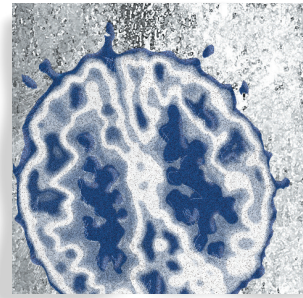


Genetics in schizophrenia: where are we and what next?

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Understanding the genetic basis of schizophrenia continues to be major challenge. The research done during the last two decades has provided several candidate genes which unfortunately have not been consistently replicated across or within a population. The recent genome-wide association studies (GWAS) and copy number variation (CNV) studies have provided important evidence suggesting a role of both common and rare large CNVs in schizophrenia genesis. The burden of rare copy number variations appears to be increased in schizophrenia patients. A consistent observation among the GWAS studies is the association with schizophrenia of genetic markers in the major histocompatibility complex (6p22.1)-containing genes including NOTCH4 and histone protein loci. Molecular genetic studies are also demonstrating that there is more overlap between the susceptibility genes for schizophrenia and bipolar disorder than previously suspected. In this review we summarize the major findings of the past decade and suggest areas of future research.

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Schizophrenia is a devastating psychiatric disease that has a lifetime prevalence of approximately 1% worldwide. It is characterized by the occurrence of delusions, hallucinations, disorganized speech, alterations in drive and volition, impaired cognition, and mood symptoms.¹ The importance of both environmental as well as genetic factors in the development of this complex disorder has been demonstrated. Growing up in an urban environment, immigration, cannabis usage, male gender and perinatal events (hypoxia, maternal infection, stress, and malnutrition) are associated with increased risk of developing schizophrenia.^{1,2} Evidence from family, twin, and adoption studies suggests a strong genetic component. A meta-analysis of twin studies estimated the heritability to be 81% (confidence interval 73% to 90%)³ and a recent Swedish study of more than 2 million families estimated it to be 64%.⁴ Of all the known risk factors for schizophrenia, genetics is the single largest one. No precise mode of inheritance is known, and less than one third of patients with schizophrenia have a family history. Elucidation of etiological factors remains the overwhelming challenge to schizophrenia researchers. In fact, the most effective method for identification of genetic risk factors for schizophrenia is not clear, and this has led to a number of approaches, including epigenetics, being adopted, in an attempt to clarify the genetic etiology. Since the initial observation that schizophrenia has a polygenic mode of inheritance and availability of polymorphic markers for genetic mapping, many attempts

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have been made to find susceptibility genes for schizophrenia using either the methods of linkage or association.^{5,6}

One of the early strategies used to begin unraveling the genetic contribution of schizophrenia was the investigation of chromosomal aberrations and familial syndromes with schizophrenia-like phenotypes. Chromosomal aberrations have been reported in families with schizophrenia and other psychiatric disorders.^{7,8} The deletion of a section of 22q11 leads to the phenotype of velocardiofacial (DiGeorge) syndrome that includes abnormalities in facial features, the palate, and midline heart defects.

Patients with 22q11 deletion syndrome exhibit psychotic symptoms resembling schizophrenia at a frequency of 18%, a rate that is much higher than the overall 1% prevalence of schizophrenia in the general population. On the other hand, when focusing on populations of patients who have the diagnosis of schizophrenia, at least 2% of these individuals are reported to have 22q11 deletions compared with the 0.025% prevalence in the general population.^{9,10} This region of 22q11 harbours the *COMT* and *PRODH* genes among others (Table I). A number of other chromosomal aberrations have been associated with schizophrenia. On chromosome

Gene	Locus	Function	Rare event ^a	Animal model ^b	Expression ^c	Meta-analysis ^d	References
<i>AKT1</i>	14q22-32	Intracellular signaling		ND	↓	-	11,12
<i>APOE</i>	19q13.2	Synapses		ND	↑	+	12,13
<i>COMT</i>	22q11	Catecholamine metabolism	+	-	^{MB} ↔	++	12,14,15-19
<i>DAOA(G72)</i>	13q32-34	D-serine metabolism		PPI def	↑	++	12,20,21
<i>DISC1</i>	1q42	Neurodevelopment and synaptic function	+	PPI/LI def ↔; FEZ↓; NUDEL↓; LIS1↓		+++	12,22-27
<i>DRD2</i>	11q23	Dopamine signaling		ND	↓	++	12,28
<i>DTNBP1</i>	6p22	Neurodevelopment and synaptic transmission		PPI def	↓	+++++	29-31
<i>ERBB4</i>	2q33.3-q34	Receptor tyrosine kinase NRG1	+	PPI def	↔/↑	ND	32,33-35
<i>GABRB2</i>	5q34	GABA signaling		ND	↓	++++	12,37
<i>GRIN2B</i>	12p12	Glutamate signaling		ND	↔	++	12,38,39
<i>HTR2A</i>	13q14-21	Serotonin signaling		ND	^{HIP} ↓	-	12,24,40
<i>IL1B</i>	2q14	Immune		ND	^{CSF} ↑	+	41-43
<i>NOTCH4</i>	6p21.3	Neurodevelopment		ND		+++	12,34,35,44-46
<i>NRG1</i>	8p12-21	Synapses		PPI/LI def	↑	Haplotype	47
<i>NRXN1</i>	2p16.3	Synapses	+	PPI def	ND	ND	44-46
<i>PDE4B</i>	1p31	Synapses		PPI def	↓	ND	48-51
<i>PRODH</i>	22q11	Glutamate synthesis	+	PPI def	↔	-	12,15,18,19
<i>RELN</i>	7q22	Synapses		<i>NPAS1/3</i> def: PPI def	↓/↔	ND	23,52,53
<i>RGS4</i>	1q23	Regulator of G-protein signaling		-	↓	-	12,54,55

Table I. Some schizophrenia candidate genes and their association with schizophrenia according to chromosomal location, genetic association, biology/animal models, expression alterations, and meta-analysis results. The number of "+" indicates the strength of association. "ND" not determined (modified from ref 126). ^a Rare events include chromosomal abnormalities, copy-number variants, mutations that have been associated with schizophrenia. ^b Data on genetically modified mouse models; PPI = prepulse inhibition in rodents; "-" = PPI similar to wildtype. ^c Expression data on postmortem tissues from the frontal/prefrontal cortex unless specified; MB = membrane-bound isoform; CSF = cerebrospinal fluid; HIP = hippocampus. ^d Results from most recent meta-analyses: "#+" = #significant polymorphisms; most updated meta-analysis of case-control studies is available SZGene (www.schizophreniaforum.org). *AKT1*, v-akt murine thymoma viral oncogene homolog 1; *APOE*, Apolipoprotein E; *COMT*, Catechol-O-methyltransferase; *DAOA(G72)*, D-amino acid oxidase activator; *DISC1*, Disrupted in schizophrenia 1; *DRD2*, Dopamine receptor 2; *DTNBP1*, Dystrobrevin binding protein 1; *ERBB4*, v-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian); *GABRB2*, Gamma-aminobutyric acid (GABA) A receptor, beta 2; *GRIN2B*, Glutamate receptor, ionotropic, N-methyl D-aspartate 2B; *HTR2A*, 5-hydroxytryptamine (serotonin) receptor 2A; *IL1B*, Interleukin-1 beta; *NOTCH4*, Notch homolog 4 (Drosophila); *NRG1*, Neuregulin 1; *NRXN1*, Neurexin 1; *PDE4B*, Phosphodiesterase 4B, cAMP-specific (phosphodiesterase E4 dunce homolog, Drosophila); *PRODH*, Proline dehydrogenase (oxidase) 1; *RELN*, Reelin; *RGS4*, Regulator of G-protein signaling 4

one, a balanced (1;11)(q42.1;q14.3) translocation, which disrupted the DISC1 gene (Table I), was found in a large Scottish family with high frequency of psychiatric disorders including schizophrenia.^{22,56,57} More recently, another family of mother-daughter pair with schizophrenia was identified with t(9;14)(q34;q13) translocation which disrupts the NPAS3 (neuronal Period Aryl hydrocarbon receptor Single-minded) gene,^{58,59} that codes for a transcription factor implicated in neurogenesis. Another cytogenetic investigation of a patient with schizophrenia revealed complex chromosomal rearrangement involving regions containing FEZ1 (which interacts with DISC1) and the kainate glutamate receptor gene *GRIK4*.⁶⁰ The gene coding for another DISC1 interacting protein, PDE4B, was found to be disrupted by a translocation in a schizophrenia proband with family history of psychiatric disorders.⁶¹ In these studies, however, the chromosomal aberrations do not fully cosegregate with the SCZ phenotype; thus these chromosomal abnormalities alone are not sufficient to cause SCZ, and they may predispose to several major psychiatric disorders as observed in some of these families, including bipolar disorder, major recurrent depression, addictions, impulse control disorders, and others.

