Psychiatric genetics: progress amid controversy

Margit Burmeister[*‡§], *Melvin G. McInnis*[‡] *and Sebastian Zöllner*[‡||]

Abstract | Several psychiatric disorders — such as bipolar disorder, schizophrenia and autism — are highly heritable, yet identifying their genetic basis has been challenging, with most discoveries failing to be replicated. However, inroads have been made by the incorporation of intermediate traits (endophenotypes) and of environmental factors into genetic analyses, and through the identification of rare inherited variants and novel structural mutations. Current efforts aim to increase sample sizes by gathering larger samples for case–control studies or through meta-analyses of such studies. More attention on unique families, rare variants, and on incorporating environment and the emerging knowledge of biological function and pathways into genetic analysis is warranted.

Heritability
The proportion of phenotypic variation that is explained by genetic variation.

*Molecular and Behavioral Neuroscience Institute, University of Michigan, 5061 BSRB, 109 Zina Pitcher Place, Ann Arbor, Michigan 48109-2200, USA.
‡Department of Psychiatry and Depression Center, University of Michigan, Rachel Upjohn Building, 4250 Plymouth Road; Ann Arbor, Michigan 48109-5734, USA.
§Department of Human Genetics, 4909 Buhl Building, 1241 East Catherine Street, Ann Arbor, Michigan 48109-5618, USA.
||Department of Biostatistics, University of Michigan, M4140 SPH II, 109 South Observatory Sreet, Ann Arbor Michigan 48019-2029, USA.
Correspondence to M.B.
e-mail: margit@umich.edu
doi:10.1038/nrg2381

The hereditary basis for psychiatric disorders was already recognized at the turn of the nineteenth century by Emil Kraepelin. Twin and adoption studies consistently demonstrate a genetic influence on all major psychiatric disorders[1], confirming work that started in the 1930s[2]. In fact, estimated heritability for bipolar disorder, schizophrenia and autism (80% to >90%)[3–7] is much higher than that of breast cancer (5% to 60%)[8,9] and Parkinson disease (13% to 30%)[10], for which several genetic risk factors are now well established[1]. In many respects, psychiatric disorders are similar to other complex traits that have been studied genetically[11,12]: studies are complicated by locus heterogeneity, imprecisely specified traits, incomplete penetrance and interaction with non-genetic factors, resulting in a low contribution of each individual risk allele (odds ratios <2). Linkage studies have low power to detect such low-risk alleles, and association studies are only powerful if the risk allele is common (that is, with a minor allele frequency >0.05), or if the odds ratio can be increased by focusing on a disease subtype or by incorporating environmental factors. Furthermore, the winner's curse leads to overestimation of effect sizes, thereby handicapping replication[13].

Here, we focus on progress that has been made in psychiatric disorders, primarily concentrating on major depression, bipolar disorder, schizophrenia and autism. The current debate centres on the nature of the specific biological influences, the best ways to identify them, and how the environment interacts with genes and their products to cause disease. We review linkage and association findings, including biological and positional candidate genes and recent genome-wide association (GWA) studies. Although it is not clear which of the hundreds of reported associations will be confirmed, throughout this Review we emphasize the lessons that emerge from some convincingly replicated findings. We demonstrate how alternative phenotypes — such as intermediate traits or endophenotypes — or unique families with rare disorders have helped gene identification. The incorporation of environmental factors from longitudinal studies has led to some significant and reproducible genetic findings, with variants in the serotonin system being implicated in depression and impulsive-aggressive behaviours. Recent reports of rare large chromosomal deletions in autism and schizophrenia suggest that rare mutations might need to be considered in common psychiatric disorders as well. We end by discussing some of the biological pathways that are involved in psychiatric disorders, and how genome-wide expression data, animal models and bioinformatics help in deciphering the biochemistry and neuroscience of the disorders. Functional knowledge in turn might be useful in the dissection of further genetic risk factors.

Because some genetic factors apparently influence behaviour or susceptibility for psychiatric disorders without falling into a specific diagnostic category, integrating genetic risk alleles with phenomenology is likely to markedly change the current classification scheme in psychiatry. In addition, we have only limited knowledge of the pathological mechanisms that lead to any psychiatric disorder, and the identification of genetic risk factors might provide a better understanding of

Locus heterogeneity
When variation in different genes affects the same phenotype; it is also known as genetic heterogeneity. This should be contrasted with allelic heterogeneity, in which multiple variants in the same gene affect the same disease.

Penetrance
The probability of observing a specific phenotype for individuals carrying a particular genotype. If this probability is smaller than 1 for all genotypes of a variant then the variant has incomplete penetrance.

Odds ratio
A measure of effect size. Defined as the ratio of the odds of a disease being observed in one group of genotypes and the odds of a disease being observed in another group.

Linkage study
A family-based method to search for a chromosomal location of a gene by demonstrating co-segregation of the disease with genetic markers of known chromosomal location.

Association study
Genetic study looking for association between a disease and a genetic locus using either a case–control design or a family-based design.

Winner's curse
Upward bias of estimates of effect sizes on data sets that have previously passed through a screening procedure for a significant test statistic.

Candidate gene
A gene that might be involved in a particular disease. A biological candidate gene might be involved in the neurotransmitter system that is implicated by the psychoactive drugs used to treat a disorder (for example, serotonin system genes for depression), whereas a positional candidate gene is any gene that maps within a chromosomal region that is implicated by linkage.

Endophenotype
Heritable phenotype that is associated with a disease but that can be measured independently of disease status.

the aetiology of psychiatric disorders. Although pharmacogenetics is not discussed here, genetic findings might lead to novel treatments and/or personalized medicine. With highly controversial genetic tests for psychiatric disorders now launched[14], but initial GWA studies of psychiatric disorders unable to confirm previous findings or each other, it is timely to take stock of the field, to outline the progress that has been made, and to candidly review the controversies and prospects for the future of psychiatric genetics.

Psychiatric diagnosis

A condition of any genetic analysis is a valid and accurate phenotype. However, there is no biochemical or physiological test available for psychiatric disorders that is equivalent to, for example, the one that measures glucose level for type 2 diabetes. A psychiatric diagnosis is made through clinical examination, usually by direct interview, and summarized on the basis of diagnostic criteria (TABLE 1; Supplementary information S1 (table)). Problems arise in the overlap of categories; for example, psychosis (illusions or hallucinations) can be part of at least three different diagnoses — schizophrenia, bipolar disorder and psychotic depression (FIG. 1). In addition, the boundaries of diagnostic categories can be blurred when symptoms in patients are not clear. For this reason, many genetic studies report on their findings using 'narrow' criteria: in the case of bipolar disorder, for example, this would indicate that the subject had documented evidence of acute mania (required for a diagnosis of bipolar I disorder), a clinical state that is strikingly similar across patients. Most studies use a 'final best estimate' process that uses data from the interview as well as clinical records and family history. Although the clinical assessment is rigorous, these diagnoses might not be reflective of genetic aetiology, and therefore can lead to diagnoses that are highly reproducible and clinically useful but not necessarily genetically valid. Genetic epidemiological studies (see TABLE 1 and Supplementary information S1 (table) for brief summaries) confirm a complex pattern of high heritability that interacts with and in addition to non-genetic factors, as well as the often unclear boundaries between disorders. As shown throughout this Review, some genetic variants might increase risk across several diagnoses, whereas others only influence risk for a subset of conditions. The impact of genetics on diagnosis will probably be large: most workers and specialists in the field agree that after replicated genetic findings are established they will be incorporated into clinical practice.

Linkage analysis

After positional cloning was used successfully to identify the genes responsible for Mendelian disorders, there was enormous optimism among psychiatrists in the early 1980s and into the 1990s that single genes involved in psychiatric disorders could be identified by the same means. The enthusiasm of the initial linkage studies, which mapped one bipolar disorder locus near the colour blindness locus on the X chromosome and another to chromosome 11p in an Amish pedigree, quickly faded when these results turned out to be false positives[15–18]. The linkage era (1980–2005) for psychiatric disorders failed to identify any single locus that was unequivocally replicated across multiple independent samples. Scientists suspected that the individual studies were affected by low power, and so it organized collaborative efforts and meta-analyses. Nevertheless, a meta-analysis of schizophrenia linkage studies found only one genome-wide significant linkage peak — in a region never before implicated in this disorder[19]. Findings in meta-analyses of bipolar disorder depended on the method that was used to combine data. Non-parametric linkage analysis using rank-based methods detected no genome-wide significant linkage findings[20], whereas joint analysis of all linkage data sets identified two genome-wide significant peaks — on chromosome 6q and on 8q[21]. Linkage studies of autism have been somewhat more consistent, with chromosomes 2q, 7q, 15q and 16p implicated reproducibly[22–24], as well as less reproducible peaks[25]. Astonishingly, the first two linkage studies of obsessive-compulsive disorder identified a peak in the same area of chromosome 9 (REFS 26,27), although this region was not confirmed by a later and much larger study[28]. The existing linkage results are now incorporated in positional candidate gene association studies and pathway analyses. Positional cloning starting with linkage can lead to gene identification in rare families with unique, essentially Mendelian forms of mental illness (see below), but this approach is not currently being pursued as the major research avenue in common heterogeneous psychiatric disorders.

Genetic association studies

In a landmark paper in 1996, Risch and Merikangas[29] demonstrated that when genetic variants have only small individual effect on risk, association studies are more powerful than linkage studies — even when properly accounting for multiple testing. The simplest type of association study tests whether the allele (a versus A) or genotype (AA versus Aa versus aa) frequency differs significantly between a sample of cases compared with a control sample. Association studies have been the major focus of psychiatric genetic studies since 1996; they were initially based on candidate genes and have more recently (since 2007) been genome wide.

Biological candidate genes. As a hypothesis, association can be tested with genetic variants in biological candidate genes in a metabolic pathway that is either known or hypothesized to be connected to the disorder, or in genes that encode protein targets of psychoactive drugs. One of the best established genetic protective variants is a functional null allele of aldehyde dehydrogenase 2 (ALDH2*2), an enzyme in the alcohol-degrading pathways. This variant of the enzyme is present at a significant frequency in Southeast Asians and protects its carriers, especially homozygotes, from risk of alcoholism; an unpleasant 'flushing' occurs in response to alcohol owing to a build-up of acetaldehyde. Milder effects are seen with variants in other alcohol dehydrogenase

