

REVIEW

Molecular genetic studies of schizophrenia

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The study of schizophrenia genetics has confirmed the importance of genes in etiology, but has not so far identified the relationship between observed genetic risks and specific DNA variants, protein alterations or biological processes. In spite of many limitations, numerous regions of the human genome give consistent, although by no means unanimous, support for linkage, which is unlikely to occur by chance. Two recent shifts have been evident in the field. First, a series of studies combining linkage and association analyses in the same family sets have identified promising candidate genes (*DTNBP1*, *NRG1*, *G72/G30*, *TRAR4*). Although a consensus definition of replication for genetic association in a complex trait remains difficult to achieve, the evidence for two of these (dystrobrevin binding protein 1 (*DTNBP1*), *NRG1*) is strong. Second, a series of studies combining association with functional investigation of changes in the associated gene in schizophrenia have also identified several candidate genes (*COMT*, *RGS4*, *PPP3CC*, *ZDHC8*, *AKT1*). Somewhat surprisingly, the loci implicated by these studies have proven less robust in replication, although the number of replication studies remains small in several cases. Assessment of the combined evidence for the *DTNBP1* gene gives some insight into the nature of the problems remaining to be solved. *European Journal of Human Genetics* (2006) 14, 669–680. doi:10.1038/sj.ejhg.5201571

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Genetic epidemiology

A large body of data collected from families, twins and adoptees over many years has consistently supported the involvement of a major, complex genetic component in liability to schizophrenia and schizophrenia spectrum disorders.

Family studies: does risk aggregate in relatives?

The lifetime morbid risks (MR) from combined results of studies published between 1921 and 1987¹ are shown in Figure 1. Early family studies were criticized for lack of proper controls, nonsystematic sampling, lack of standardized diagnostic criteria and failure to diagnose family members blind to the status of the index case (or proband).

A combined analysis of data from seven later studies that avoided these weaknesses yielded MR for narrowly defined schizophrenia in relatives of patients of 4.8%, 10 times the 0.5% average MR in relatives of controls.²

Twin studies: how large is the genetic component of risk?

Twin studies of schizophrenia, (reviewed in Cardno and Gottesman³) estimate the relative importance of genes and environment in liability and show consistently higher concordance in monozygotic (MZ, ~50%) than dizygotic (DZ, ~17%) twins. Both individual twin studies and meta-analyses of twin studies estimate the heritability of liability to schizophrenia to be approximately 80%. Some find evidence that a small proportion of liability to schizophrenia results from shared environmental risk factors. Recent studies have suggested that gene–environment interactions may be components of the overall risk.⁴ Schizophrenia is thus largely genetically *mediated* but not genetically *determined*.

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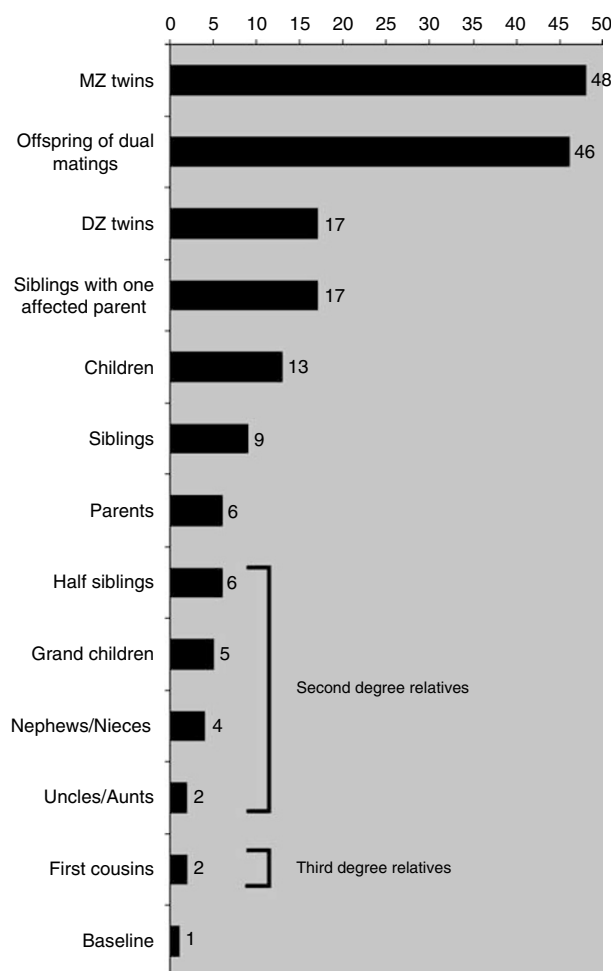


Figure 1 Lifetime MR for schizophrenia in various classes of relatives of a proband, adapted from Gottesman.¹

Adoption studies: are genetic factors evident when shared familial environment is controlled?

Across all adoption studies performed, increased risk of schizophrenia was present in the biological relatives of individuals with schizophrenia.⁵ In 361 families in Finland, 4.9% of adopted away children of schizophrenic mothers have schizophrenia and 9.1% have a schizophrenia spectrum disorder, whereas 1.1% of adopted away offspring of control mothers have schizophrenia.⁶ In Denmark, schizophrenia was significantly more common in the biological relatives of schizophrenic adoptees than in those of control adoptees.⁷ The rates of schizophrenia were low and not different in the adoptive families of both affected and control groups.