Linkage studies are family based analyses that utilize genetic markers and the information from multiple affected individuals present in a given family to identify linked regions of the genome that is, regions co-inherited or segregating with the disease. Linkage studies were initially carried out using highly informative microsatellite markers (approximately 400 markers to cover the genome). At present, pedigree studies can utilize single-nucleotide polymorphism (SNP) linkage marker sets (eg, 6056 SNPs set from Illumina Inc). Some interesting candidate genes have been identified from linkage scans and have been replicated in independent association studies. These include dystrobrevin binding protein 1 (dysbindin, DTNBP1, 6p22.3),⁶² neuregulin 1 (NRG1, 8p12),³² and D-amino acid oxidase activator (DAOA, 13q24).⁶³ A recent meta-analysis of 32 genome-wide linkage scans across 3255 pedigrees including 7413 affected individuals identified suggestive evidence of linkage based on the summed rank statistics ($P_{SR} < 0.0077$) in two regions: 5q (5q31.3-35.1; $P_{SR} = 0.0046$) and 2q (2q12.1-21.2; $P_{SR} = 0.0075$).⁶⁴ Following secondary analysis, genome wide evidence of linkage was observed on 2q ($P_{SR} = 0.00035$) after shifting the frame of the 30 centi-

morgan wide bins by 50%. The next most significant regions, in descending order were: 1p13.2-q23.3, 2q33.3-36.3, 2q21.2-31.1, 1p32.2-31.1, 5q35.1-35.3, 8p22-12, 10q26.12-26.3, and 3p14.1-q13.32. Suggestive evidence of linkage with the 8p region (8p22-12, 16-33Mb; $P_{SR} = 0.00057$) was also observed in the subsample of patients of European ancestry. The 2q, 5q, and 8p regions were found to be linked to schizophrenia in an earlier meta-analysis from a subgroup of 20 studies from this larger set.⁶⁵ Furthermore, Holmans et al⁶⁶ reported suggestive evidence of linkage with 8p21 in a subset of the above families of European ancestry (707 families, 1615 affected). Since this 8p21 region does not include the gene *NRG1*, they concluded that this linkage might be due to the presence of one or more loci with multiple rare risk-associated SNPs and/or structural variants. The utility of linkage studies was further demonstrated in a recent study where the protein kinase C alpha (*PRKCA*) gene was identified as a schizophrenia susceptibility site. Carroll et al⁶⁷ examined a linkage area that they had identified earlier on chromosome 17p11.2-q25.125 and they identified a rare four-marker haplotype in the 3' untranslated region of the *PRKCA* gene. They further demonstrated that this low-frequency haplotype showed a trend of association in a sample of unrelated schizophrenia cases and controls (661 cases, 2824 controls, $P = 0.078$, OR=1.9) and was significant in a pooled sample of schizophrenia, schizoaffective, and bipolar disorder patients ($P = 0.037$, OR=1.9). This association was more significant in a stratified sample of males with schizophrenia than in the pooled sample. However, this association was not replicated in independent samples from Ireland and Bulgaria. Carroll et al⁶⁷ also reported that common SNPs in the linkage region showed association with schizophrenia in a United Kingdom sample, although these SNPs did not replicate across other samples. A possible interpretation of this scenario is that both common and rare SNPs in the *PRKCA* gene region may be associated with schizophrenia and related disorders.

Although some interesting candidate genes have been identified using linkage methods, a major criticism of these studies is that linkage signals are observed on most of the chromosomes and cover thousands of genes. Furthermore, small effect sizes that are now expected for schizophrenia-associated polymorphisms (OR<1.2) and locus heterogeneity further reduces the chances of finding a truly significant region in linkage studies. In addi-

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tion, collection of large numbers of families with multiple affected individuals for detecting these small effect sizes is labor-intensive and expensive. However, in contrast to genome-wide association studies (GWAS), large-scale linkage studies have the advantageous ability to detect regions with multiple rare as well as common variants (allelic heterogeneity) in one or more susceptibility genes.^{64,65,68} Furthermore, focusing on families with multiple affected individuals likely enriches for transmitted genetic factors and reduces etiologic heterogeneity.

Candidate gene and genome-wide association studies

The limited power of linkage studies to identify genes of modest effect led Risch and Merikangas⁶⁹ to propose the usage of association studies for disease gene identification in disorders with complex architecture such as schizophrenia. The primary advantage of the latter strategy was the possibility to recruit a large sample size with enough power to detect loci of moderate effect. However, they recognized that an important limitation was the lack of availability of technology to assay a large number of polymorphisms across the genome (up to 100 000). This limitation was overcome by the development of prototype SNP chips containing initially only 500 SNPs,⁷⁰ however, progressing rapidly to the present-day commercially available chips containing over a million SNPs.⁷¹ Furthermore, the cost of these chips has come down dramatically over the last few years, making it possible to carry out high-throughput genotyping in very large populations (over 20 000 individuals). The completion of the human genome project,⁷² the parallel development of the HapMap database of human SNP variation, and the availability of information on more than 3.1 million SNPs across the human genome have paved the way to effectively carry out large-scale GWAS.⁷³⁻⁷⁵

Genetic association studies are based on the common disease common variant hypothesis. This hypothesis proposes that common diseases are a result of interactive contribution of common variants with small effect sizes, and the susceptibility alleles will be shared by a significant proportion of unrelated affected individuals. This is the basis of both *hypothesis-based* candidate gene association studies as well as the *hypothesis-free* GWAS method.

In the past 4 years at least 11 GWAS have attempted to identify susceptibility genes for schizophrenia by genotyping individual samples as well as using DNA pooling-based methods.

GWAS using DNA pooling

DNA pooling was initially proposed as a method to reduce genotyping costs in large-scale association studies.⁷⁶ DNA from cases and controls are pooled into two separate groups, and the differences in allele frequency between the two groups are estimated to assess association. The first pooling-based association study was gene-centric, and analyzed 25 494 SNPs present within 10 kb of each of a large set of genes⁷⁷ (*Table II*). In the initial discovery sample a significant association of the marker rs752016 in intron 11 of the Plexin A2 gene (PLXNA2, 1q32; OR = 1.49; $P=0.006$) was found. A similar association was observed in the replication case-control as well as family based samples. However, independent replications for this SNP have been mixed.⁷⁸⁻⁸⁰

Shifman et al⁸¹ conducted a pooling-based GWAS study and observed female-specific association of the SNP rs7341475 G/A, in the fourth intron of the reelin gene (RELN, OR_{GG}=2.1, 9.8×10^{-5}). This was confirmed in a replication sample of patients of European ancestry from the United Kingdom, but not in samples from three other populations (Irish, American, and Chinese). The trend in the last three samples was in the same direction, and was significant in a meta-analysis including all samples OR_{GG}=1.58 (1.31-1.89), $P=8.8 \times 10^{-7}$. However none of these observations were significant after correcting for multiple testing. The reelin protein is a serine protease that plays an important role in corticogenesis and it is associated with an autosomal recessive form of lissencephaly.⁸² It has also been implicated in neurotransmitter-related GSK3 β signaling and regulation of NMDA receptor activation.⁸³ Polymorphisms in the RELN gene have been associated with neurocognitive endophenotypes of schizophrenia (eg, working memory and executive functioning).^{84,85} Furthermore, the association of the RELN gene with schizophrenia has been replicated in an independent sample.⁸⁶

In a whole genome pooling-based study on patients of eastern European ancestry (Bulgaria) significant association of rs11064768 in intron 1 of coiled-coiled domain containing protein 60 (CCDC60, 12q24.23) was observed.⁸⁷ The second best SNP was rs11782269 that is present in an

intergenic region on 8p23.1, the closest gene being claudin 23 (CLDN23). The third best was rs893703 in the intron 2 of retinol binding protein 1 gene (RBP1, 3q23). Interestingly, the RBP1 gene has been implicated in schizophrenia pathogenesis and inhibits PI3K/Akt signaling. However these observations are not significant at the genome-wide significance level ($P=1.85 \times 10^{-7}$) and have not been investigated in other independent samples. In general, the genetic findings support the neurodevelopment hypothesis of schizophrenia.⁸⁸

GWAS with individual genotyping

In the first GWAS, Lencz et al⁹⁹ observed genome-wide significant association of a SNP rs4129148 near the colony stimulating factor 2 receptor alpha gene (CSF2RA) in the pseudoautosomal region (Table II). Homozygosity for the C-allele of this polymorphism was associated with over threefold increased risk for schizophrenia. They targeted the exonic sequences and upstream region of CSFR2A and its immediate neigh-

Study no	Ethnicity	Sample size		No of SNPs tested	Significant SNPs (P-value)	Gene	
		Discovery (cases/ controls)	Replication (cases/ controls)				
1	Mah et al, 2006 ⁷⁷	European ancestry	320/325	200/230 ^c	25 494	rs752016 (0.006)	PLXNA2
2	Lencz et al, 2007 ⁹⁹	European ancestry	178/144	71/31 ^a	439 511	rs4129148 (3.7X10 ⁻⁷)	CSF2RA and IL3RA
3	Sullivan et al, 2008 ⁹²	European/African ancestry/ others	738/733	--	492 900	--	--
4	O'Donovan et al, 2008 ⁹³	European	479 /2937	6,666/9897 ^b	362 532	rs1344706 (1.95x10 ⁻⁷)	ZNF804A
5	Shifman et al, 2008 ⁸¹	Ashkenazi Jews	241/964 ^d	768/2194 ^d		rs7341475 (2.9X10 ⁻⁵) ^d	RELN
6	Kirov et al, 2009 ⁹⁷	European ancestry	574 Trios/605	--	433 680	rs11064768(1.2X10 ⁻⁶) rs11782269 (8.4X10 ⁻⁶) rs893703 (1.6X10 ⁻⁴)	CCDC60 8p23.1 RBP1
7	Need et al, 2009 ⁹⁸	European ancestry	871/1460	863/12 995	312 565	rs2135551 (1.34X10 ⁻⁶)	ADAMTSL3
8	Internation Schizophrenia Consortium ⁹⁶	European ancestry	3322/3587	8008 /19 077 (ISC, MGS & SGENE combined)	739 995	rs13194053 (9.5X10 ⁻⁹) rs5761163 (3.4X10 ⁻⁷) rs3130297 (4.79X10 ⁻⁸) rs3130375 (3.66X10 ⁻⁷)	6p22.1 MYO18B NOTCH4 RPP21
9	Stefansson et al, 2009 (SGENE-plus) ⁴¹	European ancestry	2663/13 498	4999/15 555	314 868	rs6913660 (1.1X10 ⁻⁹) rs6932590 (1.4X10 ⁻¹²) rs13211507 (8.3X10 ⁻¹¹) rs3131296 (2.33X10 ⁻¹⁰) rs12807809 (2.4X10 ⁻⁹) rs9960767 (4.1X10 ⁻⁹)	HIST1H2BJ PRSS16 PGDB1 NOTCH4 NRGN TCF4
10	Shi et al, 2009 (MGS) ¹⁰⁹	European and African ancestry	3967/3626	8008 /19 077 (ISC, MGS & SGENE combined)	696 788 in EA 843 798 in AA	6p22.1 1.06-4.35X10 ⁻⁸	HIST1H2BJ, HIST1H2AG, HIST1H2BK, HIST1H4I and HIST1H2AH
11	AthanasIU et al, 2010 ¹⁰⁰	European ancestry	201/305	2666/13 780	572 888	rs7045881 (2.12X10 ⁻⁶) rs433598 (3.27X10 ⁻⁶) rs10761482 (3.27X10 ⁻⁶)	PLAA ACSM1 ANK3

