The **G72/G30** Gene Locus in Psychiatric Disorders: A Challenge to Diagnostic Boundaries?

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In follow-up from evidence obtained in linkage studies, systematic linkage disequilibrium mapping within chromosomal region 13q33 has led to the identification of a schizophrenia susceptibility locus which harbors the genes **G72** and **G30**. These association findings have been replicated in several independent schizophrenia samples. Association has also been found between genetic variants at the **G72/G30** locus and bipolar affective disorder (BPAD), with replication in independent studies. Results from studies of more detailed psychiatric phenotypes show that association exists with symptom clusters that are common to several disorders as well as with specific psychiatric diagnoses. These findings may indicate that the association lies not with the diagnostic categories per se but with more specific aspects of the phenotype, such as affective symptoms and cognitive effects, which cross traditional psychiatric diagnostic boundaries. At the molecular level, the picture remains far from clear. No putative functional variants have been identified in the coding regions of **G72** or **G30**, and it is therefore likely that disease susceptibility is caused by as yet unidentified variants which alter gene expression or splicing. A further complication is the fact that inconsistencies are evident in the risk alleles and haplotypes observed to be associated across different samples and studies, which may suggest the presence of multiple susceptibility variants at this locus. Functional analyses indicate that the **G72** gene product plays a role in the activation of N-methyl-D-aspartate receptors, a molecular pathway implicated in both schizophrenia and BPAD, making it the most plausible candidate gene at this locus.

**Key words:** DAOA/DAAO/DAO/schizophrenia/bipolar affective disorder/panic disorder/psychiatric disorders/13q/association

**Introduction**

**G72** (MIM 607408, also termed DAOA or D-serine amino acid oxidase activator) and **G30** (MIM 607415) are located on chromosome 13q33, a region demonstrating strong evidence for linkage with various neuropsychiatric disorders. Results from several association studies indicate that **G72/G30** plays a role in the etiology of schizophrenia and bipolar affective disorder (BPAD). In this review, we present the current state of linkage and association findings for the **G72/G30** locus and provide an overview of the possible function of both genes. Finally, we discuss the uncertainties and difficulties involved in evaluating the role of these genes in schizophrenia and BPAD.

**Linkage Findings at the **G72/G30** Locus**

Several studies report evidence for linkage between genetic markers on chromosome 13 and schizophrenia,¹⁻⁹ BPAD,¹⁰⁻²⁰ and other psychiatric phenotypes.²¹⁻²⁶ There are at least 2 regions on chromosome 13 (13q12 and 13q31-q33) for which there is accumulating evidence for linkage (see figure 1). The most widely reported evidence for linkage in schizophrenia and BPAD is for 13q31-q33. Further evidence for linkage at 13q31-q33 has been obtained from a joint analysis of 18 genome-wide studies in schizophrenia and 11 in BPAD.²¹⁻²⁶ More recently performed meta-analyses, however, have failed to find linkage on chromosome 13. Rank-based genome scan meta-analyses combining the results of 20 genome scans in schizophrenia and 18 genome-wide studies in BPAD showed no evidence for linkage on chromosome 13.²⁸⁻²⁹ The meta-analysis of McQueen et al,³⁰ which involved combined analysis of the original data from 11 BPAD genome scans, also failed to find linkage on chromosome 13. Although meta-analyses of genome-wide studies have revealed an inconsistent picture, it is nevertheless legitimate to say that the linkage evidence for

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chromosome 13 is among the most convincing across the genome.

