

Genetics of Schizophrenia Spectrum Disorders: Looking Back and Peering Ahead

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Abstract

The genetics of schizophrenia spectrum disorders have come a long way since the early demonstration of a substantial genetic component by family, twin and adoption studies. After over a decade of intensive molecular genetic studies, initially by linkage scans and candidate gene association studies, and more recently genome-wide association studies, a picture is now emerging that susceptibility to schizophrenia spectrum disorders is determined by many genetic variants of different types, ranging from single nucleotide polymorphisms to copy number variants, including rare and *de novo* variants, of pleiotropic effects on multiple diagnoses and traits. Further large-scale genome-wide association studies, and the forthcoming availability of affordable whole-genome sequencing technology, will further characterise the genetic variants involved, which in turn will be translated to improved clinical practice.

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Introduction

The hereditary nature of schizophrenia and schizophrenia spectrum disorders has been long recognised. The first systematic family study of schizophrenia dates back to 1916 by Ernst Rudin.¹ This was long before the molecular structure of the genetic material was elucidated, and long before DNA sequence could be directly measured. Early studies were therefore mainly concerned with the demonstration and quantification of genetic influences based on the pattern of disease occurrence in families. Converging lines of evidence from family, adoption and twin studies pointed to a genetic basis of schizophrenia. The risk of schizophrenia was shown to be significantly higher in the first degree relatives of affected individuals, with a risk ratio of about 10.² Elevated risk of schizophrenia was found in the biological families but not the adoptive families of affected adoptees.² Twin studies demonstrated a higher concordance rate for monozygotic than dizygotic twins. The heritability of schizophrenia was estimated at 81%, according to a meta-analysis of 12 twin studies.³ The meta-analysis also showed a small (11%) but significant contribution of shared environmental effects.

Besides schizophrenia, other psychiatric disorders and traits have been long reported to be more common in the relatives of schizophrenic probands than in the general

population. A review of 3 family studies⁴ on schizophrenia revealed significant familial clustering of schizotypal personality disorder and non-affective psychoses (including schizophreniform disorder, schizoaffective disorder, delusional disorder and atypical psychoses) in the 1st-degree relatives of schizophrenic patients. In an adoption study,⁵ schizotypal personality disorder and non-affective psychoses were also found to be significantly more prevalent in adoptees whose biological mothers had schizophrenia spectrum disorders. Schizoaffective disorder has been shown to cluster with both schizophrenia and bipolar affective disorder in families.⁶

Start of “New” Genetics: Linkage Studies

Classical family studies suffered from the limitation that they cannot inform us which specific genetic variants are associated with a disease. In the late 1980s, technology was developed to measure naturally occurring DNA “markers”, particularly simple sequence repeat polymorphisms, which made possible the screening of the whole-genome for disease-causing variants. Such genome scans aim to detect linkage (i.e. excess co-transmission) between marker variants and disease in families with multiple affected members.

The linkage approach has contributed to the discovery of

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DISC1 (disrupted in schizophrenia 1), one of the most strongly supported susceptibility genes for schizophrenia to date. The discovery rooted from a study by St Clair et al⁷ who investigated a large Scottish pedigree and found significant co-segregation of psychiatric disorders with a translocation between chromosomes 1 and 11 (maximum LOD score = 4.34). Millar et al⁸ performed positional cloning at the translocation breakpoint and identified the novel *DISC1* susceptibility gene. Other linkage studies^{9–11} also provided independent evidence for the involvement of *DISC1* in mental illnesses.

However, the linkage strategy has not been successful in revealing other strong candidate genes apart from *DISC1*. To date, at least 32 linkage scans¹² have been performed for schizophrenia and other spectrum disorders, and at least 3 meta-analyses have been completed.^{12–14} The linkage results were largely inconsistent,^{15,16} which may be due to the weak power of linkage studies to detect variants with small or modest effects. Linkage is most effective for the detection of loci with large effect sizes, as are present in Mendelian disorders. For a complex disease like schizophrenia, linkage may be successful if there exist genetic variants with large effects that can be identified in specific large pedigrees having multiple affected individuals. Such pedigrees are however rare. In addition, locus heterogeneity (i.e. loci causing the disease may be different for different families) may attenuate the power to detect linkage. Another limitation of linkage analysis is that it has low resolution, resulting in wide genomic regions that are likely to harbour disease susceptibility genes. Other techniques (such as association studies) are often required to identify the specific causal gene within the linked region.