Table II. A Summary of whole-genome association studies in schizophrenia. ^aThis sample was used for sequencing the genes identified close to the SNP rs4129148 (CSF2RA and IL3RA). ^bThe replication sample included individuals from Japan and China. ^cAn independent case-control replication sample included patients of African descent (cases/controls; 157/180), Latin Americans (111/123) and Asian Americans (63/72). Two family-based samples (294 affected sib pair nuclear families and 90 affected sib pairs and 6 multiplex families). ^dFemales only. Ethnicity refers to the ancestry of only the Discovery sample set. EA, European ancestry; AA, African ancestry

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bor, the interleukin 3 receptor alpha (IL3RA) for sequencing in an independent patient sample (n=102). They observed that intronic haplotype blocks within CSF2RA and IL3RA were significantly associated with SCZ. Interestingly, one polymorphism, rs6603272, in intron 5 of the IL3RA gene, was also found to be associated with schizophrenia in independent samples of Han Chinese patients.^{90,91} Lencz et al⁸⁹ also observed an excess of rare non-synonymous mutations in CSF2RA and IL3RA in schizophrenia patients. No further studies of these two genes in schizophrenia have been reported since the findings of Lencz et al in 2007. There may be a tendency to be noted here, that each new GWAS study highlights the top ranking markers that it finds, and does not pay much attention to previously reported findings. This tendency is compounded by the fact that there is an explosion of data available to GWAS investigators; thus, putative new associations are arising in large numbers, providing a wide array of leads to follow.

In the GWAS on schizophrenia subjects from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study (n=1471), no marker achieved genome-wide significance level.⁹² A possible reason for this was the inclusion of patients of diverse ancestry who were not adequately covered in the genotyping platform that the investigators had utilized. The genomic coverage for common SNP variation was 86% for subjects of European ancestry, 79% for East Asian ancestry and only 49% for African ancestry (with $r^2 \geq 0.8$).⁹²

One of the most interesting candidate genes for schizophrenia and psychosis came from the multistage GWAS analyzing over 20 000 cases and controls.⁹³ In the initial GWAS (479 cases, 2937 controls), O'Donovan et al⁹³ observed 12 loci to be moderately associated with schizophrenia ($P < 10^{-5}$). In the first replication sample (1664 cases, 3541 controls) association of 6 of the 12 SNPs was replicated. These six SNPs were genotyped in the second replication sample (4143 cases, 6515 controls). In the complete replication sample set (stage one + two), three loci namely: Zinc finger protein 804A (ZNF804A, rs1344706, 2q32.1, $P = 9.25 \times 10^{-5}$), intergenic regions on 11p14.1 (rs1602565, $P = 3.22 \times 10^{-4}$) and 16p13.12 (rs7192086, $P = 5.10 \times 10^{-4}$) were modestly associated with schizophrenia. In the combined sample from the initial stage plus the two replication sets, ZNF804A showed strong evidence of association (OR=1.12; $P = 1.6 \times 10^{-7}$). Furthermore, when

patients with bipolar disorder were included, the observation became more significant, suggesting that ZNF804A might be a susceptibility gene for the broader psychosis phenotype. ZNF804A is a putative transcription factor and the risk allele of the rs1344706 polymorphism (intron 2, C>A) has been recently shown to be associated with disturbed connectivity between the dorsolateral prefrontal cortex (DLPFC) and the hippocampus, as well as between left and right hemispheres. Also there was altered coupling of DLPFC with the amygdala.⁹⁴ The former may lead to disturbed executive function and the latter can affect the interaction between prefrontal and limbic structures. The association of ZNF804A marker rs1344706 with schizophrenia was recently replicated in patients from Ireland (n=1021 cases, 626 controls; $P = 0.01$).⁹⁵ Increased expression of the A allele compared with the C allele was observed in the dorsolateral prefrontal cortex of postmortem control brain samples. However, there was no difference between the two alleles in overall mRNA expression between postmortem schizophrenia cases and controls.⁹⁴ The SNP rs1344706 was also significant in the GWAS conducted by the International Schizophrenia Consortium ($P = 0.029$, OR (A-allele) = 1.08).⁹⁶ Additionally, in a large multicenter study, association of rs1344706 with schizophrenia (5164 cases and 20 709 controls; OR=1.08, $P = 0.0029$) and psychosis (OR=1.09, $P = 0.00065$) has been replicated.⁹⁷ Based on this replication by three other independent groups, and the demonstration of functional effect on brain connectivity, ZNF804A is a promising candidate gene for schizophrenia and psychosis in general. The small effect sizes (OR 1.08-1.12) account for only 1% to 2% of the variance in risk for the disease. In general these replicating small effects are consistent with the common disease common variant hypothesis.

In the GWAS conducted by Need et al⁹⁸ no SNP reached the genome-wide significance level; however the strongest association was observed for a SNP in the 3'-UTR of ADAMTS-Like protein 3 (ADAMTSL3, rs2135551, $P = 1.35 \times 10^{-7}$, OR=0.68). An effect on splicing was demonstrated for the SNP rs950169 which is in linkage disequilibrium with rs2135551. The genetic association of rs2135551 was replicated in an independent cohort of patients but was not replicated in three other sample sets. Need et al also analyzed whether association of other current candidate genes for schizophrenia was present in their study. They observed gene-wide association of polymorphisms in Fasciculation and elon-

gation protein zeta-1 (FEZ1) and Notch homolog 4 (NOTCH4).

By the middle of 2009, three GWAS of impressive scale were conducted by three large consortia for schizophrenia genetics. Each effort examined several thousand cases and controls, and they were published in the same issue of *Nature*. The study from the International Schizophrenia Consortium (ISC) analyzed 3322 schizophrenia cases and 3587 controls.⁹⁶ The most significant association was observed for rs5761163 in the first intron of myosin XVIIB (MYO18B; 22q11.2-q12.1; $P=3.4 \times 10^{-7}$). The second strongest association was with SNPs spanning the major histocompatibility complex (MHC, 6p22.1) and the most significant SNP was rs3130375. Following imputation, rs3130297, located in the MHC region 7.1 kilobases (kb) upstream from the Notch homolog 4 gene (*NOTCH4*) showed genome-wide significance ($P=4.79 \times 10^{-8}$). A trend for association of this SNP was observed in the Molecular Genetics of Schizophrenia (MGS) sample ($P=0.086$)⁹⁹ but not in the SGENE sample ($P=0.14$).⁴¹ In the combined sample from the three studies, including both imputed and genotyped SNPs in the MHC region, rs13194053 was genome-wide significant. The study also provided evidence that a large number of common variants have an important role to play in schizophrenia susceptibility, as a group explaining about one third of the total variation in risk for the disease (34% [CI=32%-36%]).⁹⁶

In the second GWAS, performed by the SGENE consortium, Stefansson et al⁴¹ did not observe any genome-wide significant SNPs in their sample of 2663 schizophrenia cases and 13 498 controls. They analyzed the top 1500 SNPs in the ISC (2602 cases and 2885 controls) and the MGS samples (2681 cases and 2653 controls). The top 25 markers ($P < 1 \times 10^{-5}$) in the combined sample were followed up in four independent samples (total of 4999 cases and 15 555 controls). They observed genome-wide significant association of SNPs in the MHC region (6p21.3-22.1, HIST1H2BJ, PRSS16, and NOTCH4), as well as with the marker rs12807809 located 3.4kb upstream of the neurogranin gene (NRGN, 11q24.2), and an intron four SNP in transcription factor 4 (TCF4, 18q21.2). The odds ratios for associated SNPs in the MHC region varied from 1.15 (HIST1H2BJ, rs6913660, $P=1.1 \times 10^{-9}$), to 1.19 (NOTCH4, rs3131296, $P=2.3 \times 10^{-10}$) to 1.24 (PGDB1, rs13211507, $P=8.3 \times 10^{-7}$). The genes associated in this study are involved in immune-related functions (MHC), impaired memory function (NRGN), and neurodevelopment (TCF4).⁴¹

