Genetic Vulnerability to Psychosis and Cortical Function:
Epistatic Effects between DAAO and G72

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Abstract
Recent studies have described G72 and DAAO as susceptibility genes for schizophrenia and bipolar disorder. Both genes modulate glutamate neurotransmission, which plays a key role in neurocognitive function and is thought to be altered in these disorders. Moreover, in vitro transcription studies indicate that the two genes interact with each other at the molecular level. However, it is unclear how these genes affect cortical function and whether their effects interact with each other. The aim of this study was therefore to examine the impact of G72 rs746187 and DAAO rs2111902 genotypes on brain function in schizophrenia, bipolar disorder and healthy volunteers. We used functional magnetic resonance imaging and an overt verbal fluency paradigm to examine brain function in a total of 120 subjects comprising 40 patients with schizophrenia, 33 patients with bipolar I disorder and 47 healthy volunteers. A significant 3 way interaction between G72, DAAO and diagnosis was detected in the right middle temporal gyrus (x=60 y=-12 z=-12; z-score: 5.32; p<0.001 after family-wise error correction), accounting for 8.5% of the individual variance in activation. These data suggest that there is a non-additive interaction between the effects of variations in the genes implicated in glutamate regulation that affects cortical function. Also, the nature of this interaction is different in patients and healthy controls, providing support for altered glutamate function in psychosis. Future studies could explore the effects of DAAO and G72 in individuals with prodromal symptoms of psychosis, in order to elucidate glutamate dysfunction in this critical phase of the disorder.
Introduction

Inheritance accounts for vulnerability to psychosis by 40% to 70% [1, 2]. The effects of risk genes may be expressed in the brain at molecular and macroscopic level even when they are not evident at behavioural level. The combination of molecular genetics and neuroimaging (“imaging genetics”) allows the investigation of the impact of genetic polymorphisms of interest on brain structure and function, with the aim of understanding the neurobiological basis of vulnerability to psychosis. Several recent linkage and association studies have identified the brain-expressed genes for G72 and D-Amino Acid Oxidase (DAAO), located in chromosomal regions 13q32-33 and 12q24 respectively, as probable susceptibility genes for schizophrenia and bipolar disorder [3-10]. The product of G72 is an activator of DAAO, which is the only enzyme that oxidises D-serine, a co-agonist for the NMDA glutamate receptor [11]. Glutamate is the most abundant excitatory neurotransmitter in the human brain and glutamate neurotransmission plays a key role in neurocognitive function [12]. In healthy volunteers, the administration of glutamatergic antagonists results in impaired performance on tests of verbal [13], and nonverbal [14] declarative memory, verbal fluency and problem solving [15]. There is also a growing body of evidence to suggest that glutamate neurotransmission is altered in psychosis [11, 16-18]. For instance, glutamate function is perturbed in people with prodromal signs of psychosis, and glutamatergic dysfunction is associated with a reduction in gray matter volume in brain regions thought to be critical to the pathogenesis of the disorder [17]. It has been proposed that hypofunction of glutamate in cortico-striatal projections may lead to the changes in striatal dopamine concentration which are thought to underlie the emergence of psychotic symptoms [19]. Consistent with this hypothesis, NMDA receptor antagonists, which are potent activators of dopamine release, can cause psychotic symptoms in healthy participants and exacerbate psychotic symptoms in patients [20].

To date, the impact of the glutamate-regulating G72 and DAAO genes on neurocognitive function have been assessed independently. Three studies have found evidence that the G72 gene moderates neuronal responses in the medial temporal cortex during verbal working memory [21], working memory [22], episodic memory [23], and semantic memory [24] tasks. Moreover, Prata and colleagues [25] demonstrated that a variation in G72 genotype modulates the activation of the left postcentral and supramarginal gyri during a verbal fluency task in a group of healthy participants. The DAAO gene has been associated with individual differences in spatial working memory in young male military
conscripts [26] but did not appear to have a significant impact on cognitive function in a different study which examined healthy controls, schizophrenic patients and their unaffected siblings [23]. We recently demonstrated that a genetic variation in DAAO is associated with differences in regional activation and functional connectivity during a verbal fluency task in schizophrenia patients compared to healthy participants, suggesting a diagnosis-dependent pattern of gene action [27].