**Association Studies at the G72/G30 Locus**

The linkage evidence obtained for chromosome 13 prompted Chumakov et al.\(^3\) to perform a systematic linkage disequilibrium (LD) mapping over a 5-Mb region on chromosome 13q33, a region for which a peak nonparametric linkage score of 4.18 was obtained in a sample of 54 affected families.\(^2\) They initially analyzed 191 single-nucleotide polymorphisms (SNPs) in a schizophrenia sample of French-Canadian descent (213 cases, 241 controls) and then used a second case-control sample of Russian descent (183 cases, 183 controls) to genotype the SNPs which had been associated in the first sample. They found significant association with disease status at the same locus in both samples (table 1). This region harbors 2 overlapping genes, G72 and G30, which are transcribed in opposite directions and which span \(\sim 29\) and \(\sim 47\) kb of genomic sequence, respectively. Because no G30 protein product was detected, Chumakov et al.\(^3\) focused on G72 and found evidence for an interaction between G72 and DAO (D-serine amino acid oxidase, MIM 124050) at the protein level using a yeast 2-hybrid screen. Following up on the hypothesis that G72 interacts with DAO, Chumakov et al.\(^3\) also found association between SNPs within DAO and the French-Canadian schizophrenia sample. When associated variants at the 2 loci were considered jointly, the estimated odds ratio of 5.02 was greater than a combination of the individual effects, a finding in support of an epistatic interaction between G72 and DAO.

The identification of G72/G30 as promising candidate genes was one of the first examples of the positional cloning approach being successfully applied to a common neuropsychiatric disorder and resulted in a wealth of replication attempts by other research groups.

**Schizophrenia**

The first replication attempt in schizophrenia was performed by Schumacher et al.\(^3\) using a case-control sample of German descent (299 patients, 300 controls). A total of 7 SNPs covering the entire G72/G30 locus were analyzed, and positive association was observed (table 1). Wang et al.\(^3\) were the first to perform an association study at the G72/G30 locus in schizophrenic patients of Asian origin. They tested 6 G72/G30 SNPs in a large case-control sample of Han Chinese origin (537 patients with schizophrenia, 538 controls) and observed significant association with disease status (table 1). Addington et al.\(^3\) genotyped 8 G72/G30 SNPs in a relatively small and ethnically diverse sample of patients with childhood-onset schizophrenia and psychosis (98 probands) and reported a significant association (table 1). Korostishevsky et al.\(^3\) tested 11 SNPs at the G72/G30 locus in a small sample.
schizophrenia sample of Ashkenazi Jewish descent (60 cases, 135 controls) and observed significant evidence for association (table 1). They also performed expression studies using the postmortem brain tissue (frontal Brodmann’ area) of 44 patients and 44 controls and found a tendency toward an overexpression of G72 in the schizophrenia brain samples. The same research group then used these 11 SNP markers to analyze a second schizophrenia sample of Palestinian origin (223 trios) and found positive association (table 1). Hall et al tested 7 SNPs at the G72/G30 locus in 2 large family samples with schizophrenia. Although they failed to detect association with individual SNPs, the results of their haplotype analysis showed the same tendency as that of Chumakov et al. Zou et al analyzed 3 SNPs in 233 trios of Han Chinese descent and found significant association with disease status (table 1). Mulle et al genotyped 11 G72/G30 SNPs in 159 trios with schizophrenia, the majority of whom were of European American descent. Association was not observed either at the single marker or at the haplotype level (table 1). In addition to a schizophrenia sample from Scotland (183 cases, 182 controls), Ma et al investigated 588 patients and 588 controls of Han Chinese descent (the third Asian sample). In total, 6 SNPs at the G72/G30 locus were genotyped, and positive association was found in both samples (table 1). Fallin et al performed an association study on 64 candidate genes for both schizophrenia and BPAD (see section below). They analyzed 323 BPAD and 274 schizophrenia trios of Ashkenazi Jewish descent. In total, 14 SNPs were tested at the G72/G30 locus, producing results that demonstrated positive association with both disorders (table 1). Although both Williams et al and Goldberg et al failed to find association between variants at the G72/G30 locus and schizophrenia in their samples, they reported a significant association with particular phenotype dimensions of the disorder (see below).