The Molecular Genetics of Schizophrenia (MGS) study⁹⁹ was not able to identify genome-wide significant markers in their sample set. The MGS study had both African-American (AA, 1 286 cases, 973 controls) as well as European ancestry (EA) patients (2681 cases, 2653 controls). In the EA sample the top SNP was an intron 10 polymorphism rs13025591 in the AGAP1 gene (ArfGAP with GTPase domain, ankyrin repeat and PH domain 1, 2q37.2, $P=4.6 \times 10^{-7}$, OR=1.22). Among the top SNPs were an intron 12 SNP, rs16941261, in NTRK3 (neurotrophic tyrosine kinase, receptor, type 3, 15q25.3, $P=8.1 \times 10^{-7}$, OR=1.25) and intron two SNP, rs10140896, in EML5 (echinoderm microtubule associated protein-like 5, 14q13.3, $P=9.5 \times 10^{-7}$, OR=1.22). In the African-American subsample, ERBB4 (v-erb-a erythroblastic leukemia viral oncogene homolog 4, 2q34, rs1851196, $P=2.4 \times 10^{-6}$, OR=0.733) and CBX2 (chromobox homolog 2, 17q25.3, rs3751954, $P=4.6 \times 10^{-6}$, OR=0.528) were associated. ERBB4 and its ligand neuregulin 1 (NRG1) have been associated with schizophrenia in earlier studies (for discussion see <http://www.schizophreniaforum.org>). *NRG1* is a very large gene that presents challenges for more detailed study due to its size. Certain areas of the gene have been highlighted by the fact that haplotypes of markers in these regions have exhibited some replication across schizophrenia studies. However, the lack of detailed information as to which markers in the gene alter its biological function leads to a need to cover the entire gene with a set of hundreds of markers, in order to be able to state that a comprehensive analysis was done. Thus, even in this single locus, one can see that multiple testing challenges arise. Overall, it can be seen that much more work by genome researchers on annotation of the functional significance of variants in any given gene is required.

Shi et al⁹⁹ further conducted a meta-analysis of the samples of European ancestry in their MGS sample as well as samples from ISC and SGENE (total sample: 8008 cases, 19 077 controls). Genome-wide significant association was observed with SNPs on chromosome 6p22.1 spanning 209kb. The strongest association was with the SNPs present within a cluster of five histone genes (*HIST1H2BJ*, *HIST1H2AG*, *HIST1H2BK*, *HIST1H4I*, and *HIST1H2AH*). The surrounding region also includes genes related to immunity, chromatin modification and G protein-coupled receptor signaling.

To summarize across these large GWAS investigations, each group of authors did not find genome-wide signif-

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icant results when they analyzed their samples individually. They found significant results only when they merged samples from several large studies and conducted pooled analysis. The overall finding was the association of SNPs in the MHC region (6p22.1) with schizophrenia. These results provide strong evidence that common variants are associated with schizophrenia; however, the effect size of the risk variants is small (<1.2). Therefore, studies with small sample size are unlikely to detect these common variants, unless these SNPs have larger effect size in more refined phenotypes. In a recent GWAS on a Norwegian sample, Athanasiu et al¹⁰⁰ also failed to find genome-wide significant results in their discovery sample ($n=506$), probably due to the small sample size. However, when they analyzed the top 1000 SNPs of their study (or the surrogates of these SNPs) in the SGENE-plus consortia sample,⁴¹ 16 loci showed marginal association ($P<0.05$). In the combined analysis they observed three markers to be significant at the genome-wide level. These were- rs7045881 in the gene phospholipase A2-activating protein (*PLAA*, 9p21, $P=2.12 \times 10^{-6}$, $OR=0.86$); rs433598 in acyl-CoA synthetase medium-chain family member 1 (*ACSM1*, 16p12.3, $P=3.27 \times 10^{-6}$, $OR=1.13$); and rs10761482 in ankyrin 3, node of Ranvier (ankyrin G) gene (*ANK3*, 10q21, $P=3.27 \times 10^{-6}$, $OR=0.86$). The function of these genes includes inflammatory response and membrane integrity (*PLAA*), endocrine function and dislipidemia (*ACSM1*), and involvement in activities such as cell motility, activation, proliferation, contact, and maintenance of specialized membrane domains (*ANK3*). Interestingly *ANK3* has also been associated with bipolar disorder in a recent meta-analysis.¹⁰¹ In addition to the above observations, Schulze et al¹⁰² also observed nominal association of genes for bipolar disorder that have been associated with schizophrenia in candidate gene as well as genome-wide association studies (*DISC1*, *NRG1*, *RELN*, and *OPCML*).

One of the major new observations from the GWAS studies is the fact that many of the positive loci for schizophrenia are also positive in bipolar, and vice-versa. This molecular-genetic overlap may have important implications for diagnostic classification of schizophrenia in the future *DSM-5* and beyond. Also in the future, the field will see a transition from SNP methodology to DNA sequencing. Thus all of the DNA variation in a given gene will be detected. For example, the sequencing will detect small insertions and deletions, as well as repeat

sequences, that largely would have been missed by the current SNP arrays. The downside of this large increase in the amount of information available will be even more multiple testing challenges, as well as considerable labor to establish the functional status of each variant of the gene in question.

Copy number variation and schizophrenia

Considering that two thirds of the cases of schizophrenia are sporadic, a role of rare variation in development of schizophrenia is not unexpected. Rare variations can include mutations as well as deletions and duplications. Copy number variations (CNV) are submicroscopic deletions or duplications stretching from a few kilobases to several megabases covering several or many genes. One of the earliest well-supported deletions relating to schizophrenia is on 22q11. This region, also known as the velocardiofacial/DiGeorge (VCFS) syndrome region, is caused by a deletion of 1.5 to 3Mb on one chromosome (thus rendering the person hemizygous for the genes involved). Besides the features observed in patients with VCFS, the 22q11 deletion region is associated with increased risk of schizophrenia and other neuropsychiatric disorders.¹⁴

Initial studies of CNVs in schizophrenia were carried out using bacterial artificial chromosome (BAC) array comparative genome hybridization (aCGH) with the resolution ranging from 2.3 Mb,¹⁰² 1.4 Mb,¹⁰³ 0.7 Mb,¹⁰⁴ to 150-200kb.¹⁰⁵ The more recent studies have utilized SNP arrays for estimation of CNVs with a resolution of approximately 30 to 100kb.^{98,106-110}

Using array CGH, several regions harboring deletions and duplications were identified in Korean schizophrenia patients.¹⁰² Two frequent CNVs were observed. First was a gain in sequence that varies in length across individuals in the Xq23 region (in 52% of schizophrenia cases). Second, was a loss at 3q13.12 (in 32% of schizophrenia cases). They also identified deletion as well as duplications at 22q11.21. Wilson et al¹⁰³ analysed 105 postmortem brain samples, $n=35$ each for schizophrenia, bipolar disorder and controls and observed CNVs at 1p34.3 (*GLUR7*), 5q21.3 (*EFNA5*), 14q23.3 (*AKAP5*), and 22q12.3 (including the *CACNG2* gene) in cases but not in controls. Furthermore, three of the CNV loci, except for 5q12.3, were replicated in an independent sample of 60 psychiatric patients.¹⁰³ Similarly, Mizuguchi et al also observed CNVs in six (10%) of the 59 schizo-

phrenia patients they analyzed. If this relatively high percentage of SCZ patients with one of a set of common deletions continues to be observed, then genetic screening for these deletions may become more useful, as discussed recently for 22q11.2 deletions.¹¹¹

The study by Kirov et al¹⁰⁵ detected 13 CNVs in 93 schizophrenia patients. These were not detected in 372 control individuals. Of these CNVs two were thought to have a possible role in schizophrenia pathogenesis. The first was a 1.4 Mb de novo duplication on chromosome 15q13.1 which includes the gene amyloid β (A4) precursor protein binding, family A, member 2 (*APBA2*), and the second was a 0.25Mb deletion on 2p16.3 that includes the Neurexin gene (*NRXN1*). Gene-gene interaction between *APBA2* and *NRXN1* is known and both play a role in synaptic development and function.¹⁰⁵

An excess of rare CNVs (both deletion and duplications) in schizophrenia and schizoaffective disorder patients (20%) versus controls (5%) was reported by Walsh et al.¹¹² Similar excess of rare CNVs was observed in young-onset cases (20%) as well as in a replication sample of childhood-onset schizophrenia. Schizophrenia patients were over three times more likely to carry deletions or duplications compared with controls. However, there was no difference in the distribution of common CNVs between cases and controls. The CNVs present in schizophrenia patients were disproportionately more likely to disrupt genes from signaling networks controlling neurodevelopment, including neuregulin and glutamate pathways. Interestingly, similarly to Kirov et al,¹⁰⁵ a deletion disrupting *NRXN1* was found in identical twins concordant for childhood-onset schizophrenia. *ERBB4*, a type I transmembrane tyrosine kinase receptor for neuregulin (*NRG1*), was also found to be disrupted by a 399 kb deletion in one schizophrenia patient. Similarly, a 503 kb deletion that disrupted *SLC1A3*, a glutamate transporter was also observed. However, this excess of rare CNVs was not replicated in an independent sample of Han Chinese schizophrenia patients (155 cases and 187 controls).¹⁰⁹

Xu et al¹⁰⁶ analyzed the presence of de novo CNVs and observed that these are approximately 8 times more frequent in sporadic cases (10%, 15 of 152) than in controls (1.3% (2 of 159)). Furthermore, they did not observe any de novo CNVs in familial cases (n=48). Additionally, sporadic cases of schizophrenia were ~1.5-times more likely to inherit a rare CNV (~30%, 46/152) than unaffected controls (~20%, 32/159). Among the de novo

CNVs observed in cases, deletions were seen at 22q11.2 (1.8%) along with deletions at 12p11.23 and 16p12.1–p12.2 which were also observed by Walsh et al.¹¹²