Chumakov and colleagues [3] presented evidence of epistatic interaction between variants at the G72 and DAAO loci in schizophrenia susceptibility using French-Canadian and Russian samples; the authors suggested that variation at the G72 and DAAO loci might influence efficiency of glutamate gating of the NMDA ion channel contributing to schizophrenia susceptibility. However, two subsequent studies have failed to replicate this epistatic effect [4, 28]. More recently, Corvin and colleagues [7] found evidence for epistasis between the G72 and DAAO markers most strongly associated with schizophrenia in an Irish sample, although these did not correspond to the single nucleotide polymorphisms (SNPs) reported in the original article by Chumakov and colleagues [3]. The existence of altered glutamate neurotransmission in psychosis suggests that any epistatic effects between the G72 and DAAO loci might differ in psychotic patients compared with healthy volunteers.

To date, no neuroimaging studies have investigated epistasis between the G72 and DAAO loci, or the extent to which this may be altered in psychosis. We therefore examined (i) the existence of a non-additive interaction between the G72 and DAAO genes on brain activation during a verbal fluency task, and (ii) the extent to which this interaction is modified in schizophrenia and bipolar disorder. We used functional Magnetic Resonance Imaging (fMRI) to study healthy volunteers and patients with schizophrenia or bipolar disorder, with genotype subgroups of sufficient size to detect interactive effects of G72 and DAAO on activation. Subjects were scanned while they performed an overt phonological verbal fluency task, which is associated with activation in a distributed network including prefrontal, cingulate and medial temporal regions in healthy volunteers and with impaired performance and altered activation in schizophrenia and bipolar disorder. On the basis of evidence that both G72 and DAAO regulate glutamate neurotransmission, which plays a key role in neurocognitive function [21-23, 26] and the topographic distribution of altered glutamate levels in patients with psychotic disorders [29], and prodromal symptoms [16], we hypothesized that there would be an epistatic interaction
between the two genes on regional activation. In addition, in view of for the putatively altered glutamate neurotransmission in schizophrenia and bipolar disorder [5], and evidence that patients with these disorders carry other genes that alter glutamate transmission [30], we predicted that the hypothesised epistatic effects on brain function would be altered in patients relative to healthy volunteers. Finally, on the basis that patients with schizophrenia and bipolar disorder are likely to share the risk variants of several other genes, we hypothesized that epistatic effects between our polymorphisms of interest would be similar in the two groups as a result of a common genetic context.

**Method**

Some of the functional neuroimaging data examined in the present investigation have been included in previous studies which investigated brain dysfunction in psychosis or the impact of other candidate genes [25, 27, 31-34].

**Subjects.** A total of 120 subjects were investigated, including 47 healthy volunteers, 40 patients with schizophrenia and 33 patients with bipolar I disorder. All participants were native English speakers and gave written informed consent in accordance with protocols approved by the Local and Multicentre Research Ethics Committee. Healthy volunteers were recruited through local advertisement and had no family history of psychiatric illness as assessed using the FIGS (Family Interview for Genetic Studies). Patients with schizophrenia and bipolar disorder were recruited through the South London and Maudsley NHS Mental Health Trusts and met the relevant DSM-IV criteria, as determined by a detailed clinical interview augmented where necessary by a systematic review of their medical records. The mean duration of illness (defined as time since the first episode) for patients with schizophrenia was 12.22 years (SD=9.54). These patients were taking regular doses of antipsychotic medication; the mean dose in chlorpromazine equivalents was 571.25 (SD=460.47). The mean duration of illness for patients with bipolar disorder (defined as time since diagnosis) was 13.39 years (SD=11.03). Only a minority of these patients (n=9) were taking regular doses of antipsychotic medication; within this subgroup, the mean dose in chlorpromazine equivalents was 300.00 (SD= 278.66). In addition, eleven bipolar patients were taking lithium medication (mean dose:836.36 mg/day; SD:196.33). Demographic data (including age, full-scale IQ, years of education, handedness, gender and ethnicity) are summarised in Table I and described in Supplementary Material S1. Within each diagnostic group,
participants were classified based on genotyping of the SNPs rs746187 for G72 and rs2111902 for DAAO. The exact number of subjects within each genotype group and their demographic and clinical characteristics are reported in Supplementary Material S2.