**Bipolar Affective Disorder**

Because chromosome region 13q33 has shown strong evidence for linkage in several BPAD family series, Hattori et al used 22 BPAD families of European American descent with preexisting evidence for linkage (total of 83 individuals) to genotype 16 SNPs within G72/G30. In total, 5 SNPs were associated with disease status (table 1). In order to replicate this finding, a second BPAD sample (152 families, 474 individuals) was investigated using 7 G72/G30 SNPs. Although none of the genotyped markers demonstrated association in the single-locus analysis, the haplotype analysis showed significant association. Schumacher et al investigated 300 BPAD patients and 300 controls of German descent. They analyzed 7 SNP markers at G72/G30 and found significant association. Chen et al analyzed a small BPAD sample of European American descent (139 patients, 113 controls) using 6 SNPs at the G72/G30 locus and found association with disease status (table 1). The candidate gene study of Fallin et al, which included 323 BPAD trios (see section above), also showed significant association between genetic variants at the G72/G30 and disease status. Williams et al found a weak association at the G72/G30 locus and BPAD. However, significant association was observed for a particular subsample of their BPAD cases (see below).

**Phenotype Subdimensions of Schizophrenia and BPAD**

Although BPAD and schizophrenia are conceptualized in modern classification systems as being distinct and exclusive diagnostic entities, they show great overlap at the level of individual symptoms. Because associations have been reported for both BPAD and schizophrenia, it follows that it should be determined if these associations are stronger in patients demonstrating symptoms that are common to both disorders. To test this hypothesis, Schulze et al reanalyzed the BPAD sample of Schumacher et al and observed strongest association in the subsample of patients with a history of persecutory delusions (90 cases). Schulze et al were able to replicate this finding in a sample of Polish BPAD patients. Whereas no association had been detected for the “total” Polish sample (294 cases, 311 controls) (table 1), a significant association was observed for this specific Polish subsample with a history of persecutory delusions which was stronger than that of the original bipolar finding of Schumacher et al. A further genotype-phenotype study was performed using samples of UK descent. Williams et al investigated 709 schizophrenic patients, 706 BPAD patients, and 1416 controls. Although they failed to find association with schizophrenia and the observed association with BPAD was only moderate, a strong association was found when the samples were stratified for a history of major mood disorders (subsample of 818 schizophrenia and BPAD patients). Finally, Goldberg et al, who observed no association with schizophrenia in 2 family samples of European American descent (217 families, 67 families), found association between variants at G72/G30 and those schizophrenia patients with cognitive impairment.

**Other Psychiatric Phenotypes**

Chromosome 13q33 proved to be among the most implicated regions in one of the largest genome-wide linkage samples of panic disorder investigated to date. Schumacher et al tested 4 SNPs at the G72/G30 locus in a case-control sample of panic disorder cases of German descent (152 cases, 208 controls) and found evidence for association. The genetic effect of G72/G30 in their panic disorder patients was even stronger than the effects observed in their schizophrenia and BPAD samples (table 1), although it just failed to reach significance.
### Table 1. Summary of Published Association Studies at the G72/G30 Locus

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<td>rs1946965 (M12) (A/G)</td>
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**Notes:** Fr Canad, French Canadian; Eur Am, European American; Ashken, Ashkenazi Jewish; Palest, Palestinian; SCZ, schizophrenia; BPAD, bipolar affective disorder; PDBP, bipolar affective disorder with persecutory delusions; EOS, childhood-onset schizophrenia; PD, panic disorder; CI, cognitive impairment; MMD, major mood disorder; PSY, psychotic phenotype; SNPs, single-nucleotide polymorphisms; NS, not significant; NI, no information; ND, not determined; —, not analyzed.

<sup>a</sup>Analyzed subsamples which had been tested in previous studies are presented independently. This is the case for the German sample analyzed by Schulze et al.<sup>46</sup> and for the UK sample analyzed by Williams et al.<sup>42</sup>

<sup>b</sup>Only those SNPs which showed association in at least one sample are presented. Information is presented in the following form: for the case-control studies—*P* value of allelic analysis (associated allele, percent of the associated allele in cases/controls) and for the family based studies—*P* value of transmission disequilibrium test analysis (associated allele, transmitted, not transmitted).