The study by Stefansson et al¹⁰⁷ tested an interesting hypothesis that regions in the genome that have a high mutation rate, but harbor rare CNVs, are likely to be selected against during evolution. These rare CNVs may be associated with disorders such as schizophrenia, autism, and mental retardation, which appear to reduce fecundity of the affected patients. Using SNP arrays to identify de novo rare CNVs in the healthy control population, they identified 66 de novo rare CNVs and tested for association with schizophrenia in two independent schizophrenia case-control samples (Phase I: 1433 cases and 33 250 controls; Phase II: 3285 cases and 7951 controls). They observed three deletions, 1q21.1, 15q11.2, and 15q13.3, to be nominally associated with schizophrenia and related psychosis in the Phase I samples. In the phase II sample, all three deletions were significantly associated with schizophrenia and related psychosis. In the combined sample, the odds ratio (OR) varied from 14.8 for 1q21.1, 11.54 for 15q13.3 to 2.73 for 15q11.2. The 15q loci were not significant if patients with psychoses other than schizophrenia were excluded. The 1q21.1 deletion which varies from 1.35Mb to 2.19Mb was present in 0.23% (11 out of the 4718) of the cases compared with 0.02% (8 of 41 199) controls. The 470 kb deletion on 15q11.2 occurred in 0.55% of cases (26 of 4 718) versus 0.19% controls (79 of 41 194). The 15q13.3 deletion spans 1.57Mb and is present in 0.17% of cases (7 of 4213) versus 0.02% of controls (8 of 39 800). They also found the 22q11.2 deletion in 0.2% of the cases (8 of 3838). The association with 1q21.1 is interesting, as this region has been associated with schizophrenia.¹¹² The 15q11.2 region has been associated with mental retardation and is also deleted in a minority of cases with Angelman syndrome or Prader–Willi syndrome. In the 15q13.3 deletion region, the alpha 7 subunit of the nicotinic receptor gene (*CHRNA7*) is present. This receptor is targeted to axons by *NRG1*, and has been implicated in schizophrenia and mental retardation.¹⁰⁷

In the study from the International Schizophrenia Consortium,¹⁰⁸ a genome-wide survey of 3391 patients with schizophrenia and in 3181 ancestrally matched controls using SNP arrays revealed 6753 rare CNVs. These rare CNVs, observed in less than 1% of the sample and more than 100Kb in length, were 1.15-fold increased in patients with schizophrenia in comparison with controls.

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They also observed deletions on 1q21.1 (0.29%, OR=6.6), 2p16.3 (0.12%, NRXN1), 7q35 (0.09%, CNTNAP2), 12p11.23 (0.12%), 15q13.3 (0.03%, APBA2), 15q13.3 (0.27%, CHRNA7, OR=17.9), 16p12.2-12.1, (0.12%) and 22q11.2 (VCFS region, 0.38%, OR=21.6).

Further support for the role of rare CNVs in schizophrenia came from a recent study that analysed 471 patients with schizophrenia or schizoaffective disorder and 2792 controls.¹¹⁰ Kirov et al observed an excess of rare CNVs larger than 1Mb in cases (OR=2.26, $P=0.00027$) compared with controls. The associations were stronger with deletions (OR=4.53, $P=0.00013$) than with duplications (OR=1.71, $P=0.04$). Similar to the abovementioned studies, these investigators also observed deletion at 22q11.2 in two schizophrenia patients. A deletion at 17p12 was also observed in two patients but not in controls. Unlike the ISC study,¹⁰⁸ Kirov et al¹¹⁰ did not observe an overall excess burden of rare CNVs in their investigation. However, rare CNVs >500kb were also enriched in schizophrenia cases (OR=2.18). On combining their results with the ISC and the SGENE study,¹⁰⁷ Kirov et al observed that the 17p12 deletion was more common in cases than controls (OR~10, 0.15% vs 0.015%) and also observed the deletion at 15q11.2 (OR=2.8, 0.62% vs 0.22). A duplication observed at 16p13.1, which includes the DISC1 interacting gene NDE1, was more common in cases than controls in the Kirov study as well as the ISC study.¹⁰⁸

Need et al¹⁰⁰ in their GWAS investigation, analysed a subset of patients for presence of copy number variations, and they identified large deletions (>2Mb) in eight cases but not in controls. Of these four CNVs, one was at 22q11.2, one at 16p13.11-p12.4 (includes the gene NDE1 a binding partner for DISC1), one at 8p22 and two at 1q21.1 (these are same as that reported by Stone et al¹⁰⁸ and Stefansson et al¹⁰⁷). Overall, similarly to Kirov et al,¹¹⁰ they did not observe an excess of rare CNVs (>100kb) in schizophrenia cases. Unlike Walsh et al¹¹¹ they did not observe an excess of rare CNVs disrupting genes from the neurodevelopmental pathways. An important difference may have been that the primary investigation samples in the study by Walsh et al were child-onset schizophrenia patients.

Similar observations have also been reported in Japanese schizophrenic patients. Ikeda et al¹¹³ did not observe an excess of large rare CNVs (>500kb) in schizophrenia patients, but observed an overall nonsignificant trend for excess of rare CNVs in schizophrenia ($P=0.087$). Similar to previous studies, they identified one deletion in a case

at 1q21.1, a deletion within NRXN1, and four duplications at 16p13.1 in cases, and one in a control subject. However no deletions were observed at 22q11.2 or 15q13.3 loci. In a reverse trend for the 15q11.2 locus, three deletions were observed in controls compared with one in cases.

Based on all the above studies, we may summarize that rare CNVs, until recently, were only thought to play an important role in disorders such as mental retardation and autism. However, it now appears that CNV make a substantial contribution to the understanding of schizophrenia etiology and pathogenesis. Deletions at 1q21.1, 15q11.2, and 15q13.3 might join the ranks of 22q11.2 as uncommon but important chromosomal aberrations that can lead to severe behavioral disturbances including schizophrenia.

What next? Conclusions and future directions

Despite decades of research effort, our understanding of the genesis of schizophrenia remains an enigma. The methods used for mapping susceptibility genes have progressed enormously over the past several years. The genome-wide studies have pointed to the role of both common variants as well as rare variants in schizophrenia susceptibility. However, the effect size associated with common variants is smaller than initially estimated (OR<1.2) and only rare variants generally have a large effect. Furthermore, the number of total susceptibility variants for schizophrenia may be in the order of thousands.¹¹⁴ Considering the low effect size observed for the associated SNPs the sample size required for replicating these associations with adequate power would theoretically be up to 100 000 each of cases and controls. To achieve such sample sizes with detailed and consistent phenotype measurement is a formidable challenge. It may be that testing broader phenotypes such as psychosis might help the field to collect these large numbers as well as detect genes which overlap between different disorders. However, the opposite approach may also be valid, that is to narrow the phenotype to a hopefully more homogeneous subgroup, for example including use of brain imaging measures, electrophysiology, or carefully defined symptom subtypes. A smaller number of genes of greater effect sizes may influence more refined, specific phenotypes.

An interesting outcome of the GWAS data analyses is that there appears to be a considerable overlap between

schizophrenia and bipolar disorder, consistent with the idea that they exist on a clinical continuum with overlapping symptom dimensions. A recent study in two million Swedish families also observed that schizophrenia and bipolar disorder share susceptibility genes.⁹³ Furthermore, the GWAS study by O'Donovan et al⁵⁰ found that inclusion of patients with bipolar disorder along with schizophrenia further strengthened their association with the SNP rs1344706 in the ZNF804A gene. The demonstration of a functional effect that alters connectivity of brain structures associated with both schizophrenia and bipolar disorder lends further support⁹⁴ to the overlap of the two diseases. Similarly, genes such as *DISC1*,¹¹⁵ *NRG1*,¹¹⁶ and *ANKK3*¹⁰⁰ are associated with both schizophrenia and bipolar disorder. A similar overlap may also exist with autism spectrum disorders wherein deletions in the Neurexin 1 gene have been associated with both autism and schizophrenia. One implication of this high degree of overlap is that combining phenotypes to build very large sample sizes may be a useful strategy to find small effect genes. These small effect genes may then be able to be assembled into neurobiological systems that would explain a significant degree of the pathological mechanisms of schizophrenia as well as other behavioral disorders.

Another complimentary strategy for detecting disease associated genetic variants will be the use of endophenotypes. These can be defined as disease-associated phenotypes that are heritable, state independent, cosegregate with illness in families, and are also found in unaffected family members.¹¹⁷ Generally the terms "alternate phenotype" or "intermediate" means a phenotype that does not meet all of the criteria for an endophenotype, but represents a different and usually more objective measure of part of the schizophrenia phenotype. One of the historically most studied endophenotypes in schizophrenia is abnormal movement of the eyes while tracking a moving object across a screen.¹¹⁸ Other endophenotypes include impairments in attention, language, and memory (neurocognitive deficits), deficits in sensory gating of auditory information (prepulse inhibition),¹¹⁹ P50 event-related potential,¹²⁰ P300 event-related potential¹²¹ and structural imaging phenotypes (for a detailed review see ref 122). Interestingly, these endophenotypes are generally applicable to both schizophrenia and bipolar disorder, and several genes have been reported to influence them. For example the catechol-o-methyl transferase gene

(COMT) and Reelin are associated with neurocognitive deficits, and the alpha 7 nicotinic receptor subunit gene variants are associated with P50 deficits. Overall there is hope that the use of endophenotypes will improve our understanding of the biology of the disease as well as creating phenotypically more homogeneous groups of patients that can reduce the number of samples required for detecting genetic signals.

