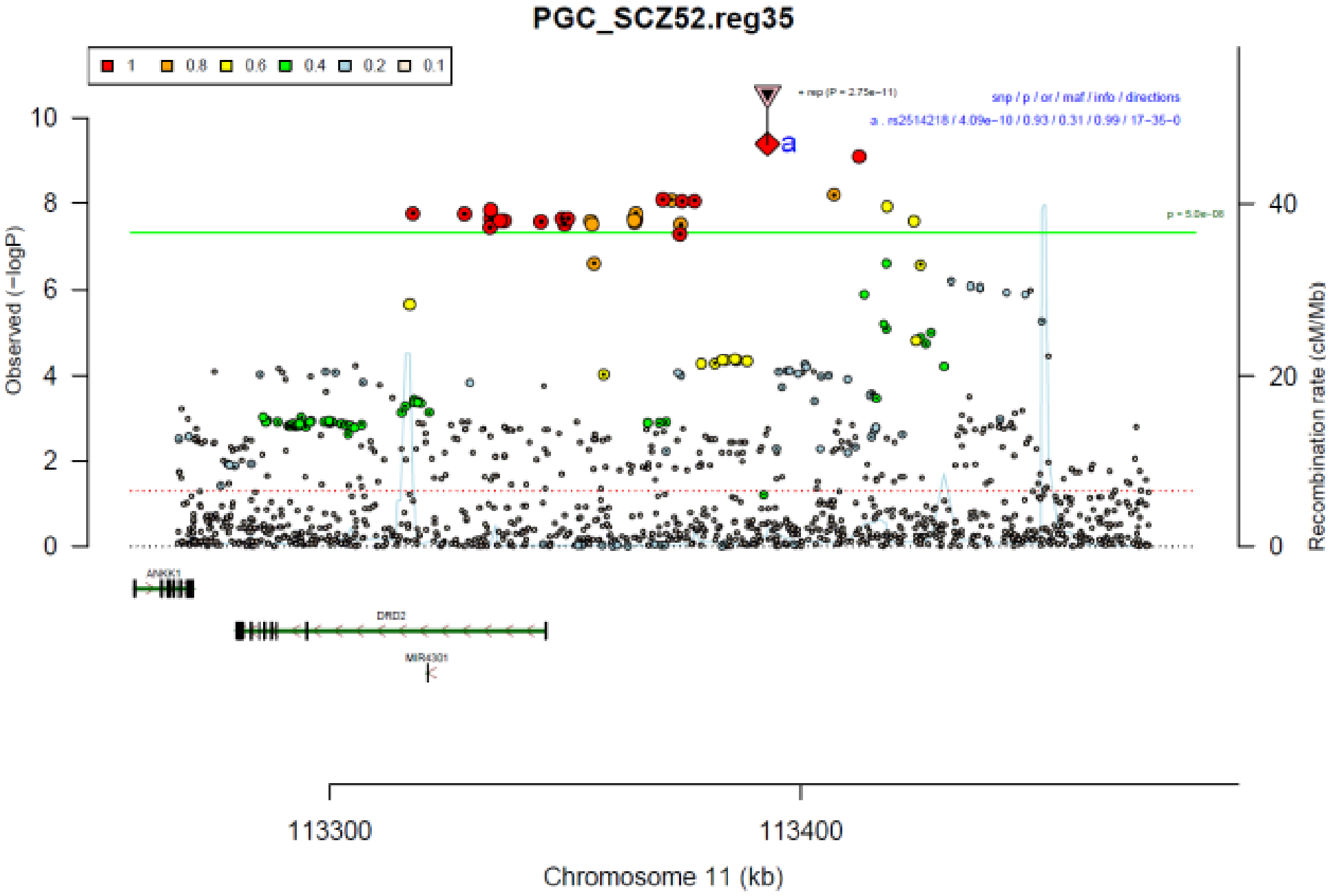
Table 4 | **Drugs, drug targets and whether the locus encoding the target was identified through GWASs**