Table 1 | **Psychiatric disorders: symptoms, diagnosis and epidemiology**

Diagnostic category	Clinical hallmarks	Genetic epidemiology			
		Population prevelance	Twin concordance	Heritability	Age of onset
Schizophrenia: a category of severe psychosis. Subtypes include paranoid, disorganized, or catatonic types	Acute: hallucinations, delusions and interference with thought processes (positive symptoms); chronic: the predominant phase of the illness and is characterized by apathy, lack of drive and social isolation (negative symptoms)	1%	MZ: 40–50%; DZ: 14%	70–85%	Late teens or early adulthood
Bipolar disorder (BP): includes BPI, BPII and BP NOS (not otherwise specified). Previously known as manic-depressive disorder	Mania (BPI): pathological elevations in energy, mood, and rates of thought and speech. Significant impairment in functioning; hypomania (BPII): milder manic symptoms with little or no impairment. Both usually (but not always) experience episodes of major depressive disorder	1% each for BPI and BPII	MZ: 70%; DZ: 19%	60–85%	Generally in late teens or early adulthood; paediatric forms are recognized
Major depressive disorder: broad category that is likely to be heterogeneous	Severely depressed mood, profound lack of energy and motivation, pathologically lowered self attitude, feelings of guilt, and slowed pessimistic thoughts	~17%	MZ: 46%; DZ: 20%	40%	Two primary waves of onset: mid-twenties and mid-forties
Autism and autism spectrum disorders: category of pervasive developmental delay disorders that are observed before 2 years of age	Deficits in reciprocal social interactions and communication. Presence of stereotypical behaviours, interests or activities. The prototype of these disorders is childhood autism, and many 'spectrum' or border-line cases are recognized	Strict: 0.04%; spectrum: 0.8%	MZ: 36–82%; DZ: 6%	90%	18–36 months
Eating disorders: includes anorexia nervosa (AN), wherein weight loss is the prominent feature; and bulimia nervosa (BN), wherein disordered eating behaviour predominates	AN: low body weight (<85% of normal range) with intense fear of weight gain and a distorted body image (overweight). Patients might either restrict intake or resort to self-induced vomiting or diarrhoea; BN: regular and recurrent binge-eating episodes with loss of control of eating behaviour and inappropriate compensatory behaviours (vomiting, abuse of laxatives, exercising and so on)	AN: 0.6%; BN 1%; tenfold more common in women	MZ: 55% (AN), 23% (BN); DZ: 7% (AN), 9% (BN)	AN: 55%; BN: 60%	Typically in the early- to mid-teen years
Alcohol use disorders: includes alcohol abuse and alcohol dependence	Abuse: maladaptive pattern of alcohol use with recurrent problems in social or interpersonal situations; dependence: a syndrome of persistent problems involving physiological tolerance, psychological cravings and behaviours centered around alcohol use or the consequences of alcohol use	Ranges from 13% (abuse) to 5% (dependence)	MZ: 40%; DZ: 10% (dependence)	50–60% (dependence)	Mid-twenties
Anxiety disorders: a broad category of heterogeneous disorders that includes panic disorder, the phobias, obsessive compulsive disorder and generalized anxiety disorder	The central and unifying feature of all anxiety disorders is a heightened sense of arousal or fear that is episodic or continuous and might be related to exposure to a specific trigger. There are psychological (worry and fear) and physiological (rapid heartbeat and sweating) components to anxiety disorders	All anxiety disorders: 29%	MZ: 23–73% (panic disorder); DZ: 0–17% (panic disorder)	40–50% (panic disorder)	Many anxiety disorders (including phobias) present in childhood. Others are variable
Obsessive compulsive disorder: a subtype of the anxiety disorders that has been studied genetically	Recurrent, intrusive and repulsive thoughts as well as repetitive purposeful behaviours (for example, checking or cleaning) that the individual feels driven to perform, all of which are recognized by the individual as excessive or unreasonable	1.6%	MZ: 50–80%; DZ: 20–40%	60–70%	~19 years, but onset can occur in later adulthood
Attention deficit-hyperactivity disorder: childhood-onset disorders that become apparent in the educational system	Excessive and impairing levels of activity and inattention. Hyperactivity, if present, adds fidgeting or physical restlessness as well as impulsive interruptive behaviour	8%	MZ: ~60%; DZ: 21%	60–90%	<7 years

A psychiatric diagnosis is a clinical examination, usually carried out by direct interview, that is summarized using diagnostic criteria: either the Diagnostic and Statistical Manual of Mental Disorders Version IV (DSM-IV)[156] or the International Classification of Disorders, Version 10 (ICD-10)[157]. Most genetic research in psychiatry worldwide uses a standardized structured instrument, the Diagnostic Interview for Genetics Studies (DIGS)[158], which allows derivation of diagnoses according to different criteria. The DSM- IV or ICD-10 categories capture the typical forms of illness; for example, an acute mania or severe episode of major depression requiring hospitalization is easily diagnosed according to either criterion with a high kappa value (>0.8) on independent evaluations (the kappa value is the chance-corrected measure of correlation between two raters and is also known as the interrater agreement). Typical descriptions in the table are consistent with either criterion. References for prevalence and concordance rates are given in Supplementary information S1 (table). The diagnostic categories listed in the table are the more common psychiatric disorders that are the subject of genetic research, listed in approximate order of severity. DZ, dizygotic; MZ, monozygotic.

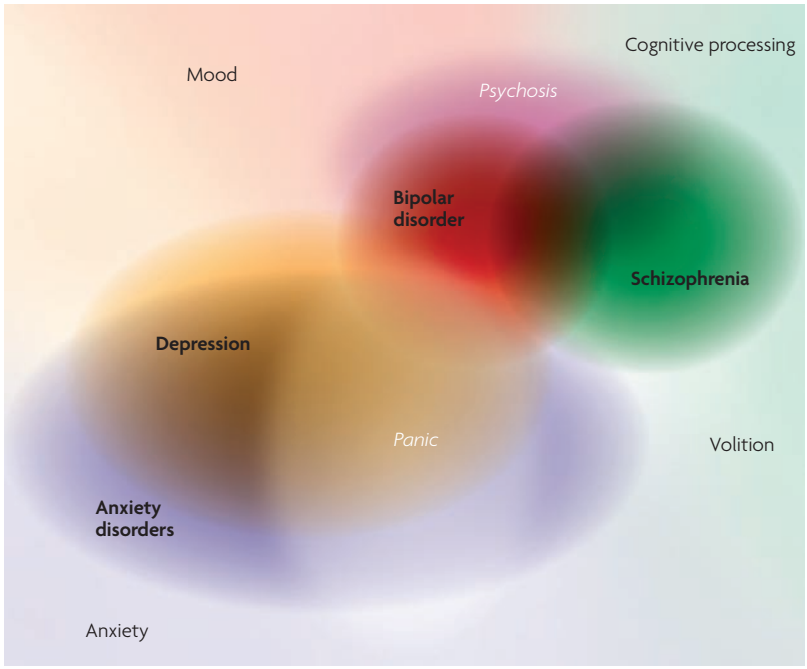


Figure 1 | **Psychiatric disorders overlap and might be extremes of personality traits.** Genetic vulnerabilities for psychiatric disorders are shown as emerging from the extreme end of normal population variations of personality, illustrated as different background shades of mood, anxiety, cognitive processing and volition. Volition, which was introduced by Kraepelin[155], combines the will or the drive to do something with energy and activity level. Genetic factors affecting levels of these underlying traits, in interaction with additional genetic and environmental factors, can lead to psychiatric disorders — shown here are bipolar disorder, schizophrenia, depression and anxiety disorders — the symptoms and genetic risk factors of which are in part unique and in part overlapping. Psychosis and panic are pathological traits and are not a formal diagnostic category, but are associated with several psychiatric diagnoses. Because not all disorders can be covered in two dimensions, interactions and overlaps exist in many more dimensions than can be represented here (for example, depression and anxiety are also present in schizophrenia).

genes in the same pathway[30]. A promoter variant, 5HTTLPR (5-hydroxytryptamine (serotonin) transporter gene-linked polymorphic region) in the serotonin transporter 5HTT (encoded by *SLC6A4*), which is the target of a major class of antidepressants, is now considered to be an established risk factor for depression (see below). Studies of association of variants in the dopamine transporter, which is the target of popular stimulants such as methylphenidate (Ritalin), as a candidate gene for attention deficit-hyperactivity disorder (ADHD) were not consistent. However, gene- and resulting protein-length variation in the C-terminal domain of the dopamine receptor 4 gene (*DRD4*) has been found to be reproducibly associated with ADHD; the longer (7 repeat) allele confers an approximately 1.4-fold increased risk[31]. Later studies suggested an additional association of promoter variants in this gene specifically with inattentive subtype[32]. In addition, there is evidence for recent selection of this variant[33] and for an accumulation of new mutations that occur on the 7-repeat haplotype[34]. Additional candidate genes with evidence for association are listed in TABLE 2 and in Supplementary information S2 (table).

Positional candidate genes. In addition to the biological candidate gene studies outlined above, several investigators took a positional candidate gene approach by testing association of SNPs under a previously identified linkage peak. In this manner, variants in the gamma-aminobutyric acid (GABA) receptor alpha 2 subunit gene (*GABRA2*) were reproducibly established[35–37] as one risk factor for alcoholism. A novel transcript, D-amino-oxidase activator (*DAOA*; previously known as *G72* or *LG72*), was identified[38] by association of SNPs in a gene poor, schizophrenia-linked interval on chromosome 13q32. *DAOA* is human specific and is expressed at extremely low levels in human brain[38], and it might be involved in glutamate signalling. Because bipolar disorder had shown linkage to the same region, it was rewarding to see that SNPs in the same gene were also found to be associated with bipolar disorder[39], although these variants are not consistently replicated. A search for schizophrenia-related variants under a linkage peak on chromosome 8p resulted in the identification of variants in the neuregulin 1 (*NRG1*) gene[40], which is involved in glutaminergic synaptic transmission. Mouse mutations in *Nrg1* show a behavioural phenotype that is related to schizophrenia[40]. Although it was confirmed in a separate study in Scots[41], the strongest finding, which was discovered in the isolated population of Iceland, might have been subject to a combination of winner's curse and genetic heterogeneity: a large study[42] and a meta-analysis[43] in mixed Caucasian samples have not confirmed the original findings. Recently, two studies of autism that were following up a linkage peak on chromosome 7q35 found association with variants in the gene encoding contactin associated protein-like 2 (*CNTNAP2*)[44,45], in addition to rare variants in some cases[46].

GWA studies. GWA studies have recently become possible through the development of high-throughput genotyping chips and the documentation by the HapMap Consortium of informative SNPs covering most of the genome[47,48]. Several genome-wide psychiatric association studies have recently been published. The first, from the Wellcome Trust Case–Control Consortium (WTCCC), was a study of 2,000 cases of bipolar disorder (as well as 2,000 cases for each of nine other disorders) compared with 3,000 population controls[49]. One SNP near the partner and localizer of BRCA2 gene (*PALB2*) on chromosome 16 achieved genome-wide significance, although it was not supported in the study's own expanded reference samples[49]. A study of pooled DNA from familial US cases and controls, which focused on genes and was followed up by replication of a subset of SNPs in a German sample[50], identified SNPs in the diacylglycerol kinase eta gene (*DGKH*). Most recently, a GWA study[51] of 1,461 bipolar cases identified SNPs in the myosin 5, tetraspanin 8, and the epidermal growth factor receptor genes, but these variants were not confirmed in the study's replication samples from Edinburgh and the United States. Combining these data with the WTCCC data resulted in the identification of SNPs within the

Phenomenology
Clinically descriptive, experiential and subjective dimensions of psychopathology.

Positional cloning
Method for identifying the location of a risk variant within a candidate region. Overlapping clones covering the candidate region are typed, and segments that co-segregate perfectly with the disease are identified. These clones are the most likely location of the risk variant.

Meta-analysis
Analysis combining the evidence of multiple data sets.