The effect of environmental factors, including maternal infection (serological evidence of influenza infection during pregnancy), and recreational drug use/abuse should also be taken into account when conducting association studies in schizophrenia.¹²³ Cannabis usage is an important risk factor aggravating psychosis, and pre-onset cannabis use hastens the onset of prodromal symptoms as well as fully developed psychosis. These and likely other environmental factors need to be considered in the design of future genetic studies. Furthermore, epigenetic changes, including both DNA methylation and histone acetylation, have also been suggested to play an important role in development of major psychoses.^{124,125} These epigenetic mechanisms can be influenced by environmental effects such as stress (cortisol) and hormonal factors. Thus we need a comprehensive systems biology approach to incorporate the effect of genetic and non-genetic factors to understand the genesis of schizophrenia and related disorders.

A possible approach that may take into account most of these variables is high-throughput whole-genome sequencing. Whole-genome sequencing has the potential to detect virtually all SNPs, CNVs (both large as well as relatively small deletions <1kb) and epigenetic modifications (DNA methylation). Small deletions and duplications are relatively common in the human genome and have been shown to affect levels of several brain expressed genes (eg, *DAT1*, *5-HTTLPR*). The cost of high-throughput sequencing at present is rather expensive but is comparable to the price of first-generation SNP chips. Further technological advances and reduction in cost of high throughput sequencing will make the discovery of each and every variant in a given person's genome feasible. While this more extensive and detailed information on each subject's DNA will provide comprehensive information at the DNA level, the data analysis, multiple testing, and other bioinformatic challenges are also greatly increased.

At this juncture in the research effort, investigations of schizophrenia have demonstrated that genetic factors

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indeed have an important role to play in its genesis. However, to progress further we need better phenotypic classification of our patients. A major area of development in this regard is the steady advance of neuroimaging technology. We now can have phenotypes of the volume of the dorsal lateral prefrontal cortex, or the connectivity between different brain regions. It should be the case that more accurate study of the target organ in schizophrenia research, that is the brain, will lead to more objective and reliable associations with genetic variants. Also, the methods to capture the complete variability of the genome (as well as the epigenome) will add to the comprehensiveness of the measurement of DNA-based information. Furthermore, a concerted effort is needed to understand the biology (the annotation) of the disease-associated genes. These genes will help us in identifying novel targets for drug development and thereby improve the efficacy of the treatment.

The discovery of genetic factors also leads to the consideration of whether or not it is useful to perform genetic testing in a clinical setting. In fact, there are direct-to-consumer genetic testing services for schizophrenia

already available on the internet. Most regulatory authorities (for example see the International Society for Psychiatric Genetics Web site: www.ispg.org) agree that genetic testing for schizophrenia is premature at this stage, given the small odds ratios and inconsistent replications reviewed above. Until some as-yet unknown approach is achieved to combine large numbers of small effect genes into meaningful prediction algorithms, we are likely facing many more years of research before acceptable testing for risk is possible. More hopefully, the uncommon but large effect CNVs on 22q, 15q, 16p, and 1q, that appear to be replicating fairly well in large datasets, may lead to tests that could identify molecular subtypes for at least a small percentage of schizophrenia cases. These molecular subtypes may in turn lead to better understanding of the pathogenesis of schizophrenia, and treatment might become more specific and individualized for these cases. Through the continued efforts of careful investigation in genetics of schizophrenia that embrace the nuances of phenotype and the latest technological developments, the field has an excellent chance to solve the etiologic puzzle of this enigmatic disorder. □

REFERENCES

1. van Os J, Kapur S. Schizophrenia. *Lancet*. 2009;374:635-645.
2. Tandon R, Keshavan MS, Nasrallah HA. Schizophrenia, "just the facts" what we know in 2008. 2. Epidemiology and etiology. *Schizophr Res*. 2008;102:1-18.
3. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry*. 2003;60:1187-1192.
4. Lichtenstein P, Yip BH, Bjork C, et al. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. *Lancet*. 2009;373:234-239.
5. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet*. 1980;32:314-331.
6. Donis-Keller H, Green P, Helms C, et al. A genetic linkage map of the human genome. *Cell*. 1987;51:319-337.
7. Shasstry BS. Recent developments in the genetics of schizophrenia. *Neurogenetics*. 1999;2:149-154.
8. Blackwood DH, Thiagarajah T, Malloy P, Pickard BS, Muir WJ. Chromosome abnormalities, mental retardation and the search for genes in bipolar disorder and schizophrenia. *Neurotox Res*. 2008;14:113-120.
9. Karayiorgou M, Morris MA, Morrow B, et al. Schizophrenia susceptibility associated with interstitial deletions of chromosome 22q11. *Proc Natl Acad Sci U S A*. 1995;92:7612-7616.
10. Murphy KC. Schizophrenia and velo-cardio-facial syndrome. *Lancet*. 2002;359:426-430.
11. Emamian ES, Hall D, Birnbaum MJ, Karayiorgou M, Gogos JA. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat Genet*. 2004;36:131-137.
12. Allen NC, Bagade S, McQueen MB, et al. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nat Genet*. 2008;40:827-834.
13. Dean B, Laws SM, Hone E, et al. Increased levels of apolipoprotein E in the frontal cortex of subjects with schizophrenia. *Biol Psychiatry*. 2003;54:616-622.
14. Murphy KC, Jones LA, Owen MJ. High rates of schizophrenia in adults with velo-cardio-facial syndrome. *Arch Gen Psychiatry*. 1999;56:940-945.
15. Gogos JA, Santha M, Takacs Z, et al. The gene encoding proline dehydrogenase modulates sensorimotor gating in mice. *Nat Genet*. 1999;21:434-439.
16. Maas JW, Bowden CL, Miller AL, et al. Schizophrenia, psychosis, and cerebral spinal fluid homovanillic acid concentrations. *Schizophr Bull*. 1997;23:147-154.
17. Matsumoto M, Weickert CS, Beltaifa S, et al. Catechol O-methyltransferase (COMT) mRNA expression in the dorsolateral prefrontal cortex of patients with schizophrenia. *Neuropsychopharmacology*. 2003;28:1521-1530.
18. Tunbridge E, Burnet PW, Sodhi MS, Harrison PJ. Catechol-o-methyltransferase (COMT) and proline dehydrogenase (PRODH) mRNAs in the dorsolateral prefrontal cortex in schizophrenia, bipolar disorder, and major depression. *Synapse*. 2004;51:112-118.
19. Stark KL, Burt RA, Gogos JA, Karayiorgou M. Analysis of prepulse inhibition in mouse lines overexpressing 22q11.2 orthologues. *Int J Neuropsychopharmacol*. In press.
20. Korostishevsky M, Kaganovich M, Cholostoy A, et al. Is the G72/G30 locus associated with schizophrenia? single nucleotide polymorphisms, haplotypes, and gene expression analysis. *Biol Psychiatry*. 2004;56:169-176.
21. Otte DM, Bilkei-Gorzo A, Filiou MD, et al. Behavioral changes in G72/G30 transgenic mice. *Eur Neuropsychopharmacol*. 2009;19:339-348.
22. Blackwood DH, Fordyce A, Walker MT, St Clair DM, Porteous DJ, Muir WJ. 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*. 2001;69:428-433.
23. Lipska BK, Peters T, Hyde TM, et al. Expression of DISC1 binding partners is reduced in schizophrenia and associated with DISC1 SNPs. *Hum Mol Genet*. 2006;15:1245-1258.
24. Shi J, Gershon ES, Liu C. Genetic associations with schizophrenia: meta-analyses of 12 candidate genes. *Schizophr Res*. 2008;104:96-107.

Genética en la esquizofrenia: ¿dónde estamos y qué viene?

La comprensión de las bases genéticas de la esquizofrenia sigue constituyendo un importante desafío. La investigación realizada durante las últimas dos décadas ha aportado algunos genes candidatos que desafortunadamente no han podido ser replicados en forma consistente a través de o dentro de una población. Los recientes estudios de asociación del genoma completo (AGC) y los estudios de variantes del número de copias (VNC) han aportado evidencia importante que sugiere un papel de numerosas VNC tanto comunes como raras en la génesis de la esquizofrenia. La carga de las variantes del número de copias raras parece estar aumentada en los pacientes esquizofrénicos. Una observación constante en los estudios de AGC es la asociación de la esquizofrenia con marcadores genéticos que contienen loci para genes que incluyen el NOTCH4 y la proteína histona en el complejo mayor de histocompatibilidad (6p22.1). Los estudios de genética molecular también están demostrando que hay más sobreposición entre los genes de susceptibilidad para la esquizofrenia y el trastorno bipolar de lo que se sospechaba previamente. En esta revisión se resumen los principales hallazgos de la última década y se sugieren áreas de investigación a futuro.

Génétique dans la schizophrénie : où en sommes-nous et où va-t-on ?

Il est encore très difficile de comprendre les bases génétiques de la schizophrénie. Ces vingt dernières années, la recherche a fourni plusieurs gènes candidats qui n'ont malheureusement pas été répliqués de façon régulière au sein ou à travers une population. Les récentes études d'association du génome entier (GWAS) et les études de variation du nombre de copies (CNV) ont donné des preuves tangibles d'un rôle de grands CNV rares et communs dans la genèse de la schizophrénie. Ainsi, la fréquence de certains CNV rares semble augmentée chez les schizophréniques. En outre, les études sur le génome entier montrent de façon régulière une association entre la schizophrénie et des marqueurs génétiques des gènes contenant le complexe majeur d'histocompatibilité (6p22.1) y compris NOTCH4 et les loci codant pour la protéine histone. Les études de génétique moléculaire ont également démontré qu'il y a plus de recoupements entre les gènes de susceptibilité de la schizophrénie et la maladie bipolaire qu'on ne le pensait auparavant. Nous résumons dans cet article les principaux résultats des 10 dernières années et proposons des pistes pour la recherche future.