**Genotyping.** Genomic DNA was extracted from blood or cheek swabs following standard methodology [35], and was resuspended in TE (Tris/EDTA) buffer (10 mM Tris/HCl, pH 7.6; 1 mM EDTA). Genotyping of G72 SNP rs746187, DAAO SNP rs2111902 and DAAO SNP rs3918346, was performed blind to status under contract by KBioscience (Herts, UK; http://www.kbioscience.co.uk/). The rs2111902 and rs3918346 SNPs were chosen for DAAO because they have been previously associated with schizophrenia and bipolar disorder studies either individually or in haplotype form [4, 7, 36, 37]. However in the present article we report the results for DAAO SNP rs2111902 only, since we did not detect any epistatic effects involving DAAO SNP rs3918346. The G72 SNP rs746187 was also chosen because it was previously associated, in haplotype and/or individual form, with schizophrenia in case-control and transmission disequilibrium test designs [3, 38, 39]. In particular this SNP was found to be associated with both schizophrenia and bipolar disorder in a case-control investigation carried out by our research group [37]. The genotyping results of our sample were in Hardy Weinberg equilibrium (p>0.05) for both DAAO SNP rs2111902 ($X^2=0.34; p=0.6291$) and G72 SNP rs746187 ($X^2=0.01; p=0.7125$).

---Table 1 around here---

**Verbal Fluency Task and Image Acquisition.** The task and image acquisition was performed as described before [40], see Supplementary Material S3 for details. In brief, during a “generation” condition, subjects were visually presented with a series of letters and required to overtly articulate a word beginning with the presented letter. This condition was contrasted with a “repetition” condition, in which subjects were presented with the word “rest” and were required to say rest out loud. The demands of the generation condition were manipulated experimentally by presenting different sets of cue letters that have previously been found to make the task relatively “easy” or “hard” [40].

**Behavioural Analysis.** The effect of task load, genotype (G72 and DAAO), diagnosis, and their interaction on the level of accuracy of verbal responses (measured by the number of incorrect responses
during scanning) were assessed by using a 3 x 2 x 2 x 2 ANOVA in SPSS (Statistical Package for Social Sciences; version 15.0), with diagnosis, G72 genotype and DAAO genotype as between-subjects factors and task load as a within-subject factor.

**Neuroimaging Analysis.** Analysis was performed with Statistical Parametric Mapping (SPM5) software ([www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)) [41], running under Matlab 6.5 (Mathworks). All inferences were made within a single statistical model, see Supplementary Material S4 for details. In order to reduce the number of experimental groups in the statistical model, we combined individuals with one or two copies of the less frequent alleles within the same group for both G72 and DAAO genotypes (see Table 2). We examined the main effects of task and diagnostic category using a standard threshold of \( p<0.05 \) after voxel-level correction for multiple comparisons across the whole brain with family-wise error rate (FWE) rate. Because we originally explored the impact of two DAAO SNPs, we examined the impact of genotype and its interaction with diagnostic category using a further Bonferroni correction resulting in a statistical threshold of \( p<0.025 \) after voxel-level FWE correction for multiple comparisons across the whole brain. To assess how much of the inter-individual variance in blood-oxygen-level-dependent activation was explained by the genetic variation, we used the \( \eta^2 \) measure of effect size in SPSS. To confirm that demographic variables (gender, ethnicity, years of education and IQ) and medication variables (dose, type and duration of antipsychotic treatment) did not bias our results, we repeated the statistical analysis modelling them as covariates of no interest and also performed a regression analysis with each medication variable as a covariate. Coordinates are reported in Montreal Neurological Institute (MNI) space.