Table 2 | **Genetic associations with psychiatric disorders (part 1)**

Gene name	Locus symbol	Evidence	Gene and variant function	Phenotypes implicated and evidence
Strong candidate genes*				
Alcohol dehydrogenase 2	*ADH2*	A, M, E, L	First step in alcohol degradation. Variants increase activity	Increased activity in ADH2 leads to unpleasant reaction and protects from alcoholism
Aldehyde dehydrogenase 2	*ALDH2*	A, M, E, L	Breaks down acetaldehyde in alcohol metabolism. Null allele common in East Asia	Individuals with little or no function experience a 'flushing' response when drinking alcohol and are thus protected from alcoholism
Catechol-O-methyltransferase	*COMT*	A, E, F, L	Degradation of neurotransmitters. Met allele is 3 times less active than val allele	Maps to VCFS deletion. Associations with cognitive processing and pain threshold are replicated; association with schizophrenia not confirmed
Dopamine receptor D4	*DRD4*	A, E, M, F	Receptor for dopamine. Length polymorphism (48 bp) in the C-terminus (intracellular loop)	The 7-repeat allele is associated with ADHD (as confirmed by meta-analyses), contains many additional mutations in patients with ADHD, was selected for during evolution
GABA A receptor alpha 2 subunit	*GABRA2*	A, E, M, L	Subunit of the receptor for the inhibitory neurotransmitter GABA	Under the linkage peak for alcoholism and electrophysiological endophenotype. Haplotypes and SNPs are associated with alcohol-use disorders in several studies; some associations were also found with endophenotypes
Monoamine oxidase A	*MAOA*	A, E, F, R, Z	Enzyme degrades serotonin. Rare null allele and common functional promoter variants (2x, 3x and 4x repeat of 30 bp) exist	Family with the null allele displayed impulsive-aggressive behaviour. Mice replicate the aggression phenotype. Functional promoter variant associated with impulsive and antisocial behaviour in interaction with maltreatment
Solute carrier family 6, member 4	*SLC6A4*	A, E, F, M, Z	Re-uptake of serotonin from synapses. The *s* allele is associated with decreased activity	The *s* allele is associated with increased neuroticism and depression symptoms in interaction with environmental factors (FIG. 2), amygdala processing as determined by fMRI, and several other behavioural traits
***Possible candidate genes**‡*				
Brain-derived neurotrophic factor	*BDNF*	A, E, F, Z	Neuronal growth and survival. Met66Val form of precursor protein is dysfunctional	Associated with eating disorders; Met66Val mouse model shows increased anxiety. Associations with neuroticism and BPD not confirmed
Calcium channel, voltage-dependent, L type, alpha 1C subunit	*CACNA1C*	G, R	Channel that mediates influx of calcium	Missense mutation (G406R) found in patients with TS (includes autism). Implicated as genome-wide significant in a combined analysis of 3 GWA data sets of BPD
Contactin associated protein-like 2	*CNTNAP2*	A, G, L, R	Member of the neurexin family. Clusters voltage-gated K^+ channels at node of Ranvier	SNPs in this gene in a linkage region are associated with autism, and mutations are found in rare autism cases
FK506-binding protein 5	*FKBP5*	A, E	Adaptive intracellular response to stress (hypothalamic–pituitary axis)	SNPs associated with anti-depressant response, MDD and possibly BPD. Also PTSD GxE interaction
Neuroligin 1, 3 and 4	*NLGN1, 3* and *4*	A, F, R	Synaptic transmembrane proteins involved in cell adhesion; interact with neurexins	Deletions of this gene in several cases are linked to autism, according to several recent association studies
Neurexin 1	*NRXN1*	A, R	Neuronal cell–cell interaction; implicated in synapse formation	Patients have a breakpoint in the chromosome as a result of a balanced translocation; one association study identified the involvement of such a translocation
Purinergic receptor P2X, ligand-gated ion channel 7	*P2RX7*	A, L, F	CNS-expressed ligand-gated metabotropic 7 transmembrane calcium channel	Found in linked 12q24 region. Different alleles associated with risk for BPD, MDD, and anxiety; some of these studies are large
Regulator of G-protein signalling 4	*RGS4*	A, L	Accelerates GTPase activities of certain G protein alpha-subunits	Decreased expression in SZ post-mortem brains. Found in the SZ-linkage region; an SNP in this gene is associated with SZ
Tryptophan hydroxylase 2	*TPH2*	A, E	The brain form of rate-limiting enzyme for serotonin synthesis	Reported association with impulsivity and suicidality, ADHD, MDD and BPD
Wolfram syndrome 1	*WFS1*	A, F, R	Transmembrane channel in ER; has a role in calcium homeostasis	Recessive null alleles associated with WFS with psychiatric illness. Heterozygotes are at increased risk of mental illness. H611R allele might be associated with suicidality

*Strong evidence is indicated when at least one of the phenotypes was confirmed by meta-analysis. ‡Possible evidence is indicated when there is congruent evidence but it is not confirmed in larger or meta-analyses, or not enough time has passed for confirmation attempts. References are given in the Supplementary information S2 (table). A, association studies with psychiatric disorder in one or more studies; E, association with endophenotype; F, functional evidence for associated variant; G, association in comprehensive study or genome-wide association study; L, in linkage region; M, meta-analysis confirms association; R, mutation in this gene is found in Mendelian and/or rare disorder(s) with a related phenotype; Z, evidence from animal model(s). ADHD, attention deficit-hyperactivity disorder; BPD, bipolar disorder; CNS, central nervous system; ER, endoplasmic reticulum; fMRI, functional magnetic resonance imaging; GABA, gamma-aminobutyric acid; GWA, genome-wide association; GxE, genotype by environment; met, methionine; MDD, major depressive disorder; PTSD, post-traumatic stress disorder; *s* allele, short promoter allele; SZ, schizophrenia; TS, Timothy syndrome; VCFS, velocardiofacial syndrome; val, valine; WFS, Wolfram syndrome.

Table 2 | **Genetic associations with psychiatric disorders (part 2)**

Gene name	Locus symbol	Evidence	Gene and variant function	Phenotypes implicated and evidence
Controversial candidate genes*				
D-amino acid oxidase activator	*DAOA*	A, L	Predicted protein of unknown function, human specific. Activates D-amino acid oxidase	SNP association study of linked region in SZ identified predicted gene sequence. Haplotypes also associated with BPD in some studies. Unclear which allele or haplotype is involved as replications are not consistent
Disrupted in schizophrenia 1	*DISC1*	A, E, R	Function is unclear. Large, cytoplasmic, ubiquitously expressed protein with protein interaction domains	Cloned from the breakpoint of a balanced translocation in a pedigree that segregated several mental illnesses. SNPs found to be associated with p300 abnormalities, SZ and BPD
Dystrobrevin binding protein 1	*DTNBP1*	A, L	Intracellular vesicle transport. Null alleles causes the rare HPS	Maps to a region linked to schizophrenia; association was found with different haplotypes in several Caucasian populations; which alleles are involved is unclear
Neuregulin 1	*NRG1*	A, L	Ligand for ERBB2; function in the nervous system is complex, many different splice forms	In region of SZ linkage. Several haplotypes associated with SZ in different populations; results from meta-analyses, and which alleles are associated, are unclear
Solute carrier family 6, member 3	*SLC6A3*	A, L, F, M, Z	Re-uptake of dopamine from synapses. Some variants are functional	Association shown, mostly by functional variant, with drug response, smoking, MDD and ADHD. Meta-analyses for MDD and ADHD gave unclear results for ADHD. Knockout mice were hyperactive
5,10-methylene-tetrahydrofolate reductase	*MTHFR*	A, F, M	Two common coding variants reduce enzyme activity and blood folate levels	Association with BPD, SZ and depression; meta-analyses exist both in favour of the association and against it
Adrenergic, beta, receptor kinase 2	*ADRBK2*	A, L	Mediates the desensitization of several neurotransmitter receptors	One group reports association with a rare variant and 2 haplotypes, and has commercialized a genetic test. No independent confirmation

*Controversial results are those with different alleles in different studies in different populations, or contradictory results from meta-analyses. There are many more controversial results, but only those genes that are much discussed in the current literature are shown. References are given in the Supplementary information S2 (table). A, association studies with psychiatric disorder in one or more studies; E, association with endophenotype; F, functional evidence for associated variant; L, in linkage region; M, meta-analysis confirms association; R, mutation in this gene is found in Mendelian and/or rare disorder(s) with a related phenotype; Z, evidence from animal model(s). ADHD, attention deficit-hyperactivity disorder; BPD, bipolar disorder; ERBB2, v-erb-b2 erythroblastic leukaemia viral oncogene homologue 2; HPS, Hermansky–Pudlak syndrome; MDD, major depressive disorder; p300, E1A binding protein p300; SZ, schizophrenia.

Non-parametric linkage analysis
Compares the observed segregation of marker alleles in affected and unaffected individuals with the expected segregation under the rules of Mendelian genetics. Deviation from the expected segregation is an indication of linkage.

Rank
Position of one result in a set of results ordered by their test statistic. If no true signal exists in the data, each rank is equally likely. Thus, studies can be combined by comparing the rank of genetic segments across studies.

Multiple testing
Usually, several genomic positions or phenotypes are tested for association or linkage in genetic mapping, and all of these tests have a probability of generating a false positive equal to α — a preset level of significance. Thus, the probability that at least one test generates a false positive is higher than α.

voltage-dependent calcium channel, L-type, alpha 1C subunit (*CACNA1C*) gene as genome-wide significant. Two GWA studies of schizophrenia with modest sample sizes (500–800 cases) found no[52] and one[53] (pseudo-autosomal, near cytokine genes) genome-wide significant finding(s).

In summary, with the possible exception of *CNTNAP2* in autism, GWA studies have not brought the field into focus: each new association study has brought new loci to the surface, and none of the previously identified loci was replicated. This is in contrast to type 2 diabetes, in which the most significant GWA studies confirmed previous associations[54]. However, no current GWA study for a psychiatric disorder has come close to the sample sizes and power of >38,000 cases and controls that were analysed for diabetes, and thus large sample sizes might bring new light in the near future.

Trusting association findings — challenges from mixed results. There is considerable debate in the field about when plausible reported association findings should be accepted and studied at a biological level[55], and when they still need to be confirmed[11,12,42]. False positives (type I errors) arise for many reasons: ethnic stratification, which can largely be dealt with statistically[56]; lack of proper correction for multiple testing in combination with publication bias favouring positive findings; and genotyping errors, which more often result in false negatives (type II errors) or non-replications but can cause false positives[57]. There is currently no generally accepted criterion in the field to declare significance or replication, although sample sizes have increased, and reasonable standards have been suggested[11,58]. The criteria and standards of significance will require careful consideration in the future.

We briefly summarize the most commonly discussed association findings in TABLE 2 and in Supplementary information S2 (table). This table does not list all the hundreds of reported associations, for which we refer to recent disorder-specific reviews[25,59–62], but rather focuses on a few definitely reproduced findings, and some of the newer and most discussed equivocal findings in the literature. One example of such ongoing controversy is association between schizophrenia and dystrobrevin binding protein 1 (*DTNBP1*, also called dysbindin)[63], which maps to chromosome 6p22.3 in a linkage region. Protein transport, in which DTNBP1 is involved, might *a priori* be considered a good candidate pathway for psychiatric disorders[64], but mice and a single patient missing this protein have no behavioural abnormalities, but rather a platelet storage pool deficiency[65]. Searching under the linkage peak in Irish pedigrees, one SNP in the *DTNBP1* gene was found to be preferentially

Box 1 | QTL mapping in population samples

Quantitative traits can be mapped by dichotomizing the sample (between individuals who have a disorder and those who do not) and applying case–control strategies. However, that approach ignores much of the available information. More powerful methods for testing association between an allele and a quantitative phenotype are analysis of variance (ANOVA) or linear regression. In basic linear regression, assuming an additive genetic model, the observed phenotype *P* is fitted to a linear equation, dependent on the genotype counts, *G*, of the minor allele (0,1,2):

$$P = a + b_1 G + \varepsilon$$

The regression coefficient b_1 is the main effect of the genotype and ε is the error term. *a* is the baseline mean phenotype. To show association between the genotype and the phenotype, b_1 has to be significantly different from 0.

Such methods can allow for different genetic models by including non-additive factors; furthermore it is straightforward to include environmental covariates by extending the linear equation:

$$P = a + b_1 G + b_2 D + b_3 E + b_4 GxE + \varepsilon$$

Here, *D* is the dominance factor (1 if the genotype is homozygote for the minor allele, 0 otherwise) and *E* is the environmental covariate; b_4 is then the interaction effect. Such methods have been further extended to include multiple markers or haplotype frequencies. However, including factors in the equation increases the degrees of freedom of the underlying test and thus might reduce the power.

It is important to point out that these methods assume normally distributed error, and therefore require a random population sample and a normally distributed trait. Applying them to quantitative traits measured in samples of cases and controls does not produce valid tests if the quantitative trait is correlated to affection status. Moreover, it is advisable to assess the distribution of the trait in the population to ensure that the trait is approximately normally distributed.

transmitted to affected offspring, as were additional nearby SNPs and haplotypes[63]. Subsequent studies reporting replication for this finding used different SNPs or haplotypes. A meta-analysis of all published studies concluded that every one of the five major haplotypes in this gene has been reported to be associated with schizophrenia in at least one study[66]. A similar situation is evident with *DAOA*: numerous SNPs that are not in linkage disequilibrium (LD) with each other have been reported as being associated with schizophrenia, resulting in ambiguous results in meta-analyses[67]. This case led to recommendations to consider haplotypes and the directions (that is, which allele is the risk allele) carefully before claiming replication[11].

On the other hand, failure of association studies might indicate a moderate level of allelic heterogeneity. Most current tests of association do not consider how to combine alleles or haplotypes in association tests to accommodate this possibility. Because linkage analysis is insensitive to allelic heterogeneity, linkage peaks might reflect the contribution of many different rare mutations in the same gene[68], as is the case in early onset breast cancer, which is caused by mutations in *BRCA1* (breast cancer 1, early onset). If this model holds true, then large-scale sequencing might be necessary[68] to identify such rare variants. Association studies will then test whether there is an overall increase in rare variants in a given candidate gene in cases compared with controls, rather than by comparing specific allele frequencies.

Haplotype
A combination of closely linked alleles that are inherited together as a unit. When the phase is unknown, then the haplotypes in an individual are unknown and need to be estimated.

Pseudo-autosomal
Regions on the sex chromosomes that are homologous between the X chromosome and the Y chromosome.