25. Rastogi A, Zai C, Likhodi O, Kennedy JL, Wong AH. Genetic association and post-mortem brain mRNA analysis of DISC1 and related genes in schizophrenia. *Schizophr Res.* 2009;114:39-49.
26. Clapcote SJ, Lipina TV, Millar JK, et al. Behavioral phenotypes of Disc1 missense mutations in mice. *Neuron.* 2007;54:387-402.
27. Pletnikov MV, Ayhan Y, Nikolskaia O, et al. Inducible expression of mutant human DISC1 in mice is associated with brain and behavioral abnormalities reminiscent of schizophrenia. *Mol Psychiatry.* 2008;13:173-186.
28. Torrey EF, Barci BM, Webster MJ, Bartko JJ, Meador-Woodruff JH, Knable MB. Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. *Biol Psychiatry.* 2005;57:252-260.
29. Weickert TW, Goldberg TE, Mishara A, et al. Catechol-O-methyltransferase val108/158met genotype predicts working memory response to antipsychotic medications. *Biol Psychiatry.* 2004;56:677-682.
30. Li D, He L. Association study between the dystrobrevin binding protein 1 gene (DTNBP1) and schizophrenia: a meta-analysis. *Schizophr Res.* 2007;96:112-118.
31. Talbot K. The sandy (sdy) mouse: a dysbindin-1 mutant relevant to schizophrenia research. *Prog Brain Res.* 2009;179:87-94.
32. Stefansson H, Sigurdsson E, Steinthorsdottir V, et al. Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet.* 2002;71:877-892.
33. Hahn CG, Wang HY, Cho DS, et al. Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. *Nat Med.* 2006;12:824-828.
34. Chong VZ, Thompson M, Beltaifa S, Webster MJ, Law AJ, Weickert CS. Elevated neuregulin-1 and ErbB4 protein in the prefrontal cortex of schizophrenic patients. *Schizophr Res.* 2008;100:270-280.
35. Hong LE, Wonodi I, Stine OC, Mitchell BD, Thaker GK. Evidence of missense mutations on the neuregulin 1 gene affecting function of prepulse inhibition. *Biol Psychiatry.* 2008;63:17-23.
36. Zhao C, Xu Z, Chen J, et al. Two isoforms of GABA(A) receptor beta2 subunit with different electrophysiological properties: Differential expression and genotypical correlations in schizophrenia. *Mol Psychiatry.* 2006;11:1092-1105.
37. Martucci L, Wong AH, De Luca V, et al. N-methyl-D-aspartate receptor NR2B subunit gene GRIN2B in schizophrenia and bipolar disorder: Polymorphisms and mRNA levels. *Schizophr Res.* 2006;84:214-221.
38. Lopez-Figueroa AL, Norton CS, Lopez-Figueroa MO, et al. Serotonin 5-HT1A, 5-HT1B, and 5-HT2A receptor mRNA expression in subjects with major depression, bipolar disorder, and schizophrenia. *Biol Psychiatry.* 2004;55:225-233.
39. Li D, Duan Y, He L. Association study of serotonin 2A receptor (5-HT2A) gene with schizophrenia and suicidal behavior using systematic meta-analysis. *Biochem Biophys Res Commun.* 2006;340:1006-1015.
40. Soderlund J, Schroder J, Nordin C, et al. Activation of brain interleukin-1beta in schizophrenia. *Mol Psychiatry.* 2009;14:1069-1071.
41. Stefansson H, Ophoff RA, Steinberg S, et al. Common variants conferring risk of schizophrenia. *Nature.* 2009;460:744-747.

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42. Glatt SJ, Wang RS, Yeh YC, Tsuang MT, Faraone SV. Five NOTCH4 polymorphisms show weak evidence for association with schizophrenia: evidence from meta-analyses. *Schizophr Res.* 2005;73:281-290.
43. Wang Z, Wei J, Zhang X, et al. A review and re-evaluation of an association between the NOTCH4 locus and schizophrenia. *Am J Med Genet B Neuropsychiatr Genet.* 2006;141B:902-906.
44. Hashimoto R, Straub RE, Weickert CS, Hyde TM, Kleinman JE, Weinberger DR. Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. *Mol Psychiatry.* 2004;9:299-307.
45. Munafo MR, Thiselton DL, Clark TG, Flint J. Association of the NRG1 gene and schizophrenia: a meta-analysis. *Mol Psychiatry.* 2006;11:539-546.
46. Li D, Collier DA, He L. Meta-analysis shows strong positive association of the neuregulin 1 (NRG1) gene with schizophrenia. *Hum Mol Genet.* 2006;15:1995-2002.
47. Etherson MR, Blais CA, Powell CM, Sudhof TC. Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. *Proc Natl Acad Sci U S A.* 2009;106:17998-18003.
48. Siuciak JA, McCarthy SA, Chapin DS, tin AN. Behavioral and neurochemical characterization of mice deficient in the phosphodiesterase-4B (PDE4B) enzyme. *Psychopharmacology (Berl).* 2008;197:115-126.
49. Fatemi SH, King DP, Reutiman TJ, et al. PDE4B polymorphisms and decreased PDE4B expression are associated with schizophrenia. *Schizophr Res.* 2008;101:36-49.
50. Kahler AK, Otnaess MK, Wirgenes KV, et al. Association study of PDE4B gene variants in Scandinavian schizophrenia and bipolar disorder multicenter case-control samples. *Am J Med Genet B Neuropsychiatr Genet.* 2010;153B:86-96.
51. Tomppoo L, Hennah W, Lahermo P, et al. Association between genes of Disrupted in schizophrenia 1 (DISC1) interactors and schizophrenia supports the role of the DISC1 pathway in the etiology of major mental illnesses. *Biol Psychiatry.* 2009;65:1055-1062.
52. Guidotti A, Auta J, Davis JM, et al. Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. *Arch Gen Psychiatry.* 2000;57:1061-1069.
53. Erbel-Sieler C, Dudley C, Zhou Y, et al. Behavioral and regulatory abnormalities in mice deficient in the NPAS1 and NPAS3 transcription factors. *Proc Natl Acad Sci U S A.* 2004;101:13648-13653.
54. Grillet N, Pattyn A, Contet C, Kieffer BL, Goridis C, Brunet JF. Generation and characterization of Rgs4 mutant mice. *Mol Cell Biol.* 2005;25:4221-4228.
55. Erdely HA, Tamminga CA, Roberts RC, Vogel MW. Regional alterations in RGS4 protein in schizophrenia. *Synapse.* 2006;59:472-479.
56. Millar JK, Wilson-Annan JC, Anderson S, et al. Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum Mol Genet.* 2000;9:1415-1423.
57. St Clair D, Blackwood D, Muir W, et al. Association within a family of a balanced autosomal translocation with major mental illness. *Lancet.* 1990;336:13-16.
58. Kamnasaran D, Muir WJ, Ferguson-Smith MA, Cox DW. Disruption of the neuronal PAS3 gene in a family affected with schizophrenia. *J Med Genet.* 2003;40:325-332.
59. Pickard BS, Christoforou A, Thomson PA, et al. Interacting haplotypes at the NPAS3 locus alter risk of schizophrenia and bipolar disorder. *Mol Psychiatry.* 2009;14:874-884.
60. Pickard BS, Malloy MP, Christoforou A, et al. Cytogenetic and genetic evidence supports a role for the kainate-type glutamate receptor gene, GRIK4, in schizophrenia and bipolar disorder. *Mol Psychiatry.* 2006;11:847-857.
61. Millar JK, Pickard BS, Mackie S, et al. DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. *Science.* 2005;310:1187-1191.
62. Straub RE, Jiang Y, MacLean CJ, 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.* 2002;71:337-348.
63. Chumakov I, Blumenfeld M, Guerassimenko O, 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 U S A.* 2002;99:13675-13680.
64. Ng MY, Levinson DF, Faraone SV, et al. Meta-analysis of 32 genome-wide linkage studies of schizophrenia. *Mol Psychiatry.* 2009;14:774-785.
65. Lewis CM, Levinson DF, Wise LH, et al. Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet.* 2003;73:34-48.
66. Holmans PA, Riley B, Pulver AE, et al. Genomewide linkage scan of schizophrenia in a large multicenter pedigree sample using single nucleotide polymorphisms. *Mol Psychiatry.* 2009;14:786-795.
67. Carroll LS, Williams NM, Moskvina V, et al. Evidence for rare and common genetic risk variants for schizophrenia at protein kinase C, alpha. *Mol Psychiatry.* In press.
68. Williams NM, Norton N, Williams H, et al. A systematic genomewide linkage study in 353 sib pairs with schizophrenia. *Am J Hum Genet.* 2003;73:1355-1367.
69. Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science.* 1996;273:1516-1517.
70. Wang DG, Fan JB, Siao CJ, Bero A, et al. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science.* 1998;280:1077-1082.
71. LaFramboise T. Single nucleotide polymorphism arrays: a decade of biological, computational and technological advances. *Nucleic Acids Res.* 2009;37:4181-4193.
72. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature.* 2001;409:860-921.
73. Sachidanandam R, Weissman D, Schmidt et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature.* 2001;409:928-933.
74. A haplotype map of the human genome. *Nature.* 2005;437:1299-1320.
75. Frazer KA, Ballinger DG, Cox DR, et al. A second generation human haplotype map of over 3.1 million SNPs. *Nature.* 2007;449:851-861.
76. Sham P, Bader JS, Craig I, O'Donovan M, Owen M. DNA pooling: a tool for large-scale association studies. *Nat Rev Genet.* 2002;3:862-871.
77. Mah S, Nelson MR, Delisi LE, et al. Identification of the semaphorin receptor PLXNA2 as a candidate for susceptibility to schizophrenia. *Mol Psychiatry.* 2006;11:471-478.
78. Fujii T, Iijima Y, Kondo H, Shizuno T, et al. Failure to confirm an association between the PLXNA2 gene and schizophrenia in a Japanese population. *Prog Neuropsychopharmacol Biol Psychiatry.* 2007;31:873-877.
79. Takeshita M, Yamada K, Hattori E, et al. Genetic examination of the PLXNA2 gene in Japanese and Chinese people with schizophrenia. *Schizophr Res.* 2008;99:359-364.
80. Betcheva ET, Mushiroda T, Takahashi A, et al. Case-control association study of 59 candidate genes reveals the DRD2 SNP rs6277 (C957T) as the only susceptibility factor for schizophrenia in the Bulgarian population. *J Hum Genet.* 2009;54:98-107.
81. Shifman S, Johannesson M, Bronstein M, et al. Genome-wide association identifies a common variant in the reelin gene that increases the risk of schizophrenia only in women. *PLoS Genet.* 2008;4:e28.
82. Hong SE, Shugart YY, Huang DT, et al. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat Genet.* 2000;26:93-96.
83. Herz J, Chen Y. Reelin, lipoprotein receptors and synaptic plasticity. *Nat Rev Neurosci.* 2006;7:850-859.
84. Wedenoja J, Loukola A, Tuulio-Henriksson A, et al. Replication of linkage on chromosome 7q22 and association of the regional Reelin gene with working memory in schizophrenia families. *Mol Psychiatry.* 2008;13:673-684.
85. Wedenoja J, Tuulio-Henriksson A, Suvisaari J, et al. Replication of association between working memory and reelin, a potential modifier gene in schizophrenia. *Biol Psychiatry.* 2010;67:983-991.
86. Kahler AK, Djurovic S, Kulle B, et al. Association analysis of schizophrenia on 18 genes involved in neuronal migration: MDGA1 as a new susceptibility gene. *Am J Med Genet B Neuropsychiatr Genet.* 2008;147B:1089-1100.
87. Kirov G, Zaharieva I, Georgieva L, et al. A genome-wide association study in 574 schizophrenia trios using DNA pooling. *Mol Psychiatry.* 2009;14:796-803.
88. Keshavan MS, Kennedy JL, Murray R, eds. *Neurodevelopment and Schizophrenia.* Cambridge, UK: Cambridge University Press; 2004.