Drug class	Drug target	Target locus identified through GWASs	Refs
Denosumab	RANKL	*RANKL*	36
Sclerostin inhibitors	Sclerostin (SOST)	*SOST*	67
Selective oestrogen receptor modulators	Oestrogen receptor	*ESR1*	83
Parathyroid hormone analogues	Parathyroid hormone receptor	Not identified, but the pathway has been highlighted through *PTHLH* (encodes PTHRP)	84,85
Bisphosphonates	Farnesyl pyrophosphate	Not identified	86
Oestrogen	Oestrogen receptor	*ESR1*	87
Cathepsin K inhibitors	Cathepsin K	Not identified	68
DKK1 inhibitors	DKK1	*DKK1*	66

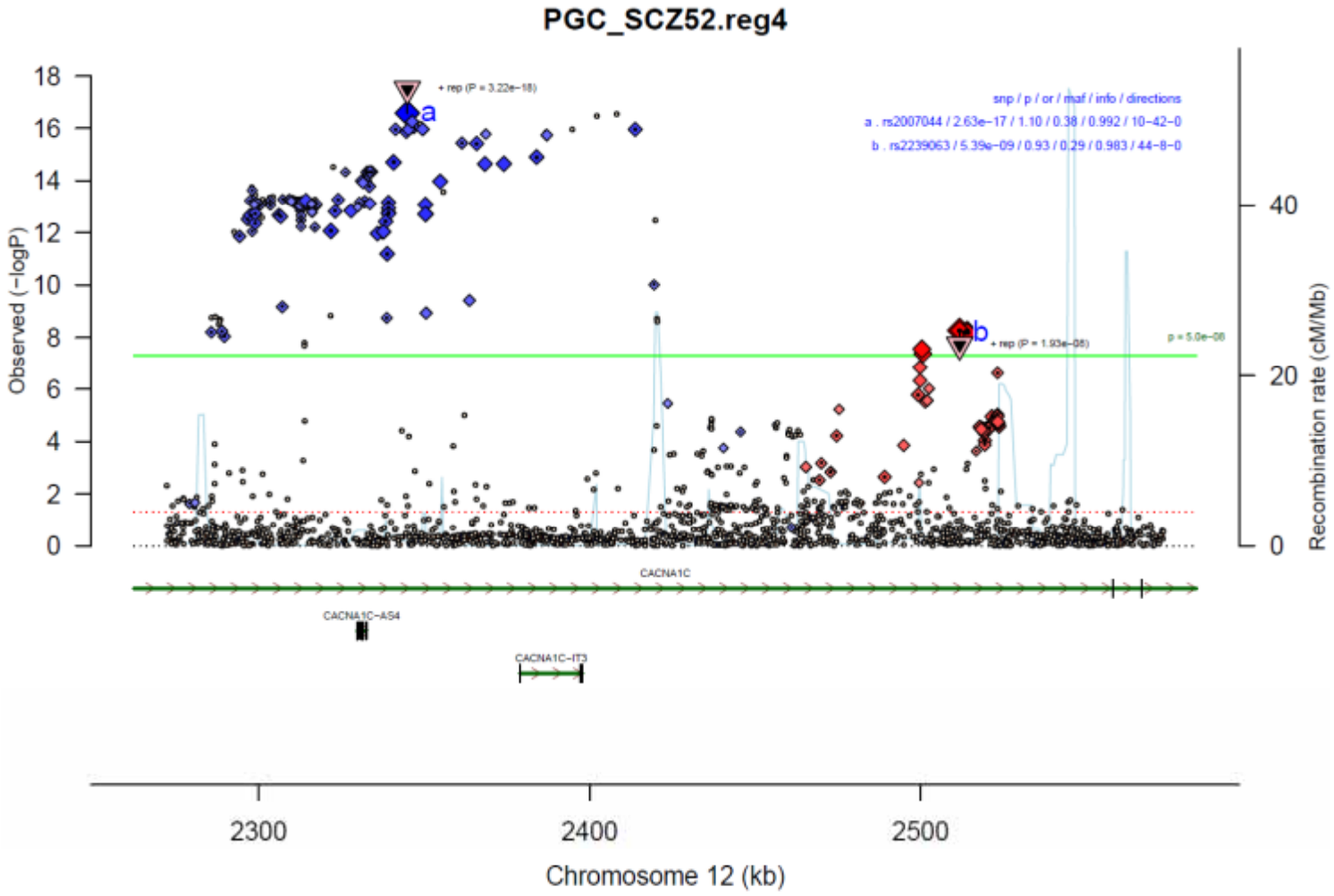
DKK1, dickkopf 1; GWASs, genome-wide association studies; *PTHLH*, parathyroid hormone-like hormone; PTHRP, parathyroid hormone-related protein.