<sup>c</sup>SNP ID (SNP designation used by Chumakov et al.<sup>31</sup>) (allele 1/allele 2).

<sup>d</sup>The presented *P* values are for the genotypic analysis.

<sup>e</sup>SNP designation used by Williams et al.<sup>42</sup>
Table 1. Extended

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<th>rs1935062 (A/C)</th>
<th>DAOA_3 (M16) (A/C)</th>
<th>rs947267 (M18) (A/C)</th>
<th>rs778294 (M19) (C/T)</th>
<th>rs3916970 (M20) (A/G)</th>
<th>rs3916971 (M21) (C/T)</th>
<th>rs778293 (M22) (A/G)</th>
<th>rs3918342 (M23) (C/T)</th>
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Results From Single-Marker Analyses

No evidence for a disease-specific association pattern for schizophrenia, BPAD, or any other psychiatric phenotype is apparent when results from all of the G72/G30 locus association studies are considered. We will now turn to discussion of G72/G30 association results obtained from single-marker and haplotype analyses that were conducted independently of the investigated phenotypes.

A total of 16 different G72/G30 SNPs have yielded significant association results at the single-marker level in at least one sample. Of these markers, 9 SNPs have shown evidence for association in more than one sample (table 1). SNP rs2391191 (M15), the most frequently tested G72 marker, showed association in 6 of 24 samples analyzed, while rs3918342 (M23) showed association in the largest number of studies (8 of 19 analyzed samples).

Attempts to identify an association pattern are complicated by the fact that different studies show opposite alleles to be associated (table 1). Of these markers, 9 SNPs have shown evidence for association in more than one sample (table 1). SNP rs2391191 (M15), the most frequently tested G72 marker, showed association in 6 of 24 samples analyzed, while rs3918342 (M23) showed association in the largest number of studies (8 of 19 analyzed samples).

Results From Haplotype Analyses

Evaluation of the G72/G30 locus-specific LD using HapMap data from individuals of European (CEU) and Asian origin (CHB) (release No. 20/phaseII Jan06, National Center for Biotechnology Information Build 35) reveals a distinctive LD pattern. Application of Haploview (version 3.248) to SNPs with a minor allele frequency of $\geq 0.05$ that are located between rs3916965 and rs1421292 (95 kb) yielded 2 blocks of high LD covering the G72/G30 region. Only a small region of ~5.5 kb (between rs3916970 and rs778293) shows no intermarker LD (figure 2). These haplotype blocks will be termed Haploblock-I (covering the genomic region of G72) and Haploblock-II (located within the 3' untranslated region of G72) in the following discussion.

Association at the haplotype level was found in samples of European, Chinese, Ashkenazi Jewish, and Palestinian descent. In all, 2 studies performed haplotype
analyses using SNP markers beyond the haplotype block structure. Chumakov et al.31 analyzed all possible 3- marker combinations across all SNPs tested. The most significant association was observed for the marker combination rs746187-rs3916965-rs3916972. Schumacher et al.32 also analyzed all possible marker combinations for the SNP markers included in their study and found rs3916965-rs2391191-rs3918342-rs1421292 to be the best marker combination. Interestingly, these best-associated haplotypes extend beyond the boundaries of a single haplotype block. Other studies have focused on marker combinations using SNPs located within Haploblock-I or -II.

Contrasting association profiles are observed at the haplotype level in both haplotype blocks, a finding consistent with results from the single-marker analyses. In addition, there is evidence that putative risk variants are located in both haplotype blocks. These results support findings from the single-marker analyses and are consistent with the existence of more than one disease-causing variant at the G72/G30 locus.