--- Table 2 around here ---

**Results**

**Performance**

Performance data are reported in Table 1. The number of errors significantly differed as a function of diagnostic group (\( F=4.368; \text{df}=2; \ p=0.015 \)). Post hoc t-tests revealed that patients with schizophrenia made significantly more errors than healthy volunteers (\( F=11.108; \text{df}=1; \ p=0.001 \)). Patients with bipolar disorder made an intermediate number of errors and did not significantly differ (\( p>0.05 \)) from either healthy volunteers or patients with schizophrenia. The number of errors did not differ as a
function of G72 or DAAO genotype (p>0.05), irrespective of whether the 3 diagnostic groups were considered separately or in combination. As expected, there was also a significant main effect of task demand on the number of errors (F=55.049; df=1; p<0.001). Finally, there were no significant 2- or 3-way interactions.

**Main Effect of Task.** In all three diagnostic groups, word generation relative to repetition (irrespective of task difficulty or genotype) was associated with activation in a bilateral network that included the inferior and middle frontal gyri, the insula, the dorsal anterior cingulate cortex, the caudate, the thalamus, the middle and superior temporal gyri and the inferior parietal cortex. Conversely, repetition relative to word generation was associated with greater activation of the rostral anterior cingulated gyrus, precuneus and occipital cortex. These data were reported in detail in an earlier study [31].

**Main Effect of Diagnostic Group.** Patients with schizophrenia expressed greater activation relative to controls in the left angular gyrus (x=-48 y=-60 z=36 Z-score =4.7 p=0.002 after FWE correction). In this region, there was also a trend for greater activation in patients with bipolar disorder than controls (x=-48 y=-60 z=36 Z-score=2.8 p=0.062 after FWE correction). In contrast, direct comparison of patients with schizophrenia and with bipolar disorder did not reveal any significant difference. These data were reported in detail in an earlier study [31].

**Individual main effects of G72 and DAAO genotypes.** There were no regions showing a significant effect of either G72 or DAAO that was expressed consistently across the three diagnostic groups.

**Individual diagnosis-dependent effects of G72 and DAAO genotypes.**
A significant diagnosis by G72 genotype interaction was detected in the left precuneus (Figure 1). Plotting of the parameter estimates revealed that, in this region, the AA genotype was associated with greater deactivation (i.e. repetition > verbal fluency) during task performance than the AG&GG genotype in patients with schizophrenia and in patients with bipolar disorder, but not in healthy volunteers. This interaction effect, which accounted for 6% of the variance in activation, was most significant for the two patient groups combined (x=-18 y=-52 z=24 Z-score=5.50 p<0.001 after FWE correction; cluster size=133), but was also evident when the schizophrenia group (x=-14 y=-54 z=24 Z-
score=5.03 p=0.002 after FWE correction) and the bipolar group (x=-18 y=-52 z=24 Z-score=4.18 p=0.066 after FWE correction) were contrasted against the control group separately. The effect of G72 in this region did not differ between the schizophrenia and bipolar groups, even at trend level (p>0.001 uncorrected). There were no regions showing a significant diagnosis by DAAO genotype interaction.

- - - Figure 1 around here - - -

**G72 x DAAO Interaction Irrespective of Diagnostic Group.** There were no regions showing epistatic effects which were expressed consistently across all three diagnostic groups.