Segregation analysis: how is the genetic risk transmitted?

Transmission models make specific predictions about risk in various classes of relatives and are tested by assessing

how well their predictions match observed patterns of risk. Neither the explicitly monogenic and deterministic generalized single major locus nor the less deterministic and explicitly polygenic multifactorial threshold (MT) models are adequate to explain observed patterns of risk.⁸ The MT model assumes that the effects of multiple genes combine additively (the total liability from n genes is equal to the sum of the n individual liabilities) and do not interact. A key predictive failure of the MT model is that the observed MZ twin concordance is too high relative to that in siblings and DZ twins. This pattern is more consistent with at least some epistatic interactions between loci, where the total liability is greater than the sum of the n individual liabilities. The inheritance model is unlikely to be fully epistatic, however, because the tetrachoric correlation in MZ relative to DZ twins should be substantially more than observed. It therefore seems that numerous kinds of influences (additive and epistatic genes, environment and $G \times E$ interactions) are involved and that the precise model reflecting an individual's or a family's risk may vary depending on the specific risk factors active in them.

Spectrum disorders: how broad is the range of psychiatric illness transmitted and who do we consider affected?

In general, the risk for all psychotic spectrum disorders (including schizoaffective, schizophreniform, delusional, paranoid personality and schizotypal personality disorders) is increased in the relatives of schizophrenics.² The risk of schizophrenia is also significantly higher in the relatives of individuals with spectrum disorders. Most studies suggest that relatives of schizophrenic patients are not at increased risk of anxiety or alcohol and drug dependence disorders.⁹ The results for bipolar affective disorder are less clear, but taken together suggest that schizophrenia and bipolar disorder might have both shared and independent genetic risk factors. It is therefore reasonably common to perform several analyses of data using a number of different definitions of illness.

Molecular genetics

Approaches: linkage and association

The issues surrounding the strengths and weaknesses of linkage and association in complex traits have been discussed at length.¹⁰ Both are impeded by unclear diagnostic boundaries, multiple loci, epistasis, clinical and etiologic heterogeneity and $G \times E$ interactions. Because of their fundamental differences, the two approaches have generally been considered separately. More and more, however, association studies are following linkage evidence. Analytic developments in family-based association testing have furthered association studies in samples originally collected for linkage. The sequential application of these two approaches has produced the most exciting

current results, including a small but growing number of specific genes, which we will focus on, for which multiple groups have found support. A second recent and important shift in the field has been towards obtaining functional evidence to support association evidence, and publishing both together. Many of these associations have proven less robust in replication studies that generally do not include replication of the functional component.

A total of 25 genome scans for schizophrenia have now been published, with no evidence for a gene of major effect. To date, eight linkage regions have identified promising candidate genes: 22q12–q13, 8p22–p21, 6p24–p22, 13q14–q32, and 6q21–q22. The interpretation of these results is controversial, particularly as the definition of replication for linkage to a complex trait remains uncertain. For brevity, we omit studies that do not find evidence for linkage in these selected regions, so there is selective bias in the results that follow. Full references for all studies mentioned can be found in Riley and Kendler.¹⁰ We do discuss both positive and negative studies of the current candidate genes.

Chromosome 22q linkage studies

In a sample of families from Maryland, three markers spanning ~23 cM in the 22q13.1 region gave evidence for linkage.¹¹ Two of a number of attempted replications were positive. A total of 11 groups contributed data for the most significant marker from the Maryland sample to the first collaborative schizophrenia linkage study. There was excess sharing of alleles in the affected pairs ($P=0.006$), particularly in those with data from both parents ($P=0.001$), but the locus accounts for no more than 2% of total variance in liability.¹² Velo-cardio-facial syndrome (VCFS) is caused by deletions nearby at 22q11. Historically, about 10% of VCFS patients were thought to present with a psychotic phenotype, but more recent studies suggest much higher rates of 25–29%.¹³ The VCFS critical region contains three genes that have been investigated in schizophrenia.

Chromosome 22q candidate genes

COMT The gene for catechol-*O*-methyl transferase (*COMT*), involved in the synthesis and degradation of catecholamines, is functionally polymorphic, with a variable amino acid, Val158Met. Val and Met alleles are of almost identical frequency. Most studies of the *COMT* gene have tested for association with the low activity (Met) allele (with mixed results). One recent report suggests that the high activity (Val) allele, through increased catabolism of dopamine in the prefrontal cortex, may slightly increase the risk of schizophrenia and may explain some of the observed differences in cognitive performance and prefrontal cortical functioning between cases and controls.¹⁴ In a large, homogeneous case–control sample of Ashkenazi Jewish individuals from Israel, the homozygous high activity genotype (Val/Val) and two other SNPs showed

significant association with schizophrenia in ~720 cases and 2000–4000 controls.¹⁵

Three recent meta-analyses of *COMT* association with schizophrenia have provided mixed (though generally negative) results. One found that *COMT* was associated only in cases of European descent.¹⁶ The others did not confirm either association or ethnic differences.¹⁷ Finally, the association between *COMT* and schizophrenia has been re-examined in a recent large-scale European study involving 709 cases, 710 controls and 488 parent–child trios.¹⁸ Markers and haplotypes positive in prior studies were tested, but despite substantial power, no evidence of significant association was found.