Shift to Association

In contrast to linkage studies, association studies relate genetic variants to disease at the population rather than the family level. Essentially, association studies compare allele or genotype frequencies of a polymorphism between cases and controls, and are analogous to conventional epidemiological studies except that the risk factor is a genetic marker rather than environmental exposure. A marker may show association if it is the true susceptibility variant (direct association) or in strong linkage disequilibrium (LD) with a true susceptibility variant (indirect association). Markers in high LD are usually within a short chromosomal segment and likely to be inherited together. The association approach is more powerful than linkage if the variant has a consistent effect (direct or indirect) in the entire population.

Association studies have become the mainstream in schizophrenia genetics research. According to SzGene¹⁷ (accessed 30 January 2009), at least 1400 association studies, which covered 761 genes and 6550 polymorphisms,

have been performed for schizophrenia and other spectrum disorders. However, discrepancies in results are typical and replications of findings are hard.¹⁸ Very few genetic variants show strong or even moderate evidence of association and most findings are inconclusive. Many reasons may be responsible for the huge difficulty in finding true susceptibility variants. One is that with few exceptions, each gene or genetic variant has a very low prior probability of association with disease. Another major reason is inadequate study power, given that the effect sizes of any common susceptibility variants are probably small. Besides, it is possible that some positive findings are in fact false positives, owing to issues like multiple testing or population stratification. Genetic and phenotypic heterogeneity may also reduce power of a study and cause inconsistency of results across samples. In addition, inconsistency of test results across populations may due to different patterns of LD structure, gene-gene or gene-environmental interactions. For instance, one genetic variant may affect the disease risk only in the presence of another variant or a particular environmental risk factor. Since the genetic and environmental backgrounds may vary across samples or populations, power of detecting the susceptibility variant may also vary.

Out of the large number of genes tested, a few have amassed relatively stronger statistical and functional evidence, such as *NRG1*,¹⁹ *DTNBPI*,²⁰ *RGS4*²¹ etc, although their associations with schizophrenia are still not unequivocal. Updated meta-analyses of association studies can be found at the SzGene database.¹⁷

Genome-wide Association Studies (GWAS)

Advances in high-throughput genotyping of single nucleotide polymorphisms (SNP) have led to increasingly high-density association studies. It is now feasible to survey up to a million SNPs using Affymetrix or Illumina arrays. These arrays have an effective coverage, through direct and indirect association, of about 90% of the common variants in the genome. A distinct advantage of GWAS is the ability to screen the genome in an unbiased and hypothesis-free manner, enabling previously unsuspected candidate genes to be revealed.

GWAS have proved to be a useful tool in dissecting the genetic basis of complex diseases. For example, GWAS have established robust associations for at least 32 novel genes for Crohn's disease²² and at least 15 genes for type II diabetes over the past 2 years.²³ Six GWAS for schizophrenia have been published.^{24–29} In the largest-scale GWAS published to date,²⁷ full genotyping was conducted on 497 cases and 2937 controls and replication sought in 16,726 additional subjects. This study revealed strong association with the gene *ZNF804A*,²⁷ but further replication by other groups is needed for confirmation of this finding. The other

studies also suggested a few candidate genes, such as *CCDC60*,²⁵ *IL3RA*²⁶ and *RELN*,²⁸ although the evidence is weaker and further replications are warranted.

Copy-number Variations

Until recently, most association studies focused on single nucleotide polymorphisms that are relatively common in the population (minor allele frequency >1%). Nevertheless, another form of genetic variation, known as copy number variation (CNV), has received increasing attention in the past 2 years. CNVs usually refer to genomic deletions and/or duplication of 1 kb to 3 MB in size.³⁰ Technologies commonly used for CNV detection include array comparative genomic hybridization (CGH) and SNP genotyping platforms like Illumina or Affymetrix. Details of the various technologies and platforms developed for CNV detection may be found in a review by Carter.³¹