Type I error
Falsely rejecting the null hypothesis. In genetic studies this is usually equivalent to erroneously attributing a genetic effect when there is no genetic effect.

Quantitative traits versus diagnostic categories

The studies discussed so far used diagnostic end-point phenotypes and compared cases and controls. If risk alleles increase or decrease a quantitative trait, then using such traits in genetic studies is more powerful than imposing an artificial threshold[69] (see BOX 1 for information on how association is tested with a quantitative trait). Psychometrics is the study of quantification of psychological traits — including extremes and abnormal traits, such as personality, drinking behaviour, anxiety levels or intelligence. Association can and has been tested with quantitative traits, such as number of drinks per week or number of depression symptoms. If, in addition, the model of psychiatric disorder is one in which the vulnerability is an extreme position on a continuum of a normal trait (FIG. 1), which needs interaction with additional environmental, developmental or genetic interacting factors to trigger a pathological end-point, one might be better off studying the marker for that vulnerability directly. This is the underlying idea of endophenotypes. Association studies with endophenotypes (BOX 2) have lead to a number of confirmed genetic association findings that are relevant for psychiatric disorders and have promise for future discoveries.

Rare Mendelian disorders

Psychiatric symptoms have a role in many inherited disorders and syndromes[70]. Well-known examples are Huntington disease, Alzheimer disease and Angelman syndrome[70], but examples can also be found in many rare disorders, such as autistic features in CHARGE syndrome[71] or psychiatric symptoms in Wolfram syndrome[72], which is a complex recessive medical syndrome. Heterozygous but otherwise unaffected parents of patients with Wolfram syndrome are also at increased risk for psychiatric disorders[73]. Several chromosomal aberrations are also associated with psychiatric impairments. The most common and best documented is velocardiofacial syndrome (VCFS)[74] — also known as 22q microdeletion syndrome, DiGeorge syndrome or CATCH22 — a complex, variably penetrant syndrome caused by a 1.5–3.0 Mb deletion on chromosome 22q11.2, with a prevalence of about 1 in 4,000. Patients clearly have behavioural abnormalities, including psychosis, which do not fit standard diagnostic criteria and might therefore be diagnosed with ADHD, psychotic depression, bipolar disorder or schizophrenia[74]. Some patients with this deletion have the psychiatric disorder and no other symptoms[75]. Some studies found linkage of schizophrenia to this region[76], and several genes within the deletion have been postulated to be the cause of the psychiatric symptoms, but none has been proven.

Identification of mutations in several unique families with rare near-Mendelian inheritance of psychiatric syndromes has also been extremely useful. A large Dutch pedigree with an X-linked mild mental retardation with violent outbursts of severe impulsive aggression, including rape and assault, was shown to have a mutation that leads to a complete lack of monoamine

Box 2 | Association studies of endophenotypes related to psychiatric disorders

Endophenotypes have been defined by Gottesman and Gould as "measurable components unseen by the unaided eye along the pathway between disease and distal genotype"[120]. Association with a phenotype that is closer to the genetic origin is expected to be stronger than with a more distal disorder. Endophenotypes typically are quantitative traits that can be measured in a general population. FIGURE 1 illustrates the idea that some psychiatric disorders might be at the extreme end of certain personality variations; genetic factors of neuroticism — a tendency to worry and to feel vulnerable and anxious — overlap by about 50% with those of depression[93]. Similarly, high impulsivity is related to drug addiction.

Association studies of endophenotypes, alone or in combination with diagnostic measures, have helped gene identification. The serotonin transporter 5HTT (encoded by *SLC6A4*) is the target of the most commonly used antidepressants, which are selective serotonin re-uptake inhibitors (SSRIs) such as paroxetine (Paxil), citalopram (Celexa) or fluoxetine (Prozac). A common functional variant in the promoter of the gene, often abbreviated to 5HTTLPR (5-hydroxytryptamine (serotonin) transporter gene-linked polymorphic region), was described 10 years ago[121], with the short promoter (*s*) allele having lower activity than the long promoter (*l*) allele. Association of the *s* allele with increased neuroticism was reported[122] and confirmed in many meta-analyses[123,124], although not all studies agree[125]. The relevance of this variant for depression in interaction with the environment is discussed in the main text. Other examples are specific electroencephalogram (EEG) brain oscillations[126] which, when combined with the propensity to drink in a variance component analysis, led to the identification[35] and replication[36,37] of variants in the gamma aminobutyric acid (GABA) receptor alpha 2 subunit gene (*GABRA2*) as a risk factor for alcoholism. Similarly, variants in the cholinergic receptor, nicotinic, alpha 7 gene (*CHRNA7*) have been associated with auditory gating in humans[127] and mice[128]: this early symptom of schizophrenia is characterized by a decrease in the normal ability of the brain to inhibit the response to unimportant stimuli[127]. Although a direct association between *CHRNA7* variants and schizophrenia is not clear[42,129], a clinical trial of a substance that binds to this receptor has already begun[130].

Another powerful technique used in genetic studies that is relevant for psychiatric disorders is functional magnetic resonance imaging (fMRI). A common coding variant (which causes a valine to methionine mutation at peptide 158; Val158Met) in the gene encoding catechol-O-methyltransferase (*COMT*) affects the stability of the enzyme and results in fourfold decreased activity in the rarer met allele[131]. The more common val allele is associated with less efficient cognitive processing by the brain[132]: more blood flow was needed to accomplish the same task in the fMRI assessment. Although this association was reproduced and physiologically explained[133], an association with schizophrenia[132] was not confirmed[42,134]. The consequence of 5HTTLPR could also be detected[135] reproducibly[136] by fMRI in the amygdala. Although imaging is expensive and only small sample sizes can be achieved, the effect sizes of genetic variants seemed larger than for association with clinical phenotypes, resulting in increased statistical power. As the example of COMT shows[133], fMRI will also have an important role in explaining the functional consequences of genetic variations within neurobiological pathways.

Case–control
Genetic study comparing genotype frequencies, allele frequencies or haplotype frequencies between a cohort carrying a disease and a control group. Significant differences between the groups might indicate LD between the screened genetic variant and a risk variant. The control group might be either screened to ensure it does not contain cases of the disease or it might be a random population sample.

Linkage disequilibrium
(LD). Preferential association of one allele of one locus with a particular allele of another locus. In the simplest case, a rare disease mutation might be in LD with alleles on nearby loci because the mutation arose only once, on a founder chromosome that carried specific alleles. Those loci close to the mutation have not been separated from the mutation during evolution. Therefore, alleles that were present in the founder chromosome are overrepresented in patients.

Allelic heterogeneity
When multiple variants in the same gene affect the same disease. This should be contrasted with genetic or locus heterogeneity, when variation in different genes affects the same phenotype.

Variance component analysis
Analysis in which the total variance is separated into the contribution from different components, such as genetic, environmental or interaction factors.

Functional magnetic resonance imaging
(fMRI). The subject is given a task (for example, recalling a sad event, remembering numbers or looking at pictures) and a measure (the blood oxygen-level dependent (BOLD) response) is taken. This measure is correlated with how much blood is used by nerve cells.

Longitudinal measurements
Repeated measurements of the same trait at multiple time points.

oxidase A (MAOA), the protein that is involved in serotonin degradation in the brain[77]; a mouse knockout model shows a similar phenotype[78]. St. Clair and colleagues reported a large pedigree with 21 individuals with a variety of psychiatric diagnoses, which included schizophrenia, bipolar disorder, recurrent depression and conduct disorder, of which 16 were carriers of a t(1;11) (q42;q14) balanced translocation[79,80]. Cloning the breakpoints led to the identification of a neuronally expressed gene, disrupted in schizophrenia 1 (*DISC1*)[81]. Both linkage[82] and association[83,84] with schizophrenia have been reported for *DISC1*, although they are not consistently replicated[42]. Nevertheless, the results are promising enough that the biological pathways of the encoded protein and its interacting proteins, as well as their associations with psychiatric illnesses, are currently being investigated by several groups[85–89].

Genetic and environmental factors

Although heritability of psychiatric disorders is high, most of the remaining risk is not due to shared environment but to unique environmental factors that also include measurement error[90]. Few environmental factors for psychiatric disorders are known: there is a small but consistently increased risk for schizophrenia in subjects born in spring[91] and in those conceived during a famine, for example, the Irish, Dutch and Chinese famine periods[92]; depression is often triggered by stressful life events[93]; the risk for ADHD is increased by maternal smoking[94]; and the risk for autism is increased by prenatal and perinatal complications[95,96] and by parental age[97].

So far, few studies have included environment in genetic analysis. Two studies by Caspi *et al.*[98,99] illustrated the importance of longitudinal measurement of environmental factors. Both studies used a birth cohort of >1,000 subjects from Dunedin, New Zealand. Investigators recorded parental behaviour and discipline throughout childhood as well as significant events that happened to subjects throughout the study, which has been ongoing for >30 years. At age 26, depressive symptoms, depressive episodes and suicidality were evaluated. Confirming previous social and psychological studies, all measures of depression increased with female gender, with parental maltreatment during childhood, and with the number of stressful life events in the years preceding evaluation. There was no main effect (BOX 1) of 5HTTLPR on any of the measures, but a significant interaction effect was observed: the short promoter (*s*) allele increased the risk of depression in interaction with childhood maltreatment (FIG. 2a) and with significant life events (FIG. 2b). Association of this same *s* allele was previously reported with high neuroticism, an endophenotype of depression (BOX 2).

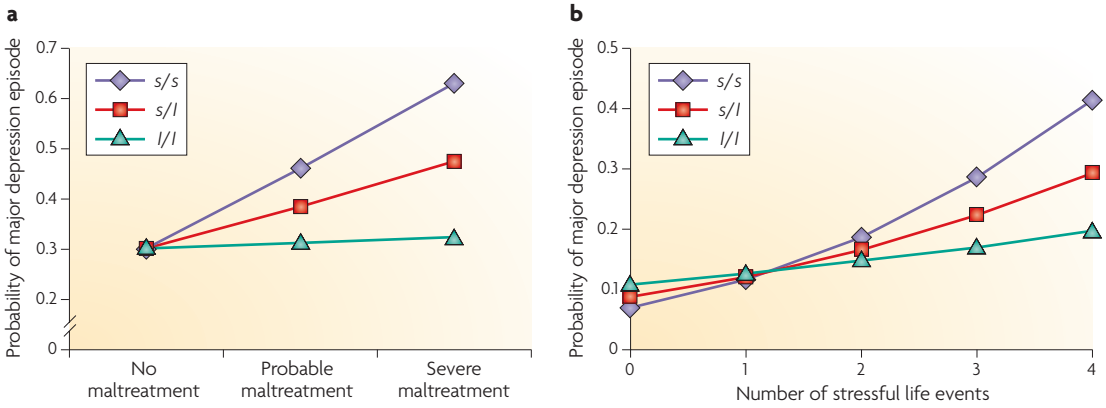


Figure 2 | **Interacting genetic and environmental risk factors for depression. a** | Depression was measured in individuals at age 26 who were evaluated throughout childhood for parental treatment. The probability of a major depressive episode increases with the extent of childhood maltreatment in subjects who have the short promoter (s) allele, a functional polymorphism in the promoter of the serotonin transporter that makes the promoter less active. By contrast, the probability of a major depressive episode in subjects who were homozygous for the more active, long promoter (*l*) allele showed no increase with the extent of childhood maltreatment. **b** | The probability of a major depressive episode increases with the number of stressful life events in the preceding 5 years; this observation occurs in interaction with genotype, with the *s* allele increasing risk in a dose-dependent manner. The graphs shown are the model calculated from a moderated regression analysis, which included additional covariates such as gender. This figure is modified, with permission, from REF. 99 © (2003) American Association for the Advancement of Science.

In another study in the same population, a common promoter variant in the X-linked *MAOA* gene was evaluated in males for association with impulsive, anti-social and criminal behaviour. The low-activity promoter form of *MAOA* interacted with childhood maltreatment such that the low-activity allele increased the risk of impulsive-aggressive or violent behaviour and of criminal activity[98].