89. Lencz T, Morgan TV, Athanasiou M, et al. Converging evidence for a pseudoautosomal cytokine receptor gene locus in schizophrenia. *Mol Psychiatry*. 2007;12:572-580.
90. Sun S, Wang F, Wei J, et al. Association between interleukin-3 receptor alpha polymorphism and schizophrenia in the Chinese population. *Neurosci Lett*. 2008;440:35-37.
91. Sun S, Wei J, Li H, et al. A family-based study of the IL3RA gene on susceptibility to schizophrenia in a Chinese Han population. *Brain Res*. 2009;1268:13-16.
92. Sullivan PF, Lin D, Tzeng JY, et al. Genomewide association for schizophrenia in the CATIE study: results of stage 1. *Mol Psychiatry*. 2008;13:570-584.
93. O'Donovan MC, Craddock N, Norton N, et al. Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat Genet*. 2008;40:1053-1055.
94. Esslinger C, Walter H, Kirsch P, et al. Neural mechanisms of a genome-wide supported psychosis variant. *Science*. 2009;324:605.
95. Riley B, Thiselton D, Maher BS, et al. Replication of association between schizophrenia and ZNF804A in the Irish Case-Control Study of Schizophrenia sample. *Mol Psychiatry*. 2010;15:29-37.
96. Purcell SM, Wray NR, Stone JL, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*. 2009;460:748-752.
97. Steinberg S, Mors O, Borglum AD, et al. Expanding the range of ZNF804A variants conferring risk of psychosis. *Mol Psychiatry*. 2010. In press.
98. Need AC, Ge D, Weale ME, et al. A genome-wide investigation of SNPs and CNVs in schizophrenia. *PLoS Genet*. 2009;5:e1000373.
99. Shi J, Levinson DF, Duan J, et al. Common variants on chromosome 6p22.1 are associated with schizophrenia. *Nature*. 2009;460:753-757.
100. Athanasiu L, Mattingsdal M, Kahler AK, et al. Gene variants associated with schizophrenia in a Norwegian genome-wide study are replicated in a large European cohort. *J Psychiatr Res*. 2010;44:748-753.
101. Schulze TG, Detera-Wadleigh SD, Akula N, et al. Two variants in Ankyrin 3 (ANK3) are independent genetic risk factors for bipolar disorder. *Mol Psychiatry*. 2009;14:487-491.
102. Moon HJ, Yim SV, Lee WK, et al. Identification of DNA copy-number aberrations by array-comparative genomic hybridization in patients with schizophrenia. *Biochem Biophys Res Commun*. 2006;344:531-539.
103. Wilson GM, Flibotte S, Chopra V, Melnyk BL, Honer WG, Holt RA. DNA copy-number analysis in bipolar disorder and schizophrenia reveals aberrations in genes involved in glutamate signaling. *Hum Mol Genet*. 2006;15:743-749.
104. Mizuguchi T, Hashimoto R, Itokawa M, et al. Microarray comparative genomic hybridization analysis of 59 patients with schizophrenia. *J Hum Genet*. 2008;53:914-919.
105. Kirov G, Gumus D, Chen W, et al. Comparative genome hybridization suggests a role for NRXN1 and APBA2 in schizophrenia. *Hum Mol Genet*. 2008;17:458-465.
106. Xu B, Roos JL, Levy S, van Rensburg EJ, Gogos JA, Karayiorgou M. Strong association of de novo copy number mutations with sporadic schizophrenia. *Nat Genet*. 2008;40:880-885.
107. Stefansson H, Rujescu D, Cichon S, et al. Large recurrent microdeletions associated with schizophrenia. *Nature*. 2008;455:232-236.
108. Rare chromosomal deletions and duplications increase risk of schizophrenia. *Nature*. 2008;455:237-241.
109. Shi YY, He G, Zhang Z, et al. A study of rare structural variants in schizophrenia patients and normal controls from Chinese Han population. *Mol Psychiatry*. 2008;13:911-913.
110. Kirov G, Grozeva D, Norton N, et al. Support for the involvement of large copy number variants in the pathogenesis of schizophrenia. *Hum Mol Genet*. 2009;18:1497-1503.
111. Bales AM, Zaleski CA, McPherson EW. Newborn screening programs: Should 22q11 deletion syndrome be added? *Genet Med*. 2010. In press.
112. Walsh T, McClellan JM, McCarthy SE, et al. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science*. 2008;320:539-543.
113. Ikeda M, Aleksic B, Kirov G, et al. Copy number variation in schizophrenia in the Japanese population. *Biol Psychiatry*. 2010;67:283-286.
114. Wray NR, Visscher PM. Narrowing the boundaries of the genetic architecture of schizophrenia. *Schizophr Bull*. 2010;36:14-23.
115. Owen MJ, Craddock N, Jablensky A. The genetic deconstruction of psychosis. *Schizophr Bull*. 2007;33:905-911.
116. Green EK, Raybould R, Macgregor S, et al. Operation of the schizophrenia susceptibility gene, neuregulin 1, across traditional diagnostic boundaries to increase risk for bipolar disorder. *Arch Gen Psychiatry*. 2005;62:642-648.
117. Gould TD, Gottesman, II. Psychiatric endophenotypes and the development of valid animal models. *Genes Brain Behav*. 2006;5:113-119.
118. Holzman PS, Proctor LR, Hughes DW. Eye-tracking patterns in schizophrenia. *Science*. 1973;181:179-181.
119. Braff D, Stone C, Callaway E, Geyer M, Glick I, Bali L. Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology*. 1978;15:339-343.
120. Freedman R, Adler LE, Myles-Worsley M, et al. Inhibitory gating of an evoked response to repeated auditory stimuli in schizophrenic and normal subjects. Human recordings, computer simulation, and an animal model. *Arch Gen Psychiatry*. 1996;53:1114-1121.
121. Roth WT, Pfefferbaum A, Kelly AF, Berger PA, Kopell BS. Auditory event-related potentials in schizophrenia and depression. *Psychiatry Res*. 1981;4:199-212.
122. Ivleva EI, Morris DW, Moates AF, Suppes T, Thaker GK, Tamminga CA. Genetics and intermediate phenotypes of the schizophrenia-bipolar disorder boundary. *Neurosci Biobehav Rev*. 2010;34:897-921.
123. van Os J, Rutten BP, Poulton R. Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. *Schizophr Bull*. 2008;34:1066-1082.
124. Mill J, Tang T, Kaminsky Z, et al. Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. *Am J Hum Genet*. 2008;82:696-711.
125. Ptak C, Petronis A. Epigenetic approaches to psychiatric disorders. *Dialogues Clin Neurosci*. 2010;12:25-35.
126. Ross CA, Margolis RL, Reading SA, Pletnikov M, Coyle JT. Neurobiology of schizophrenia. *Neuron*. 2006;52:139-153.