schizophrenia: DRD2 association




PGC_SCZ52.reg35
1
0.8
0.6
0.4
0.2
0.1
+ rep (P = 2.75e−11)
snp / p / or / maf / info / directions
a . rs2514218 / 4.09e−10 / 0.93 / 0.31 / 0.99 / 17−35−0
a
p = 5.0e−08
Observed (−logP)
Recombination rate (cM/Mb)
ANKK1
DRD2
MIR4301
113300
113400
Chromosome 11 (kb)

Voltage gated calcium channel Cav1.2



Welcome To Ricopili

Ricopili is a tool for visualizing regions of interest in select GWAS data sets.

The following data sets are currently available for free access:

(click on the consortium name for more information and on the journal for a direct link to the publication)

Schizophrenia, PGC - Psychiatric Genetics Consortium (Nature Genetics, 2011)
Bipolar disorder, PGC - Psychiatric Genetics Consortium (Nature Genetics, 2011)
Major depressive disorder, PGC - Psychiatric Genetics Consortium (Molecular Psychiatry, 2012)
ADHD, PGC - Psychiatric Genetics Consortium (JAACAP, 2010)
Psychiatric Cross Disorder Analysis PGC - Psychiatric Genetics Consortium (The Lancet, 2013)
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Inflammatory Bowel Disease:, International IBD Genetics Consortium (Nature, 2012)
Host control of HIV-1, International HIV Controllers Study (Science, 2010)
GWAS, Tobacco and Genetics (TAG) Consortium (Nature Genetics, 2010)
GWAS, rheumatoid arthritis risk: public download (Nature Genetics, 2010)
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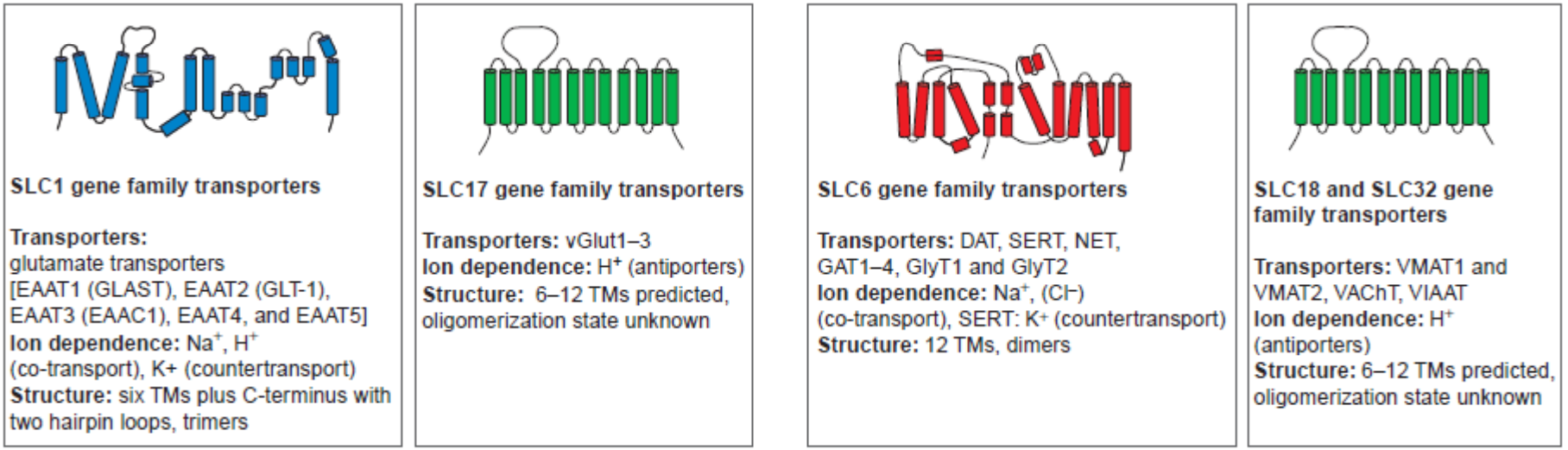
Antidepressant Efficacy in Major Depressive Disorder (here called Pharmacogenetics - PhaCuGe). (The American Journal of Psychiatry, 2013)