Novel structural mutations

Large cytogenetic aberrations[100] and smaller deletions[101] were observed rarely but consistently in autism. *De novo* mutations might also explain the contrast between the high rate of concordance (TABLE 1; Supplementary information S1 (table)) of monozygotic twins compared with that of dizygotic twins[102], leading to the suggestion that they might be 'heritable but not inherited'[103]. Sebat *et al.*[104] recently found an increase of insertions or deletions in autism compared with control families (14 in 165 compared with only 2 in 99). Several overlapping copy number variants (CNVs) were observed on chromosome 2q37, a region previously implicated in autism[105,106], and more recently, several authors implicated a 593 kb recurrent *de novo* deletion on chromosome 16p11.2 in approximately 1% of cases with autism[107–109]. A similar pattern of increased rates of structural rearrangements was recently reported also in schizophrenia (15–20%) compared with control (5%) subjects[110]. Such mutations cannot be identified by linkage, by association, or by most sequencing methods, but such highly penetrant mutations are also not expected to contribute to the inherited risk of complex disorders. Therefore, approaches to identify heritable factors in multiplex families might need to be different from those that are used to identify *de novo* mutations in sporadic cases, as was done for autism[104]. It is not yet clear what fraction of disease-associated *de novo* mutations are rare unique events and what fraction are recurrent mutations in regions of segmental duplications, which are predicted to be hot spots for such mutations[111,112].

The involvement of large structural mutations, especially recurrent ones, has been realized only recently; the numbers of these mutations are still too small to ascertain how much of an impact they will have on the field. However, the considerable progress made in the last year alone suggests that more findings can be expected, with a significant impact on the prospects for risk prediction and genetic counselling.

Addressing genetic heterogeneity

Thanks in part to genetic discoveries, we are improving our understanding of the biology of several psychiatric disorders (BOX 3). However, both the phenotypes and the genetics are complex, and we still have not identified most genetic risk alleles for any psychiatric disorder. We can conclude that major risk variants for these diseases do not exist, and that most common risk variants are likely to increase risk only marginally (that is, the odds ratio is much less than 2). This result is somewhat at odds with the high heritability estimated for these disorders, suggesting that high genetic heterogeneity or non-standard factors, such as *de novo* mutations, epigenetic changes or combinations of these factors, distort estimates of heritability.

If psychiatric disorders do exhibit genetic heterogeneity, then methods to address this heterogeneity might be the key to mapping these risk variants with GWA. At least three scenarios of this model of many genes with small marginal effect sizes can be imagined. In the first scenario, each gene contributes to the risk of

Box 3 | **Functional pathways involved in psychiatric disorders**

Ultimately, the goal of genetic investigations is to find treatments or preventive measures by furthering our understanding of the aetiology. Perhaps the best example of a novel biological pathway in a psychiatric disorder that has been provided by genetic studies is the identification of several gene families involved in the cell adhesion and regulation of glutaminergic synaptic connectivity in autism[137]. This pathway was implicated in these processes by functional mutations or deletions in the two X-linked neuroligin genes in several rare sibships or cases with autism[138–140], and also through small structural aberrations in neurexin 1 (REFS 141,142), a receptor for neuroligin. Modest association in small studies was found for three neuroligin genes[143], and the recently implicated (see main text) contactin associated protein-like 2 gene (*CNTNAP2*) encodes another neurexin-related protein, possibly in the same pathway[44–46].

Several investigators suggest that a related glutaminergic synaptic pathway is also affected in schizophrenia[60,144]; several of the schizophrenia candidate genes and some of the newly discovered large deletions[110] are involved. Pathway analysis can help to identify new candidate genes: phosphodiesterase 4B (PDE4B) was identified as a binding partner of disrupted in schizophrenia 1 (DISC1), and *PDE4B* might be associated with schizophrenia[87]. Although the exact pathways involved in bipolar disorder are not yet established, the specific efficacy of lithium in normalizing mood has suggested that protein and gene expression changes in response to lithium[145,146] will identify relevant pathways[147]. This research has so far suggested the involvement of inositol intracellular signalling and glycogen synthase kinase 3 (GSK 3)[148]. Diacylglycerol kinase eta, which showed association in one unconfirmed bipolar disorder GWA study, is involved in this pathway[50].

There is strong evidence that a lack of serotonin and of neurotrophic factors in some parts of the brain are involved in major depressive disorder[149]. Dietary depletion of serotonin can induce depressive episodes, and many anti-depressants act by blocking either the degradation of serotonin (monoamine oxidase inhibitors) or its re-uptake by selective serotonin re-uptake inhibitors (SSRIs). The neurotrophic hypothesis of depression implicates decreased neuronal growth factors in depression, and this process is counteracted by antidepressants. The fibroblast growth factor (FGF) pathway has also been implicated in major depressive disorder in a genome-wide microarray gene expression study[150]. The hypothalamic–pituitary axis (HPA) is a system of hormonal interactions affected by depression[151], although it is not clear whether HPA disturbances are only a consequence of depression or can also be causally involved. In summary, although the exact mechanism of aetiology has not yet been elucidated, progress has been made in the identification of pathways that are affected in psychiatric disorders through the use of a variety of approaches, including genetic associations.

Pathways as well as candidate genes can be studied in animal models[152]: recently, the Val66Met variant (valine to methionine mutation at peptide 66) of the brain-derived neurotrophic factor (BDNF), which is associated with depression-related traits and eating disorders, was introduced into mice and caused them to be more anxious than control mice[153]. Because some symptoms of psychiatric disorders, such as language delay and hallucinations, are difficult to model[152] in experimental animals, these mice will mostly be useful in pathway identification and in studying candidate genes and endophenotypes.

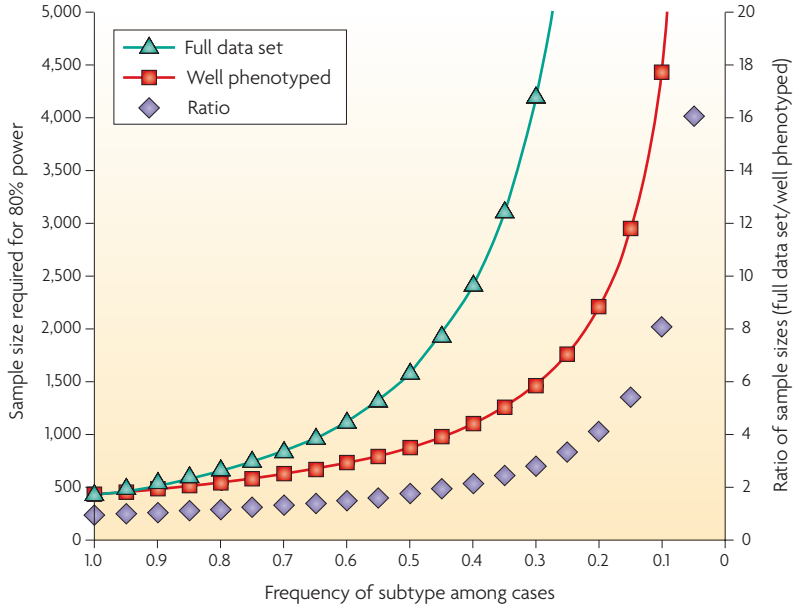
getting the same mental disorder independently of all other risk factors and of exact phenomenology. If common alleles show marginal increases of risk, the best method of identifying the risk variants is by collecting a sufficiently large sample. Therefore, several public and private repositories have been established (for example, the National Institute of Mental Health genetic study repository and the Autism Genetic Research Exchange), and many groups are collaborating in meta-analyses of their samples. Although there is the danger of increasing heterogeneity by pooling data, the successful identification of type 2 diabetes risk alleles by combining data across different populations[113] has demonstrated that this approach is well worth a try. Nevertheless, even with 38,000 samples, less than 10% of the genetic fraction of the genetic variance for type 2 diabetes has been explained. Therefore, large GWA and/or meta-analyses will probably only identify a subset of genetic variants associated with psychiatric disorders.

In the second scenario, some risk variants might be associated only with specific subtypes of a mental disorder. In this case, focusing the analysis on subtypes might allow us to improve the power of an association study. Even though the calculations presented in BOX 4 demonstrate that large samples of high quality will often result in a more powerful test, heritable subtypes might exist: breast cancer in general is mostly sporadic, yet breast cancer with onset before the age of 40 is highly heritable, although it affects only a minute fraction of the case population. At one extreme, rare families with near-Mendelian forms of disorders with psychiatric components helped to identify a small number of genes. Attention to phenotypes and the recruitment of families with rare, atypical forms of psychiatric disorders, which might constitute rare subtypes, might well be worth the effort. On the other extreme, some reproducible findings were obtained with heritable endophenotypes (BOX 2) measured at the population level, or in conjunction with clinical disorders. Whether clinical disorders or endophenotypes are used, consideration should be given to quantitative phenotypes rather than to dichotomous traits.

A third scenario is that variants only affect risk if they co-occur with other genetic or environmental risk factors. Incorporating already known genetic factors into any model is likely to improve power once first genetic effects are determined — in autism, incorporating the cytogenetic variants might be feasible in the near future. As demonstrated by the two reports from Caspi *et al.*[98,99], incorporation of environmental factors into regression models can make the difference between success and failure in detecting a genetic effect. Owing to selected recall bias, most environmental effects are best studied in large longitudinal studies, a proposal currently being discussed[114–116]. Few genetic studies of psychiatric disorders have so far incorporated the known environmental risk factors into their analysis, which is a method worth pursuing.

On the other hand, if the heterogeneity is so high that most mutations are unique to each family, as is the case with *BRCA1*, the traditional methods of comparing allele frequency differences in GWA studies will not be successful, except in isolated or inbred populations. The new results of rare large structural rearrangements in autism and schizophrenia were obtained by comparative genomic hybridization on hundreds of case and control samples. No current methods allow large-scale genome-wide screening for rare point mutations. If such rare mutations have a major role in the aetiology of most psychiatric disorders, we would not expect them to be identified by any of the current approaches. New high-throughput sequencing technologies will be needed to identify genes in which mutational load in a gene, rather than allele-frequency differences, will be tested in cases compared with controls.

Box 4 | **Sampling schemes for psychiatric disorders**



Increasing the sample size is a panacea to problems in complex disease mapping. By 2009, samples of over 10,000 cases and controls will exist for bipolar disorder and schizophrenia. However, such samples are expected to show increased heterogeneity; this is because samples from different sources are combined and because subtle biases in diagnosis might affect the composition of the sample[154]. Alternatively, assessing subtypes might allow us to identify samples that are genetically more homogeneous, increasing the marginal effect sizes of underlying variants. However, increasing power by typing more individuals is generally considered the better strategy to overcome heterogeneity. To compare the power of these two strategies, we modelled a risk variant affecting one subtype of the disorder. We contrast the strategy of using the general phenotype and analysing the entire sample with carefully phenotyping the subtype and analysing only cases with the subtype.

The results, shown in the figure, indicate that the advantage of identifying a frequent subtype is small: if the frequency of the subtype among all cases is 0.8, the sample size required to achieve 80% power is 664 cases using the broad phenotype (full data set; green triangles), whereas 550 cases have to be phenotyped for the subtype (well phenotyped; red squares) to achieve the same power. The blue diamonds and the right-hand scale indicate the ratio of sample sizes between the two strategies. On the other hand, using a general phenotype is problematic if the subtype constitutes only a small subset of the sample. If 20% of cases have a subtype, 9,200 broadly defined cases would have to be analysed to achieve the same power as 2,200 of a well-phenotyped sample.

However, here we assume the optimal scenario of a genetically distinct, heritable subphenotype that can be determined for each case. In practice, subtypes might share some genetic risk with the general phenotype, and identifying subtypes is unlikely to be error free. Furthermore, heritability of subtypes is often unknown. The power of the analysis is reduced if several different phenotypes are tested for association, because corrections have to be made for multiple testing. In addition, the cost of genotyping larger samples and of phenotyping subtypes needs to be considered. Subtypes that are derived from the Diagnostic Instrument for Genetic Studies (DIGS) (TABLE 2; Supplementary information S1 (table)) — such as rapid cycling bipolar disorder — can be obtained from the same data set that is already available for the broad phenotype, whereas some phenotypes — such as those derived from functional magnetic resonance imaging (fMRI) — substantially increase the ascertainment cost. These extra challenges might easily outweigh the modest power gains obtained from focusing on a subtype when the subtype is frequent.