Functional Analyses

Expression of G72 and G30

Chumakov et al.31 found that both G72 and G30 generate numerous splice variants in various parts of the human brain, spinal cord, and testis. The longest G72 open reading frame (ORF) encodes 153 amino acids and is termed LG72. All other observed G72 splice variants are considerably shorter. No significant homology was detected between the encoded protein and any confirmed or hypothetical protein. Chumakov et al.31 amplified all coding exons and flanking genomic DNA of LG72 in rhesus monkey and ape genomes. In the rhesus monkey genome, they found that most of the exons contain stop codons and that the majority of splice sites are mutated. Potential LG72 orthologs were identified in chimpanzee, gorilla, and gibbon genomes. The chimpanzee ORF is almost half the length of the human LG72. Chumakov et al.31 thus suggested that G72 represents a primate-specific gene with a rapidly changing sequence and protein structure, presumably connected with the rapid evolution of the underlying brain function.

Chumakov et al.31 also performed a coupled in vitro transcription/translation assay for both G72 and G30. A G72 in vitro translation was detected, but no G30 translation product was observed. Western blot analysis revealed 2 LG72 protein products of ~24 kDa and ~13.5 kDa, whereas a 18-kDa protein product, which had been anticipated on the basis of the RACE experiments, was not detected. Proteolytic cleavage of the G72 gene product and/or a specific secondary structure was proposed as a possible explanation for this.

Hattori et al.44 used Rapid Amplification of cDNA Ends (RACE) experiments on human testis tissue to analyze the expression of G72 and G30.44 They found only low levels of G72 expression, and no G30 transcription product was detectable. Complex additional G72 splice variants were observed which had not been described in the study by Chumakov et al.31 No attempts to amplify G72 transcripts in the human brain through use of RACE experiments have been successful: only polymerase chain reaction (PCR) of brain cDNA leads to the amplification of G72 products. Korostishevsky et al.35 were able to amplify G72 mRNA from human brain tissue. They analyzed postmortem brain samples and found an overexpression of G72 in schizophrenic patients. However, they did not use exon-spanning PCR primers and the possibility that genomic DNA contamination occurred therefore cannot be excluded.

Function of G72 and G30

Until now, only Chumakov et al.31 have analyzed the gene function of G72 using the corresponding in vitro translated protein of LG72, termed pLG72. They were able to localize the 24-kDa pLG72 protein at the cellular level in the Golgi complex. They showed that pLG72 forms multimers and binds to β-D-galactopyranoside residues. They performed yeast 2-hybrid experiments and screened clones from a human brain cDNA library in order to identify interacting proteins. They found the enzyme D-amino acid oxidase (DAAO) to be an interaction partner of pLG72. DAAO is of interest as a candidate in psychiatric disorders because it oxidizes D-serine, an allosteric activator of N-methyl-D-aspartate (NMDA) receptors in the synapses. Chumakov et al.31 also measured D-serine oxidation by DAAO in the presence of increasing concentrations of pLG72, a condition which stimulates DAAO enzymatic activity. These in vitro results suggest that pLG72 contributes to the regulation of NMDA type glutamate receptors in the human brain.

Because all translation assays have failed to identify a specific G30 protein product, the function of G30 remains unknown. Because G30 and G72 are partly overlapping genes and are transcribed in opposite directions, Chumakov et al.31 hypothesized that G30 transcripts could be involved in the regulation of G72 expression.

Discussion and Outlook

Existing evidence strongly supports an involvement of the G72/G30 locus in the development of schizophrenia and BPAD. This is one of the most replicated associations in the field of psychiatric genetics. Its significance is underlined by a recently published meta-analysis combining the results of various association studies at this locus. Detera-Wadleigh and McMahon49 combined P values from 10 association studies (7 samples with schizophrenia, 3 with BPAD, and 1 sample with panic disorder). When results across studies and phenotypes were combined, 8 SNPs at the G72/G30 locus showed
significant combined $P$ values (between $P < 0.0001$ and $P = 0.0413$). Joint analysis of the 7 schizophrenia samples showed 5 variants to be significantly associated with disease status ($P$ values between $P = 0.0002$ and $P = 0.0033$).