A number of recent studies have identified CNVs linked to schizophrenia.^{24,32-40} For instance, Kirov et al⁴¹ identified 13 CNVs for schizophrenia including a deletion on chr 2q involving the *Neurexin1* gene, which was confirmed by subsequent studies.^{32,38,39} Interestingly, deletions spanning *Neurexin1* are also strongly implicated in autism⁴²⁻⁴⁴ and mental retardation.⁴⁵ Other regions harboring CNVs for schizophrenia include 1q21, 15q11.2, 15q13.3 and 22q12 (region deleted in velo-cardio-facial syndrome). In contrast to linkage or association analyses, the results of CNV studies are remarkably consistent. In addition to the example of *Neurexin1*, 2 large-scale CNV screens^{34,37} both identified deletions at regions 1q21.1 and 15q13.3 associated with schizophrenia. Recently, microdeletions at 15q13.3 were also reported to increase the risk of idiopathic generalized epilepsy,⁴⁶ suggesting possible heterogeneity of phenotypic effects of CNVs. Although a number of studies show associations of CNVs with schizophrenia, a study in Chinese⁴⁷ failed to find an excess of all CNVs, all rare CNVs (frequency <1%) or rare CNVs affecting intragenic sequences in schizophrenic cases. These negative findings may be due to difficulties in the analysis of array data and limited sample sizes. The discovery of CNVs may have important clinical implications. As CNVs often have larger effects than SNPs, they may be useful for risk prediction in certain subset of individuals. In addition, the sibling of a schizophrenic proband may have a lower risk of disease if the proband carries a *de novo* CNV that has strongly contributed to the development of disease.

The Path Ahead

It should be noted that currently known genetic risk variants account for only a small portion of the overall familial risk for schizophrenia, implying that many more susceptibility variants remain to be discovered. Large sample size is crucial for detecting small-effect SNPs at the

level of genome-wide significance. Large samples are also needed for discovering structural variants due to their rarity. The small number of positive associations from GWAS to date suggests that rare variants play a significant role in schizophrenia aetiology. One may also speculate that a substantial proportion of these rare variants are recent or *de novo* mutations, which serve to maintain the high prevalence of schizophrenia despite reduced fecundity of patients. Advances in genotyping technology will enable us to interrogate the genome for rare variants at higher resolution.

Most GWAS performed on psychiatric disorders are conducted on Caucasian samples. There is a need to extend these studies to other ethnic groups. Although the true causal variants are likely to increase disease risk regardless of ancestry, the frequency and LD pattern of variants may differ across populations. Some risk variants may be more readily discovered in one population than others. A risk variant may have also different effects on populations with varied genetic and environmental backgrounds.

Study of genetic effects on intermediate and clinical phenotypes will be another important task. For example, it will be of great clinical interest to identify genes that affect the prognosis and treatment response of schizophrenic patients. In addition, as psychiatric diseases are classified mainly by symptom pattern rather than the underlying pathophysiology, the susceptibility genes may not pertain to a particular psychiatric diagnosis but more generally to physiological or cognitive dimensions. Investigation of secondary phenotypes may help to address the heterogeneity in study samples and shed light on the validity of the current classification system from a genetic point of view. An alternative way of tackling this problem is to search for genes and pathways that are shared across diagnoses and those that are specific to particular diagnoses.

How could advances in our knowledge in genetics affect clinical practice? There are 2 main ways in which patients may benefit. The first is that a more complete understanding of the pathophysiology and molecular mechanisms underlying schizophrenia may point to novel targets for intervention or biomarkers for disease diagnosis and monitoring. However, the biological functions of the few tentative risk variants suggested by association studies are largely unknown. It may require considerable effort before novel medication can be developed. The second possibility is to utilise the genetic information available for prediction of disease risk, prognosis and response to medications. However, it is likely that any common susceptibility variants for schizophrenia will have small effects and a large number of them will be required to achieve good predictive power. Rare variants (e.g. CNVs) tend to have higher penetrance and may be useful for prediction in certain

subgroups of patients. We need to have more thorough knowledge of both types of variants for genomic profiling to be applied clinically.

Research in genetics holds promise for advancing our understanding of schizophrenia, one of the most enigmatic diseases in medicine. Despite the challenges and difficulties ahead, with the expeditious growth in the field, we are hopeful that research findings in genetics could be translated into clinical practice in the foreseeable future.