In summary, if sample sizes can be increased without increasing sample heterogeneity, then concentrating on broader phenotype definitions will usually be optimal — subtypes can be explored using the same data set, and genotyping costs are usually small compared with phenotyping and recruitment costs. However, testing specific clinical subtypes is a more powerful approach if the subtype is well defined, heritable and uncommon (that is, occuring in less than a third of all cases), and also if the subtypes can be obtained without too much extra cost.

Perspective and recommendations

Currently, the fields of neuroscience, proteomics, gene expression analysis and genetics operate largely independently of each other. Once the functional pathways that are involved in psychiatric disorders and their associated traits of interest are identified, statistically sound combined analysis of genetics with gene expression and pathway analysis will be needed. Testing biologically plausible candidate genes for genetic association surely falls into this category, but this approach, although it is straightforward, has led to many false positives and irreproducible reports, probably owing to a combination of genotyping error, publication bias and insufficient correction for multiple testing. Other attempts have involved stratifying gene expression data in an animal model of bipolar disorder by human linkage peak regions[117]. Triaging association studies by candidate genes, identifying genetic variants that affect transcription levels, and studying both genetics and gene expression in populations of affected and control individuals[113] also can help in stratifying variants for genetic tests[118,119]. Merging different data types from separate fields into a common analysis that results in a joint statistical probability is a bioinformatic and statistical challenge.

In summary, psychiatric genetics exhibits complexity at many different levels — there are diverse genetic mechanisms as well as genetic, allelic and phenotypic heterogeneity, and phenotypic pleiotropy; in addition, psychiatric disorders are influenced by environmental factors. It is unlikely that a single strategy will allow the identification of all genetic risk factors, but this complexity will have to be attacked from many different angles. In addition to increasing sample size by meta-analyses and large repositories, as is already being pursued in GWA studies, we recommend increased attention to phenotypes, especially the consideration of rare heritable subtypes. Separate analysis of familial and sporadic cases is also recommended to increase detection rates of common risk factors and of novel mutations. Longitudinal and high-risk family studies are recommended to identify early symptoms and endophenotypes, and to allow the identification of interacting environmental factors. We also challenge bioinformaticians and statisticians to identify ways to incorporate the knowledge from neuroscience, proteomics, gene expression and animal models into findings in genetics in a statistically sound fashion.

Given the small individual effect sizes of the few identified risk variants and the complexities of overlapping genetic risk factors, phenotypes and environmental factors, it seems unlikely that genetic tests for diagnosing psychiatric disorders at an individual level will be informative any time soon — the launch of a test for a single unconfirmed rare variant[14] seems premature. If large numbers of rare mutations are involved in the majority of cases, we might need to wait for cheap individual re-sequencing — the '$1,000 genome', which is on the horizon. Genetic results will definitely help to shape new definitions of the standard diagnostic categories, on the basis of a better understanding of

Rapid cycling bipolar disorder
A type of bipolar disorder in which the patient experiences four or more episodes of mania and/or depression per year.

the aetiology, of phenomenology and of genetic aetiology. We will probably soon recognize dozens of bipolar disorders and schizophrenias, just as there are now dozens of genetically defined forms of deafness or spinocerebellar ataxia. We might also identify new psychiatric syndromes that are currently not recognized correctly, as has been the case with Brunner syndrome, which is defined by *MAOA* mutations, and several of the recently identified novel recurrent microdeletion syndromes. In addition to rewriting diagnostic manuals, one hope is that genetic findings will lead to recognition of new biological pathways involved in aetiology, which in turn will help in the development of new therapies and in the development of personalized treatment. The discovery of new pathways might help personalized medicine more than the discovery of individual alleles: it is unlikely that variants that only marginally increase risk will determine drug response. However, it is conceivable that the overall environmental and genetic risk patterns encountered will allow the classification of subjects with psychiatric disorders on the basis of the pathways involved in their aetiology. Treatments might then be tailored to subtypes of these disorders on the basis of the affected pathways.

1. Plomin, R., Owen, M. J. & McGuffin, P. The genetic basis of complex human behaviors. *Science* **264**, 1733–1739 (1994).
This paper reviews the evidence from family, twin and adoption studies for a genetic basis of behaviour and psychiatric disorders.
2. Slater, E. The inheritance of manic-depressive insanity. *Proc. R. Soc. Med.* **29**, 39–48 (1936).
3. McGuffin, P. *et al.* The heritability of bipolar affective disorder and the genetic relationship to unipolar depression. *Arch. Gen. Psychiatry* **60**, 497–502 (2003).
4. Sullivan, P. F., Kendler, K. S. & Neale, M. C. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch. Gen. Psychiatry* **60**, 1187–1192 (2003).
5. Gupta, A. R. & State, M. W. Recent advances in the genetics of autism. *Biol. Psychiatry* **61**, 429–437 (2007).
6. Kieseppa, T., Partonen, T., Haukka, J., Kaprio, J. & Lonnqvist, J. High concordance of bipolar I disorder in a nationwide sample of twins. *Am. J. Psychiatry* **161**, 1814–1821 (2004).
7. Bespalova, I. N. & Buxbaum, J. D. Disease susceptibility genes for autism. *Ann. Med.* **35**, 274–281 (2003).
8. Locatelli, I., Lichtenstein, P. & Yashin, A. I. The heritability of breast cancer: a Bayesian correlated frailty model applied to Swedish twins data. *Twin Res.* **7**, 182–191 (2004).
9. Schildkraut, J. M., Risch, N. & Thompson, W. D. Evaluating genetic association among ovarian, breast, and endometrial cancer: evidence for a breast/ovarian cancer relationship. *Am. J. Hum. Genet.* **45**, 521–529 (1989).
10. Wirdefeldt, K., Gatz, M., Schalling, M. & Pedersen, N. L. No evidence for heritability of Parkinson disease in Swedish twins. *Neurology* **63**, 305–311 (2004).
11. Chanock, S. J. *et al.* Replicating genotype–phenotype associations. *Nature* **447**, 655–660 (2007).
12. Newton-Cheh, C. & Hirschhorn, J. N. Genetic association studies of complex traits: design and analysis issues. *Mutat. Res.* **573**, 54–69 (2005).
This is a review of design and analysis challenges in GWA studies.
13. Zollner, S. & Pritchard, J. K. Overcoming the winner's curse: estimating penetrance parameters from case–control data. *Am. J. Hum. Genet.* **80**, 605–615 (2007).
14. Couzin, J. Science and commerce. Gene tests for psychiatric risk polarize researchers. *Science* **319**, 274–277 (2008).
15. Egeland, J. A. *et al.* Bipolar affective disorders linked to DNA markers on chromosome 11. *Nature* **325**, 783–787 (1987).
16. Kelsoe, J. R. *et al.* Re-evaluation of the linkage relationship between chromosome 11p loci and the gene for bipolar affective disorder in the Old Order Amish. *Nature* **342**, 238–243 (1989).
17. Baron, M. *et al.* Genetic linkage between X-chromosome markers and bipolar affective illness. *Nature* **326**, 289–292 (1987).
18. Gershon, E. S. Marker genotyping errors in old data on X-linkage in bipolar illness. *Biol. Psychiatry* **29**, 721–729 (1991).
19. Lewis, C. M. *et al.* Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia. *Am. J. Hum. Genet.* **73**, 34–48 (2003).
20. Segurado, R. *et al.* Genome scan meta-analysis of schizophrenia and bipolar disorder, part III: bipolar disorder. *Am. J. Hum. Genet.* **73**, 49–62 (2003).
21. McQueen, M. B. *et al.* Combined analysis from eleven linkage studies of bipolar disorder provides strong evidence of susceptibility loci on chromosomes 6q and 8q. *Am. J. Hum. Genet.* **77**, 582–595 (2005).
22. Lamb, J. A. *et al.* Analysis of IMGSAC autism susceptibility loci: evidence for sex limited and parent of origin specific effects. *J. Med. Genet.* **42**, 132–137 (2005).
23. Barnby, G. & Monaco, A. P. Strategies for autism candidate gene analysis. *Novartis Found. Symp.* **251**, 48–63; discussion 63–9, 109–11 & 281–297 (2003).
24. Monaco, A. P. & Bailey, A. J. Autism. The search for susceptibility genes. *Lancet* **358**, S3 (2001).
25. Yang, M. S. & Gill, M. A review of gene linkage, association and expression studies in autism and an assessment of convergent evidence. *Int. J. Dev. Neurosci.* **25**, 69–85 (2007).
26. Hanna, G. L. *et al.* Genome-wide linkage analysis of families with obsessive-compulsive disorder ascertained through pediatric probands. *Am. J. Med. Genet.* **114**, 541–552 (2002).
27. Willour, V. L. *et al.* Replication study supports evidence for linkage to 9p24 in obsessive-compulsive disorder. *Am. J. Hum. Genet.* **75**, 508–513 (2004).
28. Shugart, Y. Y. *et al.* Genomewide linkage scan for obsessive-compulsive disorder: evidence for susceptibility loci on chromosomes 3q, 7p, 1q, 15q, and 6q. *Mol. Psychiatry* **11**, 763–770 (2006).
29. Risch, N. & Merikangas, K. The future of genetic studies of complex human diseases. *Science* **273**, 1516–1517 (1996).
This is an opinion piece that rang in the era of association studies.
30. Ehrig, T., Bosron, W. F. & Li, T. K. Alcohol and aldehyde dehydrogenase. *Alcohol Alcohol.* **25**, 105–116 (1990).
31. Faraone, S. V., Doyle, A. E., Mick, E. & Biederman, J. Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. *Am. J. Psychiatry* **158**, 1052–1057 (2001).
This paper is one of the earliest meta-analyses of association studies of a candidate gene — a C-terminal functional protein variant in the dopamine receptor gene — and its association with ADHD.
32. Lasky-Su, J. *et al.* Family-based association analysis of a statistically derived quantitative traits for ADHD reveal an association in *DRD4* with inattentive symptoms in ADHD individuals. *Am. J. Med. Genet. B Neuropsychiatr Genet.* **147**, 100–106 (2008).
33. Ding, Y. C. *et al.* Evidence of positive selection acting at the human dopamine receptor D4 gene locus. *Proc. Natl Acad. Sci. USA* **99**, 309–314 (2002).
34. Grady, D. L. *et al.* High prevalence of rare dopamine receptor D4 alleles in children diagnosed with attention-deficit hyperactivity disorder. *Mol. Psychiatry* **8**, 536–545 (2003).
35. Edenberg, H. J. *et al.* Variations in *GABRA2*, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. *Am. J. Hum. Genet.* **74**, 705–714 (2004).
This paper shows the association of several SNPs in *GABRA2* with alcoholism — a finding that was later confirmed in references 36 and 37.
36. Lappalainen, J. *et al.* Association between alcoholism and gamma-amino butyric acid alpha 2 receptor subtype in a Russian population. *Alcohol Clin. Exp. Res.* **29**, 493–498 (2005).
37. Covault, J., Gelernter, J., Hesselbrock, V., Nellissery, M. & Kranzler, H. R. Allelic and haplotypic association of *GABRA2* with alcohol dependence. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **129**, 104–109 (2004).
38. Chumakov, I. *et al.* Genetic and physiological data implicating the new human gene *G72* and the gene for D-amino acid oxidase in schizophrenia. *Proc. Natl Acad. Sci. USA* **99**, 13675–13680 (2002).
39. Hattori, E. *et al.* Polymorphisms at the *G72/G30* gene locus, on 13q33, are associated with bipolar disorder in two independent pedigree series. *Am. J. Hum. Genet.* **72**, 1131–1140 (2003).
40. Stefansson, H. *et al.* Neuregulin 1 and susceptibility to schizophrenia. *Am. J. Hum. Genet.* **71**, 877–892 (2002).
41. Stefansson, H. *et al.* Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. *Am. J. Hum. Genet.* **72**, 83–87 (2003).
42. Sanders, A. R. *et al.* No significant association of 14 candidate genes with schizophrenia in a large European ancestry sample: implications for psychiatric genetics. *Am. J. Psychiatry* **165**, 497–506 (2008).
The authors tested, in a large sample, the genes that had shown association with schizophrenia and that had also been most highly replicated in previous studies, but could not confirm association with any of them.
43. Munafo, M. R., Attwood, A. S. & Flint, J. Neuregulin 1 genotype and schizophrenia. *Schizophr. Bull.* **34**, 9–12 (2008).
44. Arking, D. E. *et al.* A common genetic variant in the neurexin superfamily member *CNTNAP2* increases familial risk of autism. *Am. J. Hum. Genet.* **82**, 160–164 (2008).
45. Alarcon, M. *et al.* Linkage, association, and gene-expression analyses identify *CNTNAP2* as an autism-susceptibility gene. *Am. J. Hum. Genet.* **82**, 150–159 (2008).
46. Bakkaloglu, B. *et al.* Molecular cytogenetic analysis and resequencing of contactin associated protein-like 2 in autism spectrum disorders. *Am. J. Hum. Genet.* **82**, 165–173 (2008).
47. Wollstein, A. *et al.* Efficacy assessment of SNP sets for genome-wide disease association studies. *Nucleic Acids Res.* **35**, e113 (2007).
48. Eberle, M. A. *et al.* Power to detect risk alleles using genome-wide tag SNP panels. *PLoS Genet.* **3**, 1827–1837 (2007).
49. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661–678 (2007).
The WTCCC evaluated 2,000 cases each for 7 common disorders, including bipolar disorder, for GWA against a common panel of 3,000 controls. Only one SNP was found to be genome-wide significant for bipolar disorder.
50. Baum, A. E. *et al.* A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. *Mol. Psychiatry* **13**, 197–207 (2008).
51. Sklar, P. *et al.* Whole-genome association study of bipolar disorder. *Mol. Psychiatry* **13**, 558–569 (2008).
The authors identified several GWAs for bipolar disorder, although they did not confirm them in replication samples. After combining their data with those from the WTCCC, they identified association with SNPs in a novel gene, *CACNA1C*.