In the case of the BPAD samples, 3 markers showed significant association in the combined analyses ($P$ values between $P = 0.0019$ and $P = 0.0309$). Although this combined analysis of $P$ values did not take into account the fact that opposite alleles were associated in different studies and a general publication bias toward positive results has to be assumed, this meta-analysis supports the likelihood of significant association of SNPs at the G72/G30 locus in both schizophrenia and BPAD.

The observed overlap in association findings at the G72/G30 locus for schizophrenia and BPAD raises the possibility that there is no clear distinction between the 2 disorders at the biological level. It seems more likely that genetic variation at this locus contributes to a susceptibility to disease that crosses traditionally accepted psychiatric diagnostic boundaries. This hypothesis is supported by overlapping findings for 2 other candidate genes in the 2 disorders, namely NRG1 (MIM 142445) and DISC1 (MIM 605210), and by other genetic studies that have identified chromosomal regions with convergent linkage evidence for both schizophrenia and BPAD (eg, on chromosome 6q and 22q). Data from several family and twin studies offer further support for this concept of the non-independence of schizophrenia and BPAD.

In all, 3 studies on G72/G30 association data using a systematic genotype-phenotype approach have been performed to date. Results from all studies suggest that genetic variation at this locus is likely to influence susceptibility to symptoms such as affective and cognitive changes that are common to various psychiatric diagnoses. Although intriguing, this hypothesis should be treated with caution, especially given that no disease causing G72/G30 variant has so far been identified. It may prove to be that the G72/G30 locus harbors several disease-causing variants, each of them influencing a different aspect of psychopathology.

Although identification of the true causative variants at the G72/G30 locus will provide a pathway to an understanding of pathophysiology and psychopathology, the mechanism by which this locus contributes to the phenotype appears to be more complex. The observed inconsistencies between studies in associated alleles and haplotypes might suggest the presence of multiple susceptibility (and perhaps protective) variants at the G72/G30 locus. The concept of allelic heterogeneity at this locus is further supported by the fact that haplotypes in different blocks (Haploblock-I and -II) have been found to be associated. At present, the only associated nonsynonymous variant, rs2391191, does not appear to be causative because it is not the most strongly associated variant and is not associated with disease status across all studies. Because no other functional variant at the G72/G30 locus has been identified as being associated with disease, it is likely that the putative mutations at this locus influence gene regulation or splicing rather than the protein product. This poses a challenge in identifying the susceptibility alleles within the G72/G30 region, because sequence variations in regulatory regions will be more difficult to interpret, especially if there are many different mutations in the patient population. Although intriguing evidence exists that G72 and/or G30 contribute to disease susceptibility, it remains possible that it is another gene within this region that is the underlying risk factor, influenced by sequence variation at the G72/G30 locus.

The function of G72 still awaits clarification. Although the 2-hybrid system is an effective method of identifying interacting proteins, other methods such as immunoprecipitation are needed to confirm the results. The fact that the protein interacting with pLG72, DAAO, is located in the peroxisomes has already been described. Chumakov et al located pLG72 in the Golgi complex. Further analyses are therefore needed to clarify the true location of pLG72. In addition, application of methods such as animal models, which are superordinate to individual pathways, are of major importance in clarifying pathophysiological mechanisms and the phenotype. Use of animal models in investigations of G72/G30 may prove problematic, however, because the gene does not exist in rodents.

In conclusion, accumulating evidence supports the involvement of the G72/G30 locus in the development of schizophrenia and BPAD. Research has yet to identify the susceptibility (and protective) variants within this locus. This breakthrough, in addition to further functional analyses, will facilitate the understanding of underlying biochemical pathways as well as the development of more specific therapies. Current evidence suggests that the susceptibility variants influence G72, and the subsequent effects on NMDA neurotransmission seem to be implicated in both schizophrenia and BPAD. The identification of susceptibility variants at this locus will provide insights into the interaction of the various etiological factors and will have an important impact on disease classification. All preliminary results from investigation of genotype-phenotype correlations indicate that the G72/G30 locus has the potential for changing our concept of psychiatric nosology.

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