You can find some of these as whole genome downloads here: PGC - public downloads

Transporters identified by GWAS

– Four fundamentally different classes of membrane-bound transport proteins: **ion channels**; **transporters**; **aquaporins**; and **ATP-powered pumps**

- **Solute carrier superfamily (SLCs):** 300 members organized into 52 families



SLC1 gene family transporters

Transporters:
glutamate transporters
[EAAT1 (GLAST), EAAT2 (GLT-1),
EAAT3 (EAAC1), EAAT4, and EAAT5]
Ion dependence: Na^+, H^+
(co-transport), K+ (countertransport)
Structure: six TMs plus C-terminus with
two hairpin loops, trimers

SLC17 gene family transporters

Transporters: vGlut1–3
Ion dependence: H^+ (antiporters)
Structure: 6–12 TMs predicted,
oligomerization state unknown

SLC6 gene family transporters

Transporters: DAT, SERT, NET,
GAT1–4, GlyT1 and GlyT2
Ion dependence: Na^+, (Cl^-)
(co-transport), SERT: K^+ (countertransport)
Structure: 12 TMs, dimers

SLC18 and SLC32 gene family transporters

Transporters: VMAT1 and
VMAT2, VAChT, VIAAT
Ion dependence: H^+
(antiporters)
Structure: 6–12 TMs predicted,
oligomerization state unknown

SLCs in schizophrenia: GWAS and CNVs

- **SLC1A1**
  - SLC1 high affinity glutamate transporter, EAAT3
- **SLC32A1 (20q11.23)**
  - SLC32 vesicular inhibitory amino acid transporter
- **SLC38A7 (16q21)**
  - SLC38 System A & N, sodium-coupled neutral amino acid transporter
- **SLC4A10 (2q24.2)**
  - SLC4 Bicarbonate transporter
- **SLC39A8 (4q24)**
  - SLC39 metal ion transporter

Human Molecular Genetics, 2014, Vol. 23, No. 6 **1669–1676**
doi:10.1093/hmg/ddt540
Advance Access published on October 26, 2013

CNV analysis in a large schizophrenia sample implicates deletions at 16p12.1 and *SLC1A1* and duplications at 1p36.33 and *CGNL1*

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Received July 26, 2013; Revised September 26, 2013; Accepted October 24, 2013

Molecular Aspects of Medicine 34 (2013) 571–585

Contents lists available at SciVerse ScienceDirect

Molecular Aspects of Medicine

journal homepage: www.elsevier.com/locate/mam

Review

Evolutionary origin of amino acid transporter families SLC32, SLC36 and SLC38 and physiological, pathological and therapeutic aspects ☆

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Guest Editor Matthias A. Hediger
Transporters in health and disease (SLC series)

ARTICLE INFO

Article history:
Received 15 November 2011
Accepted 25 June 2012

Keywords:
SLC32
SLC36
SLC38
Amino acid transporter
Glutamate glutamine cycle
VIAAT

ABSTRACT

About 25% of all solute carriers (SLCs) are likely to transport amino acids as their primary substrate. One of the major phylogenetic clusters of amino acid transporters from the SLC family is the β-family, which is part of the PFAM APC clan. The β-family includes three SLC families, SLC32, SLC36 and SLC38 with one, four and eleven members in humans, respectively. The most well characterized genes within these families are the vesicular inhibitory amino acid transporter (VIAAT, SLC32A1), PAT1 (SLC36A1), PAT2 (SLC36A2), PAT4 (SLC36A4), SNAT1 (SLC38A1), SNAT2 (SLC38A2), SNAT3 (SLC38A3), and SNAT4 (SLC38A4). Here we review the structural characteristics and functional role of these transporters. We also mined the complete protein sequence datasets for nine different genomes to clarify the evolutionary history of the β-family of transporters. We show that all three main branches of the this family are found as far back as green algae suggesting that genes from these families existed in the early eukaryote before the split of animals and plants and that they are present in most animal species. We also address the potential of further drug development within this field highlighting the important role of these transporters in neurotransmission and transport of amino acids as nutrients.