PRODH2 and ZDHHC8 The proline dehydrogenase (*PRODH2*) gene gave evidence for association in samples of adult and childhood-onset schizophrenia.¹⁹ Marker data from a third independent sample of cases and controls did not reach conventional significance, but analysis of the childhood-onset subset of this case–control sample was significant for association of the same marker alleles with disease. However, most replication studies detect no association between psychotic phenotypes, primarily schizophrenia, and common *PRODH2* polymorphisms.²⁰ Association with a third locus in the deletion region, *ZDHHC8*, was detected by the same group that reported originally on *PRODH2*.²¹ Again, follow-up reports have been mixed, but generally have not supported this association.²²

Chromosome 8p22–p21 linkage studies

The Maryland family sample also gave the first evidence of linkage to 8p22–p21.²³ A multicenter collaborative linkage study supported this putative locus with excess allele sharing at D8S261.²⁴ Data from pedigrees from numerous different ethnic backgrounds all support a locus on 8p, but these results are spread across ~15 Mb of sequence. One of the key points to note is that although numerous samples support a locus on this chromosome, comparison between individual studies is consistent with the possibility of multiple susceptibility genes in the region.

Chromosome 8p22–p21 candidate genes

NRG1 Following linkage evidence to 8p in Icelandic families, fine mapping with 50 markers across a 30 cM interval identified two risk haplotypes spanning a region of ~1 Mb within the gene for neuregulin 1 (*NRG1*).²⁵ Case–control samples from Scotland²⁶ and Ireland²⁷ have provided additional support for this locus and for haplotypes identical or closely related to those identified in the Icelandic cases. Studies in other populations have provided support for association,²⁸ although not with the specific haplotypes seen in the Icelandic, Scottish and Irish samples. A smaller number of studies have found no evidence for association.²⁹ Neuregulin is expressed in CNS

synapses and appears to have a role in the expression and activation of neurotransmitter (including glutamate) receptors.

PPP3CC Parallel strands of evidence implicate the calcineurin A gamma ($CNA\gamma$) subunit gene (*PPP3CC*) in schizophrenia. Calcineurin is a dimeric calcium-dependent serine/threonine phosphatase composed of a regulatory (CNB) and one of three catalytic (CNA) subunits and is highly expressed in the central nervous system. First, case/control association testing of calcineurin-related genes mapping to linkage regions identified association with both individual markers and marker haplotypes in *PPP3CC* (8p21.3).³⁰ Second, conditional knockout mice lacking CNB expression in the forebrain display traits of potential relevance to schizophrenia, including decreased social interaction, impaired prepulse and latent inhibition and severe working memory deficits.³¹ Neither of the genes encoding the CNB (2p14) or $CNA\alpha$ (4q24) subunits gave evidence for association. Only one follow-up study of *PPP3CC* has been published and did not replicate the association.³²

Chromosome 6p24–p22 linkage studies

The first evidence for linkage of schizophrenia to the 6p region came from studies of Irish families with a high density of disease.³³ In data from 16 markers, evidence for linkage was modest under a narrow diagnostic model, but increased substantially as the diagnostic definition broadened to include spectrum disorders. Evidence for linkage fell when the definition was broadened further to include nonspectrum disorders. To date, nine independent reports of analyses of this region of 6p have been published, six of which are positive.¹⁰

Chromosome 6p24–p22 candidate genes

DTNBP1 Follow-up work in the Irish family set demonstrated a positive association in the dystrobrevin binding protein 1 or dysbindin (*DTNBP1*) gene.^{34,35} Replication studies have been generally supportive.^{36–43} These results are discussed in detail below. The function of *DTNBP1* in the brain is unknown, but expression of *DTNBP1* is reduced in certain brain regions of patients with schizophrenia at both RNA⁴⁴ and protein⁴⁵ levels. Reduced protein expression is associated with additional changes consistent with a role in glutamatergic neurotransmission, currently a system of great interest for schizophrenia liability. Overexpression of *DTNBP1* is associated with increased phosphorylation and activity of *AKT1* in neuronal culture, suggesting that *DTNBP1* also interacts with the *AKT* signaling pathway, which mediates cell survival. In this context, it is interesting to note that the *AKT1* gene has recently shown association with schizophrenia.⁴⁶ This association has been confirmed in two of the three published follow-up studies.^{47–49}

Chromosome 13q14–q32 linkage studies

Data from a mixed sample of UK and Japanese families initially suggested linkage to 13q14.1–q32,⁵⁰ which is of interest as the region contains the 5HT-2A receptor gene. Data from five studies of six independent samples provided support for the region, although some results were extremely distant from other findings in the region. The results from chromosome 13 are particularly difficult to interpret because of the very large distances between positive markers. Overall, the combined linkage reports are spread over a region of ~60 Mb, containing ~120 known or putative genes. On the other hand, although locations are much less certain on 13q than in other linkage regions, this chromosome has produced some of the most significant linkage evidence seen in the studies of schizophrenia.

Chromosome 13q14–q32 candidate genes

G72 and DAO An elegant recent study examined markers in the distal 5 Mb of this broad linkage region, site of one of the most significant findings on chromosome 13.⁵¹ Nearly 200 SNPs were tested across the region and identified two regions of association. In one of these regions, two genes (*G72*, now called D-amino acid oxidase activator, *DAOA*) and *G30* were investigated. Of note, the exons of these genes could not be predicted by any computational method tested, suggesting that they are highly novel in their sequence and organization. Both genes show alternative transcripts in the brain and other tissues. Association studies of SNPs within *G72* have not yet provided a clear pattern. One of these is nonsynonymous and is significant alone. The nature of the amino-acid change (lysine to arginine) is conservative, but has major functional consequences in some proteins. However, the overall pattern of results is probably most consistent with the existence of further unidentified predisposing variants in this gene.

D-amino acid oxidase (*DAO*) is activated by the protein product of *G72*. Four SNPs in the *DAO* gene on 12q24 were significantly associated. Results of this kind (showing association in two interacting genes in the same sample) are rare, so this study had a unique opportunity to test for an epistatic genetic interaction. Evidence for epistasis was observed for one pair of *DAOA* and *G72* genotypes, supporting a potential interaction between them in risk for schizophrenia. Replication studies have generally provided confirmation of a role for *G72*.^{52–57} Only one negative report has appeared.⁵⁸

Chromosome 6q21–q22 linkage studies

A sample of 53 US families provided initial evidence for a susceptibility locus on 6q21–q22.3.⁵⁹ This study is unique in that two additional independent samples of families held by this group supported the results from the first sample. An interval of ~8 Mb gave the strongest results

with an LOD of 3.82 and highly significant excess allele sharing. A collaborative study of this region was highly significant.⁶⁰

Chromosome 6q21–q22 candidate genes

TRAR4 A recent paper from the original group following up their linkage findings with linkage disequilibrium (LD) studies in the same samples has identified association in the trace amine receptor 4 (*TRAR4*) gene located at 6q23.2.⁶¹ Two replication studies of this locus did not find evidence for association,^{62,63} although neither examined samples of similar ethnicity to the original report.

Chromosome 1q32–q42 and 1q21–q22

Interest in chromosome 1 in schizophrenia began with reports of a balanced 1:11 translocation segregating with serious mental illness in a large pedigree from Scotland. The chromosome 1 breakpoint lies at 1q42.1, and two groups reported suggestive linkage findings in this region, in a population isolate from Finland and in the Maryland sample. Genome scans of families from a mixed sample of US/UK families⁶⁴ and Canada⁶⁵ provided evidence for a 1q21–q22 locus. The latter provided very strong LOD scores but this region was not replicated by a large collaborative study.⁶⁶ Unlike the results discussed above, candidate loci on 1q have not been identified through family-based association follow-up of linkage data, but have come instead from studies of chromosomal rearrangements and microarray data.

Chromosome 1q42 candidate genes

DISC1 Ongoing work in the Scottish pedigree has now identified three genes disrupted by the breakpoint, one of which, disrupted in schizophrenia 1 (*DISC1*), has been intensively studied.⁶⁷ Studies of this locus in samples with no translocation have been mixed, but positive results^{68–71} outnumber negative ones.^{72–74} A frameshift mutation has also been identified in a single multiply affected family.⁷⁵ *DISC1* appears to have a role in cytoskeletal regulation, and thus may affect neuronal migration, neurite outgrowth and intracellular transport.^{76,77}

Chromosome 1q21–q22 candidate genes

RGS4 Microarray studies of post-mortem schizophrenic brain suggested that the regulator of G-protein signalling 4 (*RGS4*) gene showed altered expression in schizophrenia.⁷⁸ *RGS4* maps to the 1q21–q22 linkage region. A study of US and Indian pedigrees showed association with the same markers in both, although with different specifics.⁷⁹ One replication study supported the *RGS4* association with schizophrenia liability,⁸⁰ whereas others have not.^{81–84}

Meta-analyses of genome screen data

Meta-analysis of whole genome screen data represents a first approximation of a very large, multisample genome

screen. The strongest method ranks 30cM bins of the genome from most positive to least positive for each study, sums the ranks for each bin and calculates significance by simulation. As this method uses the actual marker LOD scores from each component study, it can identify regions of the genome showing modest positive results across many samples but which may have been overlooked in individual studies. Results of this approach supported linkage to chromosomes 6p, 8p and 10p.⁸⁵ However, the strongest evidence for a potential locus was on 2q, a region suggested by only a few studies and not widely followed up, and on 3p, the site of an early linkage finding which could never be replicated. Finally, significant evidence of linkage was also detected for two regions never previously implicated by an individual study, on chromosomes 11q and 14p.

Discussion

Current linkage regions and candidate genes

The best supported linkage regions have been replicated in other samples in addition to the one in which they were first reported but have not been replicated in all studies. Collaborative replication has been at least suggestive of an effect. The collected evidence for current candidate genes displays both similarities and contrasts to the linkage evidence. The reported associations for several of these candidates, *DTNBP1*, *NRG1*, *G72*, *RGS4*, *DISC1* and *AKT1* have been replicated in other samples. Critically, positive replications outnumber negative ones for all these loci. Some candidates, such as *TRAR4*, await the collection of sufficient data to interpret the validity of the original studies, whereas others, such as *COMT*, have not yielded conclusive results despite intense study, including large sample association and meta-analysis.

One exciting feature of most of the candidates discussed above is that they can be related to potential pathophysiology through dysfunction in glutamatergic neurotransmission, which may be an important systemic element in the etiology of schizophrenia. Detailed discussion of this theory is outside the scope of this review, but recent reviews of the genetic⁸⁶ and neurobiological⁸⁷ evidence discuss the positions of the gene products of *NRG1*, *COMT*, *DAAO*, *G72* and *RGS4*, among others, in the biochemical and functional pathways influencing the glutamatergic system. Currently, all of the regions and candidate genes discussed in the sections above are promising, but further assessment of each is still needed, both to clarify patterns of linkage or association and to elucidate their contribution to the neurobiology of schizophrenia.

Limitations of current knowledge

No DNA variant widely associated with schizophrenia has been shown to produce a distinct change in expression, structure or function of any of the candidates. Even in the

data from the two genes (*DTNBP1* and *NRG1*) where most replication studies detect evidence for association, important questions remain. No single high-risk haplotype is associated across all samples. It is unclear whether we should expect to observe association with the same specific haplotype, or only with the gene, across samples, as we have no idea of the age or number of liability variants. A single, evolutionarily recent variant would be expected to yield association with the same haplotype across samples, whereas a single ancient variant may have been extensively shuffled by recombination, yielding haplotypes that are differentially represented in, or even specific to, different populations. As we show below (Table 3), there is evidence that such differences in haplotypes exist for some genes even between closely related Northern European groups. Multiple variants of any age would be expected to occur on different haplotypes with or without recombination.

Furthermore, there is no reason to expect that a single variant will be responsible for all risk in a particular gene: multiple mutations are already well documented in the Mendelian cystic fibrosis gene, for example, and multiple variants in a gene may also cause liability to complex traits. If multiple liability variants of any age do exist, population genetic effects and stochastic sampling variation would then determine which variants a particular sample could detect. The complex patterns of association also provide the basis for specific hypotheses about variants that might be shared across multiple backgrounds. We have chosen

DTNBP1 as an example, as this locus has the largest number of positive replications, and therefore the most data to assess.

Patterns of association in *DTNBP1*

The initial report of association between schizophrenia and *DTNBP1* in 270 multiply affected Irish families included 12 SNPs within the gene, of which six showed some degree of nominally significant association ($0.00004 < P < 0.0466$).³⁴ Subsequent reanalysis of this data set showed that a set of eight of these markers (p1792–p1635) satisfied criteria for a haplotype block. The eight markers cover 30.1 kb from intron 1 to intron 4 and 6 common haplotypes (frequency > 1%) define 94.7% of haplotypes observed (Table 1). The six common haplotypes fit a stable clade (Figure 2) with only low-frequency haplotype 6 apparently arising from historical recombination based on alleles at p1325 and p1635.³⁵ Although there is some variation in the haplotype complement observed across different samples, the pattern of specific haplotypes and their relative frequencies are generally maintained.

Results from the original study and eight published positive replications are shown in Table 2. The replications include 13 independent (two family, three triad, and eight case/control) samples and use different but overlapping subsets of the markers from the original study. In our original haplotype analyses, the association in the Irish family sample is limited to haplotype 2 in Table 1 and

Table 1 *DTNBP1* haplotypes reconstructed in the Irish family sample

Haplotype	p1792 rs1474605	p1578 rs1018381	p1763 rs2619522	p1320 rs760761	p1757 rs2005976	p1765 rs2619528	p1325 rs1011313	p1635 rs3213207	Frequency
1	1	1	1	1	1	1	1	1	0.733
2	2	1	2	2	2	2	1	2	0.058
3	1	1	1	1	1	1	2	1	0.071
4	1	1	1	2	1	1	1	1	0.010
5	2	2	2	2	2	2	1	1	0.060
6	2	1	1	2	1	1	2	2	0.015
Rest									0.053

Haplotypes 1, 2 and 5 (in bold) show evidence of association in different samples.

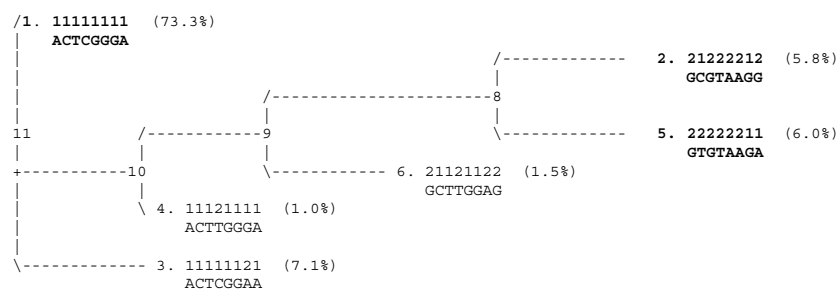


Figure 2 The clade produced from *DTNBP1* haplotypes in Table 1.

Table 2 Specific *DTNBP1* alleles and haplotypes associated with schizophrenia across studies

Study	1	2	3	4	5	6	7	8	9	10	Reference
Irish	-	-	G	T	A	A	-	G	-	-	Straub <i>et al</i> , 2002 ³⁴
German	-	-	G	C	G	T	A	A	G	G	Van den Oord <i>et al</i> , 2003 ³⁵
Swedish	5	-	T	-	T	A	-	G	A	-	Schwab <i>et al</i> , 2002 ³⁶
Polish	-	-	-	-	-	-	-	-	-	-	Van den Bogaert <i>et al</i> , 2003 ³⁸
Chinese	1	A ^a	C	T	-	-	-	G	-	T ^a	Tang <i>et al</i> , 2003 ³⁷
UK	1	A	-	-	-	-	-	-	-	-	Williams <i>et al</i> , 2004 ⁴²
Irish	1	A	-	-	-	-	-	A	C	-	
Bulgarian	-	-	-	G	-	-	A	-	-	-	Kirov <i>et al</i> , 2004 ⁴⁰
US (C)	-	-	T	G	-	A	-	-	-	-	Funke <i>et al</i> , 2004 ³⁹
US (H)	-	-	T	G	T	A	-	-	-	-	
Japanese	A	G ^a	T	G	T	A	G	-	-	-	Numakawa <i>et al</i> , 2004 ⁴¹
Chinese	-	-	-	-	-	G	-	A	-	-	
Scottish ^b	-	-	-	-	-	G	G	-	-	-	Li <i>et al</i> , 2005 ⁴³
	-	-	-	-	-	-	-	-	-	-	

Adapted from Funke *et al*, 2004³⁹

SNP A: rs2619538; p1583: rs909706; p1792: rs1474605; p1578: rs1018381; p1763: rs2619522; p1320: rs760761; p1757: rs2005976; p1765: rs2619528; p1325: rs1011313; p1635: rs3213207; p1655: rs2619539; p1287: rs760666; p1333: rs742105; p1328: rs742106;

All SNPs are shown as the variable base on the coding strand. Associated alleles (if reported) are shown first, and associated haplotypes are shown shaded. Haplotype identities (as in Table 1 and Figure 2) are shown as a shaded number to the left of the haplotype. Blank cells indicate that a marker was not tested in the specific sample, - indicates that a marker was tested but was not associated.

^aMarkers 1583 and 1328 were not included in the haplotype reconstruction in Figure 2, so the allele distribution on the Irish risk haplotype is unknown.

^bThe 'C' allele for marker 1320 in the results from the Scottish sample was not observed in conjunction with the 'A' allele at 1757 in the haplotype reconstruction in Figure 2, nor in subsequent sequencing in the Irish family sample. The haplotype associated in the Scottish sample is not consistent with any observed haplotype from Figure 2, but is consistent with a recombinant version of haplotype 2 or 5.

Figure 2.³⁵ Two other haplotypes (1 and 5 in Table 1 and Figure 2) show evidence for association in different samples. Significance levels in the following summaries are haplotype-specific unless otherwise noted.

Three (and possibly four) studies observe association with the common haplotype 1. A study of a German, Israeli and Hungarian sample (78 sib-pair families and 125 triads, six shared markers) observed allelic association with five of the markers tested and numerous associated haplotypes (depending on the number of markers included). These were always composed of the common alleles and thus consistent only with haplotype 1 in Table 1 and Figure 2.³⁶

The most significant single haplotype (shown in Table 2) was composed of the common alleles of p1320 (rs760761), p1765 (rs2619528) and p1325 (rs1011313), $P=0.00002$. This study includes the most shared markers with the original study, and thus is the most useful for assessing the stability of the haplotype picture shown in Table 1 and Figure 2. Comparative haplotype reconstructions from the Irish and German/Israeli/Hungarian sample are shown in Table 3.

Five of the six common Irish haplotypes are present in both samples at comparable frequencies. One low-frequency haplotype from the Irish sample and two from

the German/Israeli/Hungarian sample are not observed in the other group at frequencies above our 1% cutoff. Assessment of the allele patterns in Table 1 and Figure 2 shows that all three of these are inconsistent with origins from SNP mutation alone, and thus reflect historical recombination events. As noted in Table 3, haplotype 8 may represent the result of the first of the two events giving rise to haplotype 6. The second of these events and that giving rise to haplotype 9 appear to be sample or population-specific recombinations.

The first study of a Han Chinese sample (233 triads, five shared markers) observed association with a haplotype containing the minor allele at p1583 (rs909706), the common alleles of p1578 (rs1018381) and p1763 (rs2619522) and the G allele (common in the Han sample) at p1655 (rs2619539), $P=0.01$.³⁷ Considering only p1578 (rs1018381) and p1763 (rs2619522), which appear in the data in Table 1 and Figure 2, this pattern is consistent with any of haplotypes 1, 3, 4 or 6. However, two additional pieces of information suggest strongly that the principal

Table 3 Haplotype comparisons between the eight marker Irish family and German/Israeli/Hungarian family and trio sample

Haplotypes (numbered as in Figure 2)								
1	2	3	4	5	6 ^a	Not seen	Not seen	
1	2	1	1	2	2			
1	1	1	1	2	1			
1	2	1	1	2	1			
1	2	1	2	2	2			
1	2	1	1	2	1			
1	2	1	1	2	1			
1	1	2	1	1	2			
1	2	1	1	1	2			
0.733	0.058	0.071	0.010	0.06	0.015			
1	2	3	4	5	Not seen	8 ^a	9 ^a	
1	1	1	1	2		1	1	
1	2	1	1	2		1	2	
1	2	1	2	2		2	1	
1	2	1	1	2		1	2	
1	1	2	1	1		2	1	
1	2	1	1	1		1	2	
0.64	0.12	0.09	0.02	0.06		0.01	0.02	

Numbering is as shown in Figure 2. Haplotype 7 (not shown) is a low-frequency intermediate between haplotypes 2 and 5 in the Irish sample.