REFERENCES

- Kendler KS, Zerbini-Rudin E. Abstract and review of "Studien Über Vererbung und Entstehung Geistiger Störungen. I. Zur Vererbung und Neuentstehung der Dementia praecox." (Studies on the inheritance and origin of mental illness: I. To the problem of the inheritance and primary origin of dementia praecox) 1916. *Am J Med Genet* 1996;67:338-42.
- Lieberman JA, Stroup TS, Perkins DO, American Psychiatric Publishing. *The American Psychiatric Publishing Textbook of Schizophrenia*. 1st ed. Washington DC: American Psychiatric Pub, 2006.
- Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry* 2003;60:1187-92.
- Kendler KS, Gardner CO. The risk for psychiatric disorders in relatives of schizophrenic and control probands: a comparison of three independent studies. *Psychol Med* 1997;27:411-9.
- Tienari P, Wynne LC, Laksy K, Moring J, Nieminen P, Sorri A, et al. Genetic boundaries of the schizophrenia spectrum: evidence from the Finnish Adoptive Family Study of Schizophrenia. *Am J Psychiatry* 2003;160:1587-94.
- Laursen TM, Labouriau R, Licht RW, Bertelsen A, Munk-Olsen T, Mortensen PB. Family history of psychiatric illness as a risk factor for schizoaffective disorder: a Danish register-based cohort study. *Arch Gen Psychiatry* 2005;62:841-8.
- St Clair D, Blackwood D, Muir W, Carothers A, Walker M, Spowart G, et al. Association within a family of a balanced autosomal translocation with major mental illness. *Lancet* 1990;336:13-6.
- Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA, et al. Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum Mol Genet* 2000;9:1415-23.
- Ekelund J, Lichtermann D, Hovatta I, Ellonen P, Suvisaari J, Terwilliger JD, et al. Genome-wide scan for schizophrenia in the Finnish population: evidence for a locus on chromosome 7q22. *Hum Mol Genet* 2000;9:1049-57.
- Ekelund J, Hovatta I, Parker A, Paunio T, Varilo T, Martin R, et al. Chromosome 1 loci in Finnish schizophrenia families. *Hum Mol Genet* 2001;10:1611-7.
- Hamshere ML, Bennett P, Williams N, Segurado R, Cardno A, Norton N, et al. Genomewide linkage scan in schizoaffective disorder: significant evidence for linkage at 1q42 close to DISC1, and suggestive evidence at 22q11 and 19p13. *Arch Gen Psychiatry* 2005;62:1081-8.
- Ng MYM, Levinson DF, Faraone SV, Suarez BK, DeLisi LE, Arinami T, et al. Meta-analysis of 32 genome-wide linkage studies of schizophrenia. *Mol Psychiatry*; in press.
- Badner JA, Gershon ES. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 2002;7:405-11.
- Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, et al. Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 2003;73:34-48.
- Crow TJ. How and why genetic linkage has not solved the problem of psychosis: review and hypothesis. *Am J Psychiatry* 2007;164: 13-21.
- Sullivan PF. The genetics of schizophrenia. *PLoS Med* 2005;2:e212.
- Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ, et al. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nat Genet* 2008;40:827-34.
- Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat Genet* 2001;29:306-9.
- Mei L, Xiong WC. Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat Rev Neurosci* 2008;9:437-52.
- Williams NM, O'Donovan MC, Owen MJ. Is the dysbindin gene (DTNBP1) a susceptibility gene for schizophrenia? *Schizophr Bull* 2005;31:800-5.
- Levitt P, Ebert P, Mirnics K, Nimgaonkar VL, Lewis DA. Making the case for a candidate vulnerability gene in schizophrenia: Convergent evidence for regulator of G-protein signaling 4 (RGS4). *Biol Psychiatry* 2006;60:534-7.
- Lette G, Rioux JD. Autoimmune diseases: insights from genome-wide association studies. *Hum Mol Genet* 2008;17:R116-21.
- Mohlke KL, Boehnke M, Abecasis GR. Metabolic and cardiovascular traits: an abundance of recently identified common genetic variants. *Hum Mol Genet* 2008;17:R102-8.