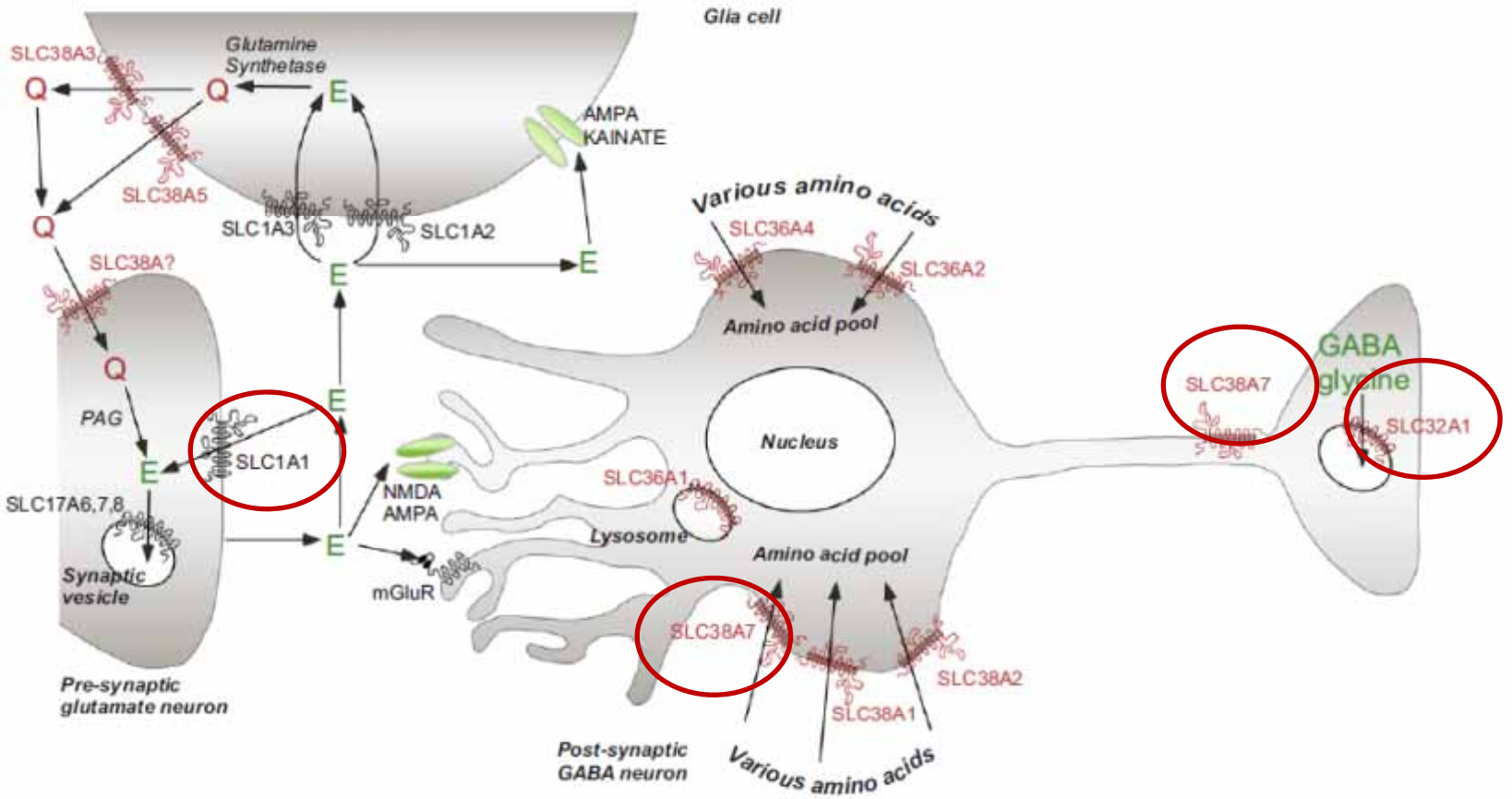

Fig. 4. The neuronal function of the transporters from the SLC32, SLC36 and SLC38 families are drawn in red in the figure. The glutamate/glutamine cycle is shown in the left side of the figure, with the amino acids transported by members from the SLC32, 36 or 38 family shown in red (One letter abbreviations).