52. Sullivan, P. F. *et al.* Genomewide association for schizophrenia in the CATIE study: results of stage 1. *Mol. Psychiatry* **13**, 570–584 (2008).
53. Lencz, T. *et al.* Converging evidence for a pseudoautosomal cytokine receptor gene locus in schizophrenia. *Mol. Psychiatry* **12**, 572–580 (2007).
54. Scott, L. J. *et al.* A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* **316**, 1341–1345 (2007).
This is a landmark paper that, together with two others, identified >10 common variants associated with type 2 diabetes, 3 of them confirming previous findings. Joint analysis with other groups, including the WTCCC, evaluated >38,000 samples, demonstrating that large sample sizes can overcome the challenges of small effect sizes.
55. Ross, C. A., Margolis, R. L., Reading, S. A., Pletnikov, M. & Coyle, J. T. Neurobiology of schizophrenia. *Neuron* **52**, 139–153 (2006).
56. Pritchard, J. K., Stephens, M., Rosenberg, N. A. & Donnelly, P. Association mapping in structured populations. *Am. J. Hum. Genet.* **67**, 170–181 (2000).
57. Mitchell, A. A., Cutler, D. J. & Chakravarti, A. Undetected genotyping errors cause apparent overtransmission of common alleles in the transmission/disequilibrium test. *Am. J. Hum. Genet.* **72**, 598–610 (2003).
58. Sullivan, P. F. Spurious genetic associations. *Biol. Psychiatry* **61**, 1121–1126 (2007).
59. Lopez-Leon, S. *et al.* Meta-analyses of genetic studies on major depressive disorder. *Mol. Psychiatry* 16 Oct 2007 (doi:10.1038/sj.mp.4002088).
This paper presents a meta-analysis that identified six different SNPs that were reproducibly associated with major depressive disorder.
60. Lang, U. E., Puls, I., Muller, D. J., Strutz-Seebohm, N. & Gallinat, J. Molecular mechanisms of schizophrenia. *Cell. Physiol. Biochem.* **20**, 687–702 (2007).
61. Hayden, E. P. & Nurnberger, J. I. Jr. Molecular genetics of bipolar disorder. *Genes Brain Behav.* **5**, 85–95 (2006).
62. Craddock, N., O'Donovan, M. C. & Owen, M. J. Genes for schizophrenia and bipolar disorder? Implications for psychiatric nosology. *Schizophr. Bull.* **32**, 9–16 (2006).
63. Straub, R. E. *et al.* Genetic variation in the 6p22.3 gene *DTNBP1*, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. *Am. J. Hum. Genet.* **71**, 337–348 (2002).
64. Newell-Litwa, K., Seong, E., Burmeister, M. & Faundez, V. Neuronal and non-neuronal functions of the AP-3 sorting machinery. *J. Cell Sci.* **120**, 531–541 (2007).
65. Li, W. *et al.* Hermansky–Pudlak syndrome type 7 (HPS-7) results from mutant dysbindin, a member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). *Nature Genet.* **35**, 84–89 (2003).
66. Mutsuddi, M. *et al.* Analysis of high-resolution HapMap of *DTNBP1* (dysbindin) suggests no consistency between reported common variant associations and schizophrenia. *Am. J. Hum. Genet.* **79**, 903–909 (2006).
This is a sobering paper that shows that several claimed replications of the findings of *DTNBP1* association with schizophrenia (including those in reference 63) were in fact not with the same haplotypes in the gene. This demonstrates that much care is needed to untangle the LD patterns when different SNPs are used in different 'replication' studies.
67. Shi, J., Badner, J. A., Gershon, E. S. & Liu, C. Allelic association of *G72/G30* with schizophrenia and bipolar disorder: a comprehensive meta-analysis. *Schizophr. Res.* **98**, 89–97 (2008).
68. Gershon, E. S., Liu, C. & Badner, J. A. Genome-wide association in bipolar. *Mol. Psychiatry* **13**, 1–2 (2008).
69. Allison, D. B. Transmission-disequilibrium tests for quantitative traits. *Am. J. Hum. Genet.* **60**, 676–690 (1997).
70. Cassidy, S. B. & Morris, C. A. Behavioral phenotypes in genetic syndromes: genetic clues to human behavior. *Adv. Pediatr.* **49**, 59–86 (2002).
71. Stromland, K. *et al.* CHARGE association in Sweden: malformations and functional deficits. *Am. J. Med. Genet. A* **133**, 331–339 (2005).
72. Swift, M. & Swift, R. G. Psychiatric disorders and mutations at the Wolfram syndrome locus. *Biol. Psychiatry* **47**, 787–793 (2000).
73. Swift, R. G., Polymeropoulos, M. H., Torres, R. & Swift, M. Predisposition of Wolfram syndrome heterozygotes to psychiatric illness. *Mol. Psychiatry* **3**, 86–91 (1998).
74. Gothelf, D. Velocardiofacial syndrome. *Child Adolesc Psychiatr. Clin. N. Am.* **16**, 677–693 (2007).
75. Karayiorgou, M. *et al.* Schizophrenia susceptibility associated with interstitial deletions of chromosome 22q11. *Proc. Natl Acad. Sci. USA* **92**, 7612–7616 (1995).
This is the first demonstration that some subjects with the 22q deletion typical of a medical condition, VCFS or diGeorge syndrome, might only carry the diagnosis of schizophrenia. This demonstrates the importance of rare medical conditions and careful phenotyping — the subjects had subtle facial abnormalities typical of the 22q deletion.
76. Mowry, B. J. *et al.* Multicenter linkage study of schizophrenia loci on chromosome 22q. *Mol. Psychiatry* **9**, 784–795 (2004).
77. Brunner, H. G., Nelen, M., Breakefield, X. O., Ropers, H. H. & van Oost, B. A. Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. *Science* **262**, 578–580 (1993).
Using a unique large X-linked pedigree this paper shows that inactivation of MAOA leads to a syndrome of impulsive-aggressive behaviour.
78. Cases, O. *et al.* Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. *Science* **268**, 1763–1766 (1995).
79. St. Clair, D. *et al.* Association within a family of a balanced autosomal translocation with major mental illness. *Lancet* **336**, 13–16 (1990).
This paper reports the study of a large extended pedigree in which >20 members carry a balanced translocation and have many different psychiatric diagnoses. This demonstrates pleiotropy of some genetic risk factors and, together with the later cloning, the importance of such rare unique cases in gene identification.
80. Blackwood, D. H. *et al.* Schizophrenia and affective disorders — cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. *Am. J. Hum. Genet.* **69**, 428–433 (2001).
81. Millar, J. K. *et al.* Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum. Mol. Genet.* **9**, 1415–1423 (2000).
82. Ekelund, J. *et al.* Chromosome 1 loci in Finnish schizophrenia families. *Hum. Mol. Genet.* **10**, 1611–1617 (2001).
83. Hodgkinson, C. A. *et al.* Disrupted in schizophrenia 1 (DISC1): association with schizophrenia, schizoaffective disorder, and bipolar disorder. *Am. J. Hum. Genet.* **75**, 862–872 (2004).
84. Kockelkorn, T. T. *et al.* Association study of polymorphisms in the 5′ upstream region of human *DISC1* gene with schizophrenia. *Neurosci. Lett.* **368**, 41–45 (2004).
85. Pickard, B. S. *et al.* The *PDE4B* gene confers sex-specific protection against schizophrenia. *Psychiatr. Genet.* **17**, 129–133 (2007).
86. Millar, J. K. *et al.* Disrupted in schizophrenia 1 and phosphodiesterase 4B: towards an understanding of psychiatric illness. *J. Physiol.* **584**, 401–405 (2007).
87. Millar, J. K. *et al. DISC1* and *PDE4B* are interacting genetic factors in schizophrenia that regulate cAMP signaling. *Science* **310**, 1187–1191 (2005).
After *DISC1* was found to be associated with schizophrenia in previous work, the authors of this paper show that it interacts with another protein that might also be associated with schizophrenia.
88. Li, C. *et al.* An essential role for DYF-11/MIP-T3 in assembling functional intraflagellar transport complexes. *PLoS Genet.* **4**, e1000044 (2008).
89. Brandon, N. J. Dissecting DISC1 function through protein–protein interactions. *Biochem. Soc. Trans.* **35**, 1283–1286 (2007).
90. McGue, M. & Bouchard, T. J. Jr. Genetic and environmental influences on human behavioral differences. *Annu. Rev. Neurosci.* **21**, 1–24 (1998).
This is a review of genetic and environmental factors on behaviour, based on the results of twin studies. This review not only discusses genetic aetiology, but also unique versus shared environmental effects on behaviour.
91. Davies, G., Welham, J., Chant, D., Torrey, E. F. & McGrath, J. A systematic review and meta-analysis of northern hemisphere season of birth studies in schizophrenia. *Schizophr. Bull.* **29**, 587–593 (2003).
92. St. Clair, D. *et al.* Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959–1961 *JAMA* **294**, 557–562 (2005).
93. Kendler, K. S., Kessler, R. C., Neale, M. C., Heath, A. C. & Eaves, L. J. The prediction of major depression in women: toward an integrated etiologic model. *Am. J. Psychiatry* **150**, 1139–1148 (1993).
This papers shows that neuroticism and depression share about half the genetic variance.
94. Langley, K., Rice, F., van den Bree, M. B. & Thapar, A. Maternal smoking during pregnancy as an environmental risk factor for attention deficit hyperactivity disorder behaviour. A review. *Minerva Pediatr.* **57**, 359–371 (2005).
95. Kolevzon, A., Gross, R. & Reichenberg, A. Prenatal and perinatal risk factors for autism: a review and integration of findings. *Arch. Pediatr. Adolesc. Med.* **161**, 326–333 (2007).
96. Brimacombe, M., Ming, X. & Lamendola, M. Prenatal and birth complications in autism. *Matern. Child Health J.* **11**, 73–79 (2007).
97. Croen, L. A., Najjar, D. V., Fireman, B. & Grether, J. K. Maternal and paternal age and risk of autism spectrum disorders. *Arch. Pediatr. Adolesc. Med.* **161**, 334–340 (2007).
98. Caspi, A. *et al.* Role of genotype in the cycle of violence in maltreated children. *Science* **297**, 851–854 (2002).
This is a landmark paper, the results of which have already used been in a court case. It used a >30 year ongoing longitudinal study to show that a genetic variant in MAOA interacts with childhood environmental factors in influencing impulsivity and violent behaviour.
99. Caspi, A. *et al.* Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* **301**, 386–389 (2003).
In this paper, the same longitudinal study as that reported in reference 98 was used to demonstrate that risk for depression is increased by interaction between variation in the promoter of the serotonin transporter and stressful life events. Both references demonstrate the importance of longitudinal studies in assessing risk.
100. Vorstman, J. A. *et al.* Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. *Mol. Psychiatry* **11**, 18–28 (2006).
101. Yu, C. E. *et al.* Presence of large deletions in kindreds with autism. *Am. J. Hum. Genet.* **71**, 100–115 (2002).
102. Lauritsen, M. & Ewald, H. The genetics of autism. *Acta Psychiatr. Scand.* **103**, 411–427 (2001).
103. Beaudet, A. L. Autism: highly heritable but not inherited. *Nature Med.* **13**, 534–536 (2007).
104. Sebat, J. *et al.* Strong association of *de novo* copy number mutations with autism. *Science* **316**, 445–449 (2007).
This paper demonstrates that non-familial cases of autism have an increased number of novel large chromosomal deletions.
105. Smith, M. *et al.* Molecular genetic delineation of 2q37.3 deletion in autism and osteodystrophy: report of a case and of new markers for deletion screening by PCR. *Cytogenet. Cell Genet.* **94**, 15–22 (2001).
106. Ghaziuddin, M. & Burmeister, M. Deletion of chromosome 2q37 and autism: a distinct subtype? *J. Autism Dev. Disord.* **29**, 259–263 (1999).
107. Weiss, L. A. *et al.* Association between microdeletion and microduplication at 16p11.2 and autism. *N. Engl. J. Med.* **358**, 667–675 (2008).
Together with references 108 and 109, this paper shows that a significant fraction of autism (approximately 1%) is caused by a recurrent deletion on chromosome 16.
108. Kumar, R. A. *et al.* Recurrent 16p11.2 microdeletions in autism. *Hum. Mol. Genet.* **17**, 628–638 (2008).
109. Eichler, E. E. & Zimmerman, A. W. A hot spot of genetic instability in autism. *N. Engl. J. Med.* **358**, 737–739 (2008).
110. Walsh, T. *et al.* Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science* **320**, 539–543 (2008).
This paper shows that rare structural variants are increased in cases compared with controls, suggesting that a subset of schizophrenia cases might be due to *de novo* mutations.
111. Bailey, J. A. *et al.* Recent segmental duplications in the human genome. *Science* **297**, 1003–1007 (2002).
112. Sharp, A. J. *et al.* Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome. *Nature Genet.* **38**, 1038–1042 (2006).