- Need AC, Ge D, Weale ME, Maia J, Feng S, Heinzen EL, et al. A genome-wide investigation of SNPs and CNVs in schizophrenia. *PLoS Genet* 2009;5:e1000373.
- Kirov G, Zaharieva I, Georgieva L, Moskvina V, Nikolov I, Cichon S, et al. A genome-wide association study in 574 schizophrenia trios using DNA pooling. *Mol Psychiatry*; in press.
- Lencz T, Morgan TV, Athanasiou M, Dain B, Reed CR, Kane JM, et al. Converging evidence for a pseudoautosomal cytokine receptor gene locus in schizophrenia. *Mol Psychiatry* 2007;12:572-80.
- O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V, et al. Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat Genet* 2008;40:1053-5.
- Shifman S, Johannesson M, Bronstein M, Chen SX, Collier DA, Craddock NJ, 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.
- Sullivan PF, Lin D, Tzeng JY, van den Oord E, Perkins D, Stroup TS, et al. Genomewide association for schizophrenia in the CATIE study: results of stage 1. *Mol Psychiatry* 2008;13: 570-84.
- Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, et al. Global variation in copy number in the human genome. *Nature* 2006;444: 444-54.
- Carter NP. Methods and strategies for analyzing copy number variation using DNA microarrays. *Nat Genet* 2007;39:S16-21.
- Rujescu D, Ingason A, Cichon S, Pietilainen OP, Barnes MR, Touloupoulou T, et al. Disruption of the neurexin 1 gene is associated with schizophrenia. *Hum Mol Genet* 2009;18:988-96.
- Kirov G, Grozeva D, Norton N, Ivanov D, Mantripragada KK, Holmans P, et al. Support for the involvement of large CNVs in the pathogenesis of schizophrenia. *Hum Mol Genet* (In press).
- Rare chromosomal deletions and duplications increase risk of schizophrenia. *Nature* 2008;455:237-41.
- Friedman JI, Vrijenhoek T, Marx S, Janssen IM, van der Vliet WA, Faas BH, et al. CNTNAP2 gene dosage variation is associated with schizophrenia and epilepsy. *Mol Psychiatry* 2008;13:261-6.
- St Clair D. Copy number variation and schizophrenia. *Schizophr Bull* 2009;35:9-12.
- Stefansson H, Rujescu D, Cichon S, Pietilainen OP, Ingason A, Steinberg S, et al. Large recurrent microdeletions associated with schizophrenia. *Nature* 2008;455:232-6.
- Vrijenhoek T, Buizer-Voskamp JE, van der Stelt I, Strengman E, Sabatti C, Geurts van Kessel A, et al. Recurrent CNVs disrupt three candidate genes in schizophrenia patients. *Am J Hum Genet* 2008;83:504-10.
- Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, et al. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science* 2008;320:539-43.
- 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;40:880-5.
- Kirov G, Gumus D, Chen W, Norton N, Georgieva L, Sari M, et al. Comparative genome hybridization suggests a role for NRXN1 and APBA2 in schizophrenia. *Hum Mol Genet* 2008;17:458-65.
- Kim HG, Kishikawa S, Higgins AW, Seong IS, Donovan DJ, Shen Y, et al. Disruption of neurexin I associated with autism spectrum disorder. *Am J Hum Genet* 2008;82:199-207.
- Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, et al. Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet* 2008;82: 477-88.
- Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, Liu XQ, et al. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. *Nat Genet* 2007;39:319-28.
- Friedman JM, Baross A, Delaney AD, Ally A, Arbour L, Armstrong L, et al. Oligonucleotide microarray analysis of genomic imbalance in children with mental retardation. *Am J Hum Genet* 2006;79:500-13.
- Helbig I, Mefford HC, Sharp AJ, Guipponi M, Fichera M, Franke A, et al. 15q13.3 microdeletions increase risk of idiopathic generalized epilepsy. *Nat Genet* 2009;41:160-2.
- Shi YY, He G, Zhang Z, Tang W, Zhang J, Jr., Zhao Q, et al. A study of rare structural variants in schizophrenia patients and normal controls from Chinese Han population. *Mol Psychiatry* 2008;13:911-3.