Helgi B. Schiöth, Sahar Roshanbin, Maria G.A. Hägglund, Robert Fredriksson Evolutionary origin of amino acid transporter families SLC32, SLC36 and SLC38 and physiological, pathological and therapeutic aspects. Molecular Aspects of Medicine Volume 34, Issues 2–3, April–June 2013, Pages 571–585

High affinity glutamate transporter EAAT3

- High affinity L-glutamate and DL-aspartate transporter; also transports cysteine.
- Expressed in the majority of neurons throughout the CNS, but is selectively targeted to somata and dendrites avoiding axon terminals; present on the soma and processes of DA neurons; not expressed in glia
- Highest concentration in the hippocampus, but total tissue content in young adult rat brains is about 100 times lower than that of EAAT2
- Amphetamine modulates excitatory neurotransmission through endocytosis of the glutamate transporter EAAT3 in dopamine neurons
- Knockout mice have deficits in learning and memory

Vesicular GABA transporter

- **SLC32A1 (20q11.23)** The vesicular GABA transporter SLC32A1 (VGAT, VIAAT) is involved in the transport of GABA and glycine, the major fast inhibitory neurotransmitters, which play a role in neurogenesis, neuronal migration and synaptogenesis.

- VGAT is localised to vesicles of inhibitory terminals of GABAergic and glycinergic neurons (symmetric synapses). It has high affinity for GABA, and cooperates with the neuronal transporter GlyT2 to determine the vesicular glycinergic phenotype (Aubrey et al., 2007).

- Symptoms of schizophrenia have been postulated to arise from an imbalance in the normal excitatory (glutamate)/inhibitory (GABA) ratio (Cline, 2005).

- A deficit in GABAergic inhibitory neurotransmission has been indicated by postmortem studies with both the synthesis and reuptake of GABA impaired in a subset of DLPFC neurons in schizophrenia (Lewis and Gonzales-Burgos, 2008).

N-type amino acid transporters

- **SLC38A7 (16q21)** The SLC38 family of sodium-coupled amino acid transporters (SNATs) has 12 members. N-type transporters (SLC38A3, SLC38A5 and SLC38A7) show the highest transport for glutamine and alanine.

- SLC38A7 (SNAT7) prefers L-glutamine but also transports other amino acids with polar side chains, as well as L-histidine and L-alanine. In the mouse brain SLC38A7 is expressed in all neurons, but not in astrocytes, and is SLC38A7 is unique in being the first system N transporter expressed in GABAergic and also other neurons.

- The preferred substrate and axonal localization of SLC38A7 close to the synaptic cleft indicates that SLC38A7 could have an important function for the reuptake and recycling of glutamate (Haggelund et al., 2011).

Zinc transporter ZIP8

- **SLC39A8 (4q24)** also known as ZIP8, is a transmembrane ion transporter for iron, zinc and manganese as well as toxic heavy metals such as cadmium. Prepartum maternal iron deficiency has previously been associated with offspring risk of schizophrenia (Sørensen et al., 2011; Insel et al., 2008).

- A non-synonymous SNP (Ala391Thr) has previously been associated with schizophrenia (Carrera et al., 2012). Body mass index, diastolic blood pressure and HDL-cholesterol is also associated with this locus, with the same SNPs as in the present study (van Vliet-Ostaptchouk et al., 2013). It is ubiquitously expressed.

Sodium-coupled bicarbonate transporter

- **SLC4A10 (2q24.2)** Solute carrier family 4, sodium bicarbonate transporter, member 10 is a sodium-coupled bicarbonate transporter that regulates the intracellular pH of neurons, the secretion of bicarbonate ions across the choroid plexus, and the pH of the brain extracellular fluid, and may contribute to the secretion of cerebrospinal fluid.