113. Dixon, A. L. *et al.* A genome-wide association study of global gene expression. *Nature Genet.* **39**, 1202–1207 (2007).
114. Manolio, T. A., Bailey-Wilson, J. E. & Collins, F. S. Genes, environment and the value of prospective cohort studies. *Nature Rev. Genet.* **7**, 812–820 (2006).
115. Collins, F. S. & Manolio, T. A. Merging and emerging cohorts: necessary but not sufficient. *Nature* **445**, 259 (2007).
116. Collins, F. S. The case for a US prospective cohort study of genes and environment. *Nature* **429**, 475–457 (2004).
117. Niculescu, A. B. 3rd & Kelsoe, J. R. Convergent functional genomics: application to bipolar disorder. *Ann. Med.* **33**, 263–271 (2001).
118. Li, J. & Burmeister, M. Genetical genomics: combining genetics with gene expression analysis. *Hum. Mol. Genet.* **14**, R163–R169 (2005).
119. Myers, A. J. *et al.* A survey of genetic human cortical gene expression. *Nature Genet.* **39**, 1494–1499 (2007).
120. Gottesman, I. I. & Gould, T. D. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am. J. Psychiatry* **160**, 636–645 (2003). **This is a review of the endophenotype concept for psychiatry.**
121. Heils, A. *et al.* Allelic variation of human serotonin transporter gene expression. *J. Neurochem.* **66**, 2621–2624 (1996). **This is the first publication to demonstrate a common polymorphism in the promoter of the serotonin transporter and its effect on expression — the association of this variant with behavioural traits has now >500 published associations.**
122. Lesch, K. P. *et al.* Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* **274**, 1527–1531 (1996). **This is the first demonstration of an association between variation in the serotonin transporter and neuroticism and other behavioural traits.**
123. Schinka, J. A., Busch, R. M. & Robichaux-Keene, N. A meta-analysis of the association between the serotonin transporter gene polymorphism (5-HTTLPR) and trait anxiety. *Mol. Psychiatry* **9**, 197–202 (2004).
124. Sen, S., Burmeister, M. & Ghosh, D. Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. *Am. J. Med. Genet.* **127B**, 85–89 (2004). **The meta-analysis presented in this paper confirms the findings of reference 117 and explains previous non-replications as being due to small effect size (not replicating in small studies) and to the use of different measures of anxiety.**
125. Willis-Owen, S. A. *et al.* The serotonin transporter length polymorphism, neuroticism, and depression: a comprehensive assessment of association. *Biol. Psychiatry* **58**, 451–456 (2005).
126. Almasy, L. *et al.* Heritability of event-related brain potentials in families with a history of alcoholism. *Am. J. Med. Genet.* **88**, 383–390 (1999).
127. Freedman, R. *et al.* The genetics of sensory gating deficits in schizophrenia. *Curr. Psychiatry Rep.* **5**, 155–161 (2003).
128. Adams, C. E., Yonchek, J. C., Zheng, L., Collins, A. C. & Stevens, K. E. Altered hippocampal circuit function in C3H alpha7 null mutant heterozygous mice. *Brain Res.* **15**, 138–145 (2007).
129. Martin, L. F. *et al.* Sensory gating and alpha-7 nicotinic receptor gene allelic variants in schizoaffective disorder, bipolar type. *Am. J. Med. Genet. B Neuropsychiatr Genet.* **144**, 611–614 (2007).
130. Olincy, A. *et al.* Proof-of-concept trial of an alpha 7 nicotinic agonist in schizophrenia. *Arch. Gen. Psychiatry* **63**, 630–638 (2006).
131. Lachman, H. M. *et al.* Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* **6**, 243–250 (1996).
132. Egan, M. F. *et al.* Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc. Natl Acad. Sci. USA* **98**, 6917–6922 (2001).
133. Meyer-Lindenberg, A. *et al.* Midbrain dopamine and prefrontal function in humans: interaction and modulation by COMT genotype. *Nature Neurosci.* **8**, 594–596 (2005). **Building on references 131 and 132, these authors put forward a neurobiological hypothesis explaining the action of the catechol-O-methyltransferase (COMT) val–met variant in the brain.**
134. Munafo, M. R., Bowes, L., Clark, T. G. & Flint, J. Lack of association of the *COMT* (Val158/108 Met) gene and schizophrenia: a meta-analysis of case–control studies. *Mol. Psychiatry* **10**, 765–770 (2005).
135. Hariri, A. R. *et al.* Serotonin transporter genetic variation and the response of the human amygdala. *Science* **297**, 400–403 (2002).
136. Munafo, M. R., Brown, S. M. & Hariri, A. R. Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. *Biol. Psychiatry* **63**, 852–857 (2008).
137. Lise, M. F. & El-Husseini, A. The neuroligin and neurexin families: from structure to function at the synapse. *Cell. Mol. Life Sci.* **63**, 1833–1849 (2006). **This paper reviews the biological pathways of neuroligin and neurexin proteins, which were implicated in autism by several studies.**
138. Comoletti, D. *et al.* The Arg451Cys-neuroligin 3 mutation associated with autism reveals a defect in protein processing. *J. Neurosci.* **24**, 4889–4893 (2004).
139. Jamain, S. *et al.* Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nature Genet.* **34**, 27–29 (2003).
140. Chocholska, S., Rossier, E., Barbi, G. & Kehrer-Sawatzki, H. Molecular cytogenetic analysis of a familial interstitial deletion Xp22.2–22.3 with a highly variable phenotype in female carriers. *Am. J. Med. Genet. A* **140**, 604–610 (2006).
141. Szatmari, P. *et al.* Mapping autism risk loci using genetic linkage and chromosomal rearrangements. *Nature Genet.* **39**, 319–328 (2007).
142. Feng, J. *et al.* High frequency of neurexin 1 beta signal peptide structural variants in patients with autism. *Neurosci. Lett.* **409**, 10–13 (2006).
143. Ylisaukko-oja, T. *et al.* Analysis of four neuroligin genes as candidates for autism. *Eur. J. Hum. Genet.* **13**, 1285–1292 (2005).
144. Harrison, P. J. & Weinberger, D. R. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol. Psychiatry* **10**, 40–68 (2005). **This paper reviews the glutaminergic hypothesis of schizophrenia, which is based on some of the previous genetic findings.**
145. Chetcuti, A., Adams, L. J., Mitchell, P. B. & Schofield, P. R. Microarray gene expression profiling of mouse brain mRNA in a model of lithium treatment. *Psychiatr. Genet.* **18**, 64–72 (2008).
146. Seelan, R. S., Khalyfa, A., Lakshmanan, J., Casanova, M. F. & Parthasarathy, R. N. Deciphering the lithium transcriptome: microarray profiling of lithium-modulated gene expression in human neuronal cells. *Neuroscience* **151**, 1184–1197 (2008).
147. Alda, M., Grof, P., Rouleau, G. A., Turecki, G. & Young, L. T. Investigating responders to lithium prophylaxis as a strategy for mapping susceptibility genes for bipolar disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **29**, 1038–1045 (2005).
148. Jope, R. S. Lithium and GSK-3: one inhibitor, two inhibitory actions, multiple outcomes. *Trends Pharmacol. Sci.* **24**, 441–443 (2003).
149. Levinson, D. F. The genetics of depression: a review. *Biol. Psychiatry* **60**, 84–92 (2006).
150. Evans, S. J. *et al.* Dysregulation of the fibroblast growth factor system in major depression. *Proc. Natl Acad. Sci. USA* **101**, 15506–15511 (2004).
151. Lopez, J. F., Akil, H. & Watson, S. J. Neural circuits mediating stress. *Biol. Psychiatry* **46**, 1461–1471 (1999).
152. Seong, E., Seasholtz, A. F. & Burmeister, M. Mouse models for psychiatric disorders. *Trends Genet.* **18**, 643–650 (2002).
153. Chen, Z. Y. *et al.* Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science* **314**, 140–143 (2006).
154. Saunders, E. H., Scott, L. J., McInnis, M. G. & Burmeister, M. Familiality and diagnostic patterns of subphenotypes in the National Institutes of Mental Health bipolar sample. *Am. J. Med. Genet. B Neuropsychiatr Genet.* **147**, 18–26 (2008).
155. Zec, R. F. Neuropsychology of schizophrenia according to Kraepelin: disorders of volition and executive functioning. *Eur. Arch. Psychiatry Clin. Neurosci.* **245**, 216–223 (1995).
156. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Version IV (DSM-IV)* (American Psychiatric, 1994).
157. World Health Organization. The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Descriptions and Diagnostic Guidelines. *WHO* [online], <http://www.who.int/substance_abuse/terminology/ICD10ClinicalDiagnosis.pdf> (1992).
158. Nurnberger, J. I. Jr. *et al.* Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH Genetics Initiative. *Arch. Gen. Psychiatry* **51**, 849–864 (1994). **The Diagnostic Interview for Genetic Studies (DIGS), described in this paper, has become the main instrument worldwide to assess subjects for psychiatric genetic studies.**

Acknowledgements

We thank J. Li, M. Boehnke, S. Sen and M. Meisler for comments on the manuscript. Funding of our work in psychiatric disorders is provided by the Nancy Pritzker Psychiatric Disorders Fund, the Heinz Prechter Bipolar Disorders fund, NARSAD, and the National Institutes of Health (MH070775, MH070793).

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
CNTNAP2 | *DAOA* | *DISC1* | *DTNBP1* | *SLC6A4*
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Alzheimer disease | Angelman syndrome | attention deficit-hyperactivity disorder | autism | bipolar disorder | CHARGE syndrome | Huntington disease | Parkinson disease | schizophrenia | velocardiofacial syndrome | Brunner syndrome | Wolfram syndrome
UniProtKB: http://ca.expasy.org/sprot
MAOA

FURTHER INFORMATION

Autism Genetic Research Exchange: http://www.agre.org
Depression Center, University of Michigan: http://www.depressioncenter.org
National Institute of Mental Health: http://www.nimh.nih.gov
National Institute of Mental Health (NIMH) genetics initiative study: http://nimhgenetics.org

SUPPLEMENTARY INFORMATION

See online article: S1 (table) | S2 (table)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF