

## Rapid review

## Genes for schizophrenia? Recent findings and their pathophysiological implications

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**Context** Schizophrenia is highly heritable, but the genes have remained elusive. Identifying the genes is essential if the pathogenesis and pathophysiology of schizophrenia is finally to be understood, and to give the prospect of more effective treatment.

**Starting point** H Stefansson and colleagues (*Am J Hum Genet* 2002; **71**: 877–92) showed association of the neuregulin (*NRG1*) gene with schizophrenia. Other recent papers describe six additional susceptibility genes. Replications are already being reported for some of them. The genes are biologically plausible, and may have convergent effects on glutamatergic and other synapses. We review the evidence for each gene, the possible pathogenic mechanisms, and the implications of the findings.

**Where next?** Given earlier failures to replicate apparent breakthroughs, the results should be viewed with caution. Unequivocal replications remain the top priority. The respective contributions of each gene, epistatic effects, and functional interactions between the gene products, all need investigation. Confirmation that any of the genes is a true susceptibility gene for schizophrenia could trigger the same rapid therapeutic progress as has occurred recently in Alzheimer's disease.

Schizophrenia has a heritability of around 80%.<sup>1</sup> However, the search for chromosomal loci and genes has been slow and frustrating, probably because there are multiple susceptibility genes, each of small effect, which act in conjunction with epigenetic processes and environmental factors. Research has also been hampered by practical problems, notably the lack of monogenic (Mendelian) forms and the absence of a diagnostic neuropathology or other biological marker of the syndrome(s).<sup>1</sup> Nevertheless, replicated linkages to several chromosomal regions are accumulating. Two meta-analyses highlight loci at 8p and 22q, as well as at 2, 3p, 5q, 6p, 11q, 13q, and 20p.<sup>2,3</sup> Several papers published in the past few months describe seven genes which may warrant the title “schizophrenia genes”. One commentator has called the progress “truly a landmark event in the history of psychiatry”.<sup>4</sup> Certainly, the evidence for the genes is, at first sight, statistically robust, and also neurobiologically plausible, with all the genes involved in processes implicated in the pathogenesis of the disorder.

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### Recent studies

Most of the studies (table) focused on chromosomal regions implicated by earlier data, and used various methods to refine the region of linkage, identify single nucleotide polymorphisms (SNPs) within the area, find the SNPs associated with schizophrenia, and identify the candidate gene(s) containing the associated SNPs and haplotype (a combination of SNPs).

The Icelandic deCODE Genetics group first did a genome-wide scan which replicated findings of linkage of schizophrenia to chromosome 8p. They then identified several markers in the neuregulin 1 (*NRG1*) gene making up a core haplotype that showed a significant association (relative risk 2.1) with schizophrenia.<sup>5</sup> An almost identical pattern was found in a Scottish population.<sup>6</sup>

Straub and colleagues<sup>7</sup> picked an area within the broad 6p region linked in Irish families and used family-based association analysis of SNPs and haplotype analyses to identify the dysbindin (*DTNBP1*) gene. Highly significant associations were found for SNPs in introns 4, 5, and 6. A German study<sup>8</sup> replicated the association with one of the SNPs, and with a haplotype consisting of the three SNPs. However, the studies differed as to whether it was the rare<sup>5</sup> or the common alleles of each marker which were being preferentially transmitted.

Chumakov and colleagues<sup>9</sup> constructed an SNP map of the 13q22–34 region and identified several associations which implicated *G72*, a novel primate-specific gene, for which both SNPs and haplotypes were associated with schizophrenia. The yeast two-hybrid system was then used to identify a protein, D-aminoacid oxidase (*DAAO*), which interacted with *G72*. *DAAO* was itself associated with schizophrenia, and combinations of *G72/DAAO* genotypes had a synergistic effect on disease risk.

The study of the regulator of G-protein signalling-4 (*RGS4*)<sup>10</sup> was prompted by a microarray finding of decreased *RGS4* expression in schizophrenia,<sup>11</sup> and by the location of *RGS4* at chromosome 1q21–22.<sup>12</sup> 26 SNPs spanning *RGS4* were identified, and several in the upstream sequence and first intron were associated with schizophrenia. Evidence for association was seen in three family samples, although the pattern varied between them.<sup>10</sup>

Liu and colleagues<sup>13</sup> reported a complex pattern of associations between SNPs in the proline dehydrogenase (*PRODH*) gene and schizophrenia. The gene was studied because it is located on chromosome 22q11 (this region is deleted in velocardiofacial syndrome [VCFS], in which there is a high rate of psychosis<sup>14</sup>) and mice with inactivated *PRODH* have abnormalities of sensorimotor gating similar to those in schizophrenia.<sup>15</sup> The *PRODH* finding has not been replicated.<sup>16</sup>

Shifman and coworkers,<sup>17</sup> extending earlier work,<sup>18</sup> found significant associations between schizophrenia and SNPs in the gene for catechol-O-methyltransferase (*COMT*), another gene within the VCFS-deleted region. *COMT* degrades catecholamines, with a particular role in cortical dopamine metabolism.<sup>19</sup> As disturbances in dopaminergic transmission have long been implicated in schizophrenia, *COMT* is a candidate gene in its own right. Interestingly

Gene	Locus	Populations studied	Prior linkage	Replication	Relevant transgenic mouse phenotype
<i>NRG1</i>	8p12-p21	Icelandic	Yes	Yes	Yes
<i>DTNBP1</i>	6p22	Irish	Yes	Yes	No
<i>G72</i>	13q34	French Canadian, Russian	Yes	Yes, within study	No
<i>DAAO</i>	12q24	French Canadian	No	No	No
<i>RGS4</i>	1q21-22	USA ×2, Indian	Yes	Yes	No
<i>COMT</i>	22q11	USA, Israel, Chinese	Yes	Yes*	Yes
<i>PRODH</i>	22q11	USA	Yes	Failed	Yes

*NRG1*=neuregulin-1, *DTNBP1*=dysbindin, *DAAO*=D-aminoacid oxidase, *RGS4*=regulator of G-protein signalling-4, *COMT*=catechol-O-methyltransferase, *PRODH*=proline dehydrogenase. \*Some case-control studies have been negative.

**Genes for schizophrenia**

one of the *COMT* SNPs, a codon 158 (Val/Met) polymorphism, affects activity of the enzyme and influences frontal lobe function<sup>18</sup> and presynaptic dopaminergic activity,<sup>20</sup> providing biological correlates of the association with schizophrenia.

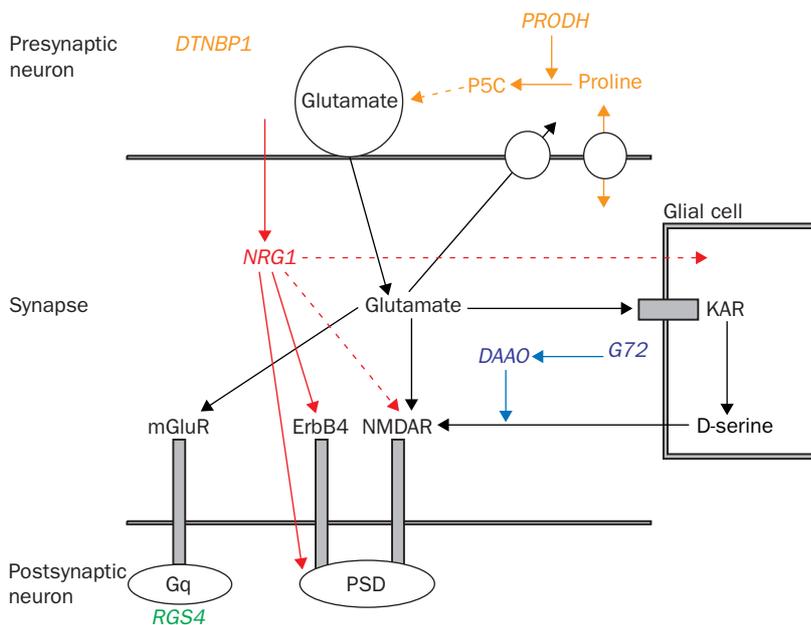
**Interpretation of genetic findings**

The genetic findings are potentially very important but should be viewed with caution. First, for each gene, more than one SNP shows association with schizophrenia, but rarely are data from individual SNPs highly significant. The findings rely for their impact on the strength of associations seen with haplotypes inferred from estimations which are extremely sensitive to even low rates of genotyping error. Second, in some cases (*DTNBP1* and *RGS4*) the associations, although well replicated, are not with the same allele or haplotype, which complicates interpretation but is not unprecedented.<sup>8</sup> Third, with the possible exception of *COMT*, none of the findings can be explained by an association between schizophrenia and variants with manifest functional consequences—ie, those which are predicted to alter the aminoacid sequence of the protein or which affect expression or splicing of the gene. Fourth, none

of the associations are of sufficient magnitude to explain fully the linkage finding which prompted each study. These observations could be accounted for if the polymorphisms are in linkage disequilibrium with pathogenic variants lying within the non-coding regions, or if disease susceptibility results not from a single variant in the gene but from the combined effect of several variants. Both of these situations can apply to complex traits.<sup>21</sup>

**Pathophysiological mechanisms**

In the prevailing pathogenic model, schizophrenia is a neurodevelopmental disorder<sup>22,23</sup> leading to abnormal synaptic connectivity.<sup>24</sup> Glutamatergic transmission via N-methyl-D-aspartate (NMDA) receptors may be especially involved.<sup>25</sup> All the genes relate to one or more of these interlinked processes. *G72* and *DAAO* impact most directly on NMDA receptors, since *DAAO* metabolises D-serine, an endogenous modulator of the receptor,<sup>26</sup> and *G72* is probably an activator of *DAAO*.<sup>9</sup> Neuregulin is present in glutamatergic synaptic vesicles, and affects NMDA receptors via actions on ErbB receptors and regulation of NMDA-receptor expression.<sup>5,27</sup> Neuregulin has roles in neurodevelopment, promoting neuronal migration and cellular differentiation; it also modulates synaptic plasticity by interacting with the postsynaptic density proteins with which ionotropic glutamate receptors are associated.<sup>27</sup> In addition, a neuregulin hypomorph mouse reproduces behavioural features relevant to schizophrenia.<sup>5</sup> *RGS4* is a negative regulator of G-protein-coupled receptors, including the metabotropic glutamate receptor,<sup>28</sup> and may have neurodevelopmental roles.<sup>29</sup> Dysbindin is localised to presynaptic terminals, and may participate in the formation and maintenance of synapses, and in signal transduction.<sup>7,30</sup> *PRODH* potentially affects glutamatergic synapses via several mechanisms.<sup>13,15</sup> Finally, *COMT* acts directly on monoaminergic neurotransmission,<sup>19</sup> and will likely affect other synaptic populations, including glutamatergic ones, via the many links between dopamine and aminoacid transmitter systems.<sup>22,31</sup>



**Schizophrenia-susceptibility genes and synaptic plasticity**

Hypothetical scenario in which genes (*italics*) may have shared effect on synapses, via influences on their formation, plasticity, or signalling properties. Only glutamatergic synapse is shown, but GABAergic, cholinergic, and monoaminergic synapses (especially relevant to *COMT*) are also likely involved. Also omitted is the issue of localisation of pathology.<sup>24</sup> Solid arrows=direct interactions, dotted arrows=indirect ones. Gq=subtype of GTP-binding proteins, KAR=kainate receptor, mGluR=metabotropic glutamate receptor, NMDAR=N-methyl-D-aspartate receptor, P5C=Δ<sup>1</sup>-pyrroline-5-carboxylate, PSD=post-synaptic density proteins.

The putative functions of the seven genes resonate with the view of schizophrenia outlined above, and implicate glutamatergic synapses as a common site of action. The figure shows a unifying scenario, which is consistent with the data on gene expression and function. Three caveats apply. First, the genes may have additional and distinct roles which prove

more relevant to their involvement in schizophrenia. Second, most genes probably influence these fundamental processes of neurodevelopment, synaptic plasticity, and glutamate neurotransmission; the model is thus weak and will be hard to disprove. Third, the evidence that these processes are abnormal in schizophrenia is incomplete, and neglects other aspects of the disease process, which range from GABAergic dysfunction to retroviruses and cerebral asymmetry. Evaluating this speculative integration of genetics and neurobiology will require sophisticated molecular neuropathological studies, including microarrays and proteomics, as well as appropriate transgenic and in-vitro characterisations of the genes, their functions, and their interactions.

## Conclusions

Salutary past experience requires that stringent criteria are applied when evaluating reports of schizophrenia susceptibility genes. The recent findings are a major step forward in the quality and quantity of evidence. The apparent commonalities and relevance of putative functions, and the fact that the findings for neuregulin, dysbindin, and RGS4 have been replicated, are impressive. However, the case for each gene remains to a greater or lesser extent incomplete, and further independent replications are essential before firm acceptance. Equally, some negative results are also likely, given the lack of power of many replication samples and the probable genetic and allelic heterogeneity of the disorder.

As the evidence for a particular gene grows, the effects of the associated SNPs on expression and function of the gene products will need to be investigated. In parallel, the search for other schizophrenia genes must continue,<sup>32</sup> using both linkage and association strategies, and paying due attention to epigenetic factors. The issue of genotype-phenotype correlations also remains to be resolved, including whether schizophrenia and bipolar disorder are genetically distinct.<sup>2,3</sup>

What would the discovery of one or more confirmed susceptibility genes for schizophrenia mean? First and foremost, it would finally provide an unambiguous clue about the pathophysiology. Even if the gene proved to be of minor effect, the biochemical pathways and molecular mechanisms which it implicated might prove relevant to the disorder in general. A precedent is given by Alzheimer's disease, for which mutations in the  $\beta$ -amyloid precursor protein are a vanishingly rare cause, but aberrant  $\beta$ -amyloid metabolism is central to the whole disease process. Alzheimer's disease also illustrates how a genetic breakthrough can lead rapidly to treatment approaches aimed at pathogenesis rather than at symptoms: the first mutation was described in 1991 and various  $\beta$ -amyloid therapeutic strategies are already at an advanced stage.<sup>33</sup> It is not unreasonable to hope that a similar timescale might apply to schizophrenia.

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