- Slc4a10 is predominantly expressed in the central CNS, especially in neurons in the prefrontal cortex and hippocamus. Within the hippocampus, it is abundant in CA3 pyramidal cells.

- Mice with targeted Slc4a10 gene disruption have small brain ventricles and show reduced neuronal excitability (Jacobs et al., 2008). Disruption in humans is associated with complex partial epilepsy and mental retardation.

SERCA2 (ATP2A2)

- **ATP2A2 (12q24.11)** ATP2A2 encodes one of the SERCA Ca^{2+}-ATPases (SERCA2), which are intracellular pumps located in the sarcoplasmic or endoplasmic reticula of muscle cells.

- Mutations in ATP2A2 cause Darier-White disease, also known as keratosis follicularis, an autosomal dominant skin disorder (Sakuntabhai et al., 2010). These mutations result in constitutive ER stress with increased sensitivity to ER stressors and reduced intercellular adhesion strength which can be rescued by the orphan drug Miglustat, a pharmacological chaperone (Savignac et al., 2014).

- Darier disease has high prevalence of mood disorders (50%), including schizophrenia, depression, bipolar disorder, suicidal thoughts and suicide attempts, suggesting a possible genetic link (Gordon-Smith et al., 2010; Tang et al., 2010).

CLINICAL AND LABORATORY INVESTIGATIONS

BJD
British Journal of Dermatology

The neuropsychiatric phenotype in Darier disease

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Accepted for publication
20 April 2010

Key words
Darier disease, neuropsychiatric phenotype

Conflicts of interest
None declared.

DOI 10.1111/j.1365-2133.2010.09834.x

Summary

Background Darier disease (DD) is a rare autosomal dominantly inherited skin disorder in which co-occurrence of neuropsychiatric abnormalities has been frequently reported by dermatologists. It is caused by mutations in a single gene, *ATP2A2*, which is expressed in the skin and brain.

Objectives To conduct the first systematic investigation of the neuropsychiatric phenotype in DD.

Methods One hundred unrelated individuals with DD were assessed using a battery of standardized neuropsychiatric measures. Data were also obtained on a number of clinical features of DD.

Results Individuals with DD were found to have high lifetime rates of mood disorders (50%), specifically major depression (30%) and bipolar disorder (4%), and suicide attempts (13%) and suicidal thoughts (31%). These were more common in DD when compared with general population data. The prevalence of epilepsy (3%) in the sample was also higher than the prevalence in the general population. There was no consistent association of specific dermatological features of DD and presence of psychiatric features.

Conclusions These findings highlight the need for clinicians to assess and recognize neuropsychiatric symptoms in DD. The results do not suggest that neuropsychiatric symptoms are simply a psychological reaction to having a skin disease, but are consistent with the pleiotropy hypothesis that mutations in the *ATP2A2* gene, in addition to causing DD, confer susceptibility to neuropsychiatric features. Further research is needed to investigate genotype–phenotype correlations between the types and/or locations of pathogenic mutations within *ATP2A2* and the expressed neuropsychiatric phenotypes.

Summary

- GWAS and copy number variant analysis has identified >100 independent genetic loci for schizophrenia
- These include many genes involved in neurodevelopment and neurotransmission, especially those expressed in CNS neurons, including synaptic proteins
- Several transporters have been identified as probable genetic risk factors for schizophrenia, especially neurotransmitter reporters for glutamate, glutamine and GABA
- While there are druggable targets, there is a lack of specific compounds and more work is needed to develop a therapeutic hypothesis.

Acknowledgements

Psychiatric Genomics Consortium

- SGENE consortium
  - Hreinn Stefansson
  - David St Clair
  - Leena Peltonen
  - Dan Rujescu
- CRESTAR consortium
- GAP study
- Maudsley Family Study
- UK10K
- WTCCC
- European Commission FP6, FP7 Programs
- Medical Research Council
- Wellcome Trust

King's College London

- King's College London
  - Cathy Fernandes
  - Hannah Grayton
  - Katherinne Navarette
  - Evangelos Vassos
  - Cathryn Lewis
  - Robin Murray
  - Maria Tropeano
  - Gerome Breen
  - Richard Dobson
- And many others