^aHaplotypes marked (6 in the Irish sample and 8 and 9 in the German/Israeli/Hungarian sample) reflect obligate historical recombination events. Note that Irish haplotype 6 is the product of two obligate recombinations. German haplotype 8 results from one obligate recombination consistent with the earlier of the two giving rise to Irish haplotype 6, and so may represent the ancestor of Irish haplotype 6.

contributor to the observed association is haplotype 1. First, our sequence data show that both major and minor alleles of p1583 (rs909706) and p1655 (rs2619539) are present on multiple haplotypes (including haplotype 1), consistent with recombination since the SNPs arose and our observed block structure which excludes them. Second, the associated haplotype is the most common observed in the Han sample. P1328 (rs742106) was also included in the analysis and the minor allele was present on the associated haplotype. In our data, P1328 (rs742106) shows almost no LD with the more 5' SNPs, so it does not assist in relating the identity of the associated haplotype to those in Table 1 and Figure 2.

Independent studies of samples of 708/711 UK cases/controls⁴² and 219/231 Irish case/controls⁸⁸ observed no evidence for association with any of the markers defining the haplotype associated with the Irish family sample. However, when these samples were analyzed together for a different set of SNPs in *DTNBP1*, association with the same haplotype was observed in both.⁴² The associated haplotype is composed of common alleles of SNP A (rs2619538), p1635 (rs3213207) and p1655 (rs2619539), $P=0.000056$. Both major and minor alleles of SNP A (rs2619538) also occur on the most common background, and this associated haplotype is only consistent with a subgroup (defined by the alleles at SNP A (rs2619538) and p1655 (rs2619539)) of haplotype 1 in Table 1 and Figure 2. This may explain why the UK/Irish sample did not detect association with haplotype 1 using the original marker set.

Some important features of the data from these two studies must be noted. Based on the Irish haplotype structure, these last two studies appear to detect association on completely nonoverlapping subgroups of haplotype 1 in Table 1 and Figure 2 defined by the allele at p1655 (rs2619539). However, the sample populations are very different, and we cannot assume that the haplotype structures or their frequencies are the same in Han Chinese as in Europeans. For example, the associated alleles of p1655 (rs2619539) reported at this marker in the studies of Tang *et al*³⁷ and Williams *et al*⁴² are different, but are the common alleles in both samples. The allele frequencies are very close in European samples ($C\sim 0.52$, $G\sim 0.48$) but appear to be inverted in the Han sample data ($G>0.63$). Critically, both studies support the conclusion of the German study that a liability variant may be present on the oldest, highest frequency background haplotype. The implications of this are discussed further below.

A study of a Bulgarian sample (488 trios, four overlapping markers) identifies association with the common alleles of p1757 (rs2005976) and p1635 (rs3213207).⁴⁰ Numerous haplotypes gave evidence of association, and the authors were unable to identify a specific risk haplotype in this sample. The combination of these two

common alleles is consistent with haplotypes 1, 3 or 4 in Table 1 and Figure 2. Although ambiguous, it seems likely that this sample also provides evidence for association of the common haplotype 1.

Two further replication studies identify association with haplotype 5 in Table 1 and Figure 2. These consist of four independent samples (142/272 Swedish, 418/285 German and 294/113 Polish cases/controls, five shared markers³⁸) and 524/573 US cases/controls of various ethnicities (258/467 Caucasian, 215/74 African-American and 51/32 Hispanic cases/controls, six shared markers³⁹). The Swedish, US Caucasian and US Hispanic samples unambiguously detect association with haplotype 5 in Table 1 and Figure 2. The African-American, German and Polish samples show no evidence for association with any *DTNBP1* haplotype.

One replication study identifies association with the same risk haplotype observed in the Irish family sample.⁴¹ This study of 670/588 Japanese cases/controls gave nominal evidence of association with several single markers tested, particularly the minor allele of p1635 (rs3213207). This allele tags the Irish risk haplotype, and is only observed on that specific background. Other associated alleles and haplotypes are all consistent with the haplotype associated in the Irish family sample. Haplotypes of various lengths from two to six markers were assessed, and many produced significant evidence of association. Case and control frequencies for the associated haplotype vary only slightly depending on the number of SNPs included, with an average value of 0.01 in controls and 0.03 in cases. The maximum observed case-control difference is for the two-marker haplotype of p1635 (rs3213207) and p1325 (rs1011313), $P=0.00028$.

Finally, one further replication study in two independent samples (638 Han Chinese families and 580/620 Scottish cases/controls, six shared markers) also detects evidence for association.⁴³ The Chinese sample shows individual marker association with p1765 (rs2619528), $P=0.002$, and p1635 (rs3213207), $P=0.02$. No individual markers were associated in the Scottish sample. Only haplotypes of two markers were analyzed in this study. In the Chinese sample, the associated haplotype is composed of the common alleles of p1757 (rs2005976) and p1765 (rs2619528), $P=0.00005$. In the Scottish sample, a haplotype composed of the common allele at p1320 (rs760761) and the rare allele at p1757 (rs2005976) is associated, $P=0.0006$. The reporting of only two-marker haplotypes and lack of marker overlap with the other study of Han samples makes interpretation ambiguous, but the associated haplotype is again the most frequent, so seems likely to support the previous Han Chinese results, and to represent haplotype 1. The haplotype associated in the Scottish sample is not observed in Table 1 and Figure 2. One additional study observed no evidence for association in any of three separate samples tested.⁸⁹

In summary, association between schizophrenia and *DTNBP1* has been replicated in seven of eight studies (excluding the negative study of the Irish case/control sample) or in 10 of 15 independent samples (the US sample described in Funke *et al*³⁹ was analyzed separately by Caucasian, Hispanic and African-American ethnicity, but was collected, and is treated here, as a single independent sample).

Two very different hypotheses can be advanced given the data outlined above. First, and perhaps most intuitive, these results may reflect the presence of more than one liability variant in the *DTNBP1* gene. As discussed above, there is no biological reason why this may not be the case for at least some genes in complex traits. The evolutionary distance between haplotype 1 and haplotypes 2 and 5 argues in favor of this idea. It remains unclear whether the same or different variants might be responsible for the associations observed on haplotypes 2 and 5; the close evolutionary relationship between them would argue in favor of a variant common to both. However, it would also suggest that these two haplotypes (and markers common to both) should be associated to the same degree in the samples where one or other is associated, and this has not generally been reported. More recent conditional analyses of the Irish families, excluding those transmitting haplotype 2 to affected offspring, suggest that there may be some residual association with haplotype 5 not detected in our original assessment. Interpretation of this is complicated by the fact that, in our sample, both the sequence data and the LD pattern show this haplotype to be the product of ancestral recombination. In contrast, the HAPMAP data in this region show no such recombination. Until further markers are analyzed, it remains unclear whether the reported associations are on the recombinant or non-recombinant version of this haplotype. Our sequence study of haplotype 2 identified numerous polymorphisms specific to or enriched on our associated haplotype. One coding variant enriched on this background is not associated with schizophrenia. A number of variants occur in evolutionarily conserved regions, and are being followed up currently.

However, a second hypothesis is that there may be variation common to the different associated backgrounds. We observe a number of such variants (with alleles shared by haplotypes 1, 2 and 5) in our sequence data, so their existence is not in question. They are somewhat less intuitive as putative liability factors for disease, however. We tend to think of disease predisposing alleles as changes from a functional 'common' norm to a dysfunctional 'rare' variant, analogous to Mendelian mutations. The alleles shared across evolutionarily distant haplotypes are almost certainly ancestral rather than variant. This is consistent with the idea (based on reduction in fertility in patients) that predisposing alleles may be advantageous individually, outweighing the higher risk in the smaller number

of individuals carrying multiple such alleles. As expected, they tend to have higher minor allele frequencies and evidence for substantial recombination (suggesting greater age); p1655 (rs2619539) is one such marker. A number of these variants also occur in evolutionarily conserved regions, and are also being followed up currently.

Conclusions

Certainly the most important development in the last several years has been the emergence of a number of replicated linkage findings and the identification of associations within genes in these target regions. Replication is critical, but there are many reasons why a 'true' finding might not be replicated including genetic variation between populations and differences in statistical power, diagnostic methods and statistical approaches. Given the evidence of linkage replication across several groups for regions on 22q, 8p, 6p, 13q, 5q, 10p, 6q, 1q and 15q, and association replication for *DTNBP1*, *NRG1* and other genes, it seems increasingly unlikely that all of these findings represent false positives. It is difficult to conceive of an inherent bias that would produce spuriously positive results across multiple groups (especially given the wide differences between studies described above) in the same chromosomal region.

We have focused only on the molecular genetic study of schizophrenia in this review. In generalizing to other psychiatric (and other complex) phenotypes, the broad conclusions from the study of schizophrenia seem likely to hold: (1) such phenotypes are genetically influenced but not genetically determined, (2) a number of genes (which may even vary between individual family members) are likely to be involved, (3) the liability variants in these genes are generally expected to be within the range of normal human variation and to have low risk associated with them individually, (4) some of the variants may interact with others or with environmental risk factors.

An emerging number of identified positional candidate genes are, in some cases, now being replicated in independent samples. Given the major focus on this area of multiple research groups throughout the world, it is likely that within several years these or other loci might emerge as widely replicated susceptibility genes for schizophrenia. If this occurs, it will represent a true watershed event in the history of schizophrenia research. Although the step of gene-identification will itself represent a major advance, it will also represent the beginning of several new lines of research including (1) rational drug design based on knowledge of basic pathophysiology, (2) characterization of genotype-phenotype relationships based on knowledge of specific pathogenic mutations, (3) identification of environmental risk factors that interact with specific genes and (4) realistic prevention research given our ability to identify high-risk individuals.

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