**G72 x DAAO x Diagnostic Group Interaction.** A significant interaction between G72, DAAO and diagnosis was detected in the right middle temporal gyrus (x=60 y=-12 z=-12; z-score=5.32; p<0.001 after FWE correction; cluster size=22 voxels). In this region, the GG&GT DAAO genotype was associated with less activation than the TT DAAO genotype in patients with bipolar disorder and patients with schizophrenia, but not in healthy volunteers; furthermore, this DAAO x diagnostic group interaction was more pronounced in individuals with the AA genotype for G72 than in those with one or two copies of the G allele, resulting in a 3 way interaction (Figure 2). This G72 x DAAO x diagnostic group interaction accounted for 8.5% of the variance in activation in this region. Although this 3 way interaction was significant when the two patient groups combined were contrasted against healthy controls, plotting of the parameter estimates suggested that it was more pronounced in the patients with bipolar disorder than in those with schizophrenia (Figure 2). Consistent with this observation, the 3 way interaction survived correction for multiple comparisons when the bipolar group alone was contrasted against healthy controls (x=60 y=-12 z=-12; z-score=4.78; p=0.006 after FWE correction), but was only expressed at an uncorrected level when the schizophrenia group alone was contrasted against healthy controls (x=60 y=-12 z=-10; z-score=3.78; p=0.001 uncorrected). However a direct comparison which contrasted the strength of the G72 x DAAO interaction in one patient group against the other was not significant, even at trend level (p>0.001 uncorrected), suggesting that the strength of the interaction between G72 and DAAO did not differ significantly between the two patient groups.
Effects of Potentially Confounding Factors on Activation. When the dose, type (first vs. second generation) and duration of antipsychotic treatment were entered into the statistical analysis as covariates of no interest, they did not change the foci of maximal significance or reduce the associated Z-scores. Furthermore, whole brain analysis indicated that the activation in the left precuneus and the right middle temporal gyrus, where significant effects of genotype were detected, was not related to either the dose, type (first vs. second generation), or the duration of antipsychotic treatment, even at a liberal statistical threshold (p<0.05 uncorrected). Likewise, the inclusion of dose of lithium medication as covariate of no interest did not affect the significance of the results and the amount of activation in the left precuneus and the right middle temporal gyrus was not related to this variable (p<0.05 uncorrected).

Discussion

Psychotic disorders are likely to result from the interaction of multiple genes, each of which has a small effect on its own [42]. Thus, looking for interactions between the effects of genes implicated in psychotic disorders may be more useful than focussing on the effects of a given gene in isolation. Similarly, because patients with these disorders are likely to carry the risk variants of several other genes, it is potentially useful to examine the effect of gene-gene interactions on brain function in patients, as well as in healthy controls, as these effects may differ as a result of altered genetic context [42]. Previous studies have implicated G72 and DAAO in the aetiology of schizophrenia and bipolar disorder. The product of G72 is thought to activate DAAO, which in turn is the only enzyme that oxidises D-serine, an important co-agonist for the NMDA glutamate receptor, which is implicated in the pathogenesis of schizophrenia [3, 42]. We therefore examined the interaction between the G72 and the DAAO polymorphisms on neurocognitive function in healthy participants and patients with schizophrenia and bipolar disorder.

We first characterized the individual diagnosis-dependent effects of G72 and DAAO genotypes separately. While we found no evidence for an interaction between DAAO genotype and diagnosis, we detected a significant G72 genotype by diagnosis interaction in the left precuneus. In this region, the G allele was associated with greater activation than the T allele in patients with schizophrenia and in
patients with bipolar disorder, but not in healthy volunteers, irrespective of DAAO genotype. The left precuneus is implicated in executive and working memory processes and is structurally and functionally altered in patients with schizophrenia and their non-psychotic relatives [43-45]. The finding of a G72 genotype by diagnosis interaction in this region indicates that this gene has a diagnosis-dependent impact on brain function. The mechanisms that lead to a different effect of G72 in controls and patients, or in different diagnostic categories, are unclear. One possibility is that the effects of variation in a given gene depend on the genetic context [42]. Patients with schizophrenia and bipolar disorder are likely to carry a number of different risk genes in addition to the gene of interest, and these may interact with the gene of interest, such that its effect is modified. Similarly, the effect of a gene may also vary with differences in environmental exposure [46], which again may differ between patients and controls. The effects of a given gene may also interact with the effects of schizophrenia and bipolar disorder on the function of the brain.

We then examined the interaction between the G72 and DAAO genes, irrespective of, and dependent on diagnostic group. There were no significant interaction effects between G72 and DAAO independent of diagnostic group. However, we detected a significant interaction between G72, DAAO and diagnosis in the right middle temporal gyrus. In this region, the G allele for DAAO was associated with less activation than the T allele for DAAO allele in patients with bipolar disorder, but not in patients with schizophrenia or healthy volunteers; critically, this interaction effect was more pronounced in individuals with the AA genotype for G72 than in those with one or two copies of the G allele. The right middle temporal gyrus is thought to play a key role in multimodal and higher sensory processing, and has also been implicated in the processing of complex, socially relevant stimuli including the human voice [47] and audiovisual speech [48]. Neuroimaging studies have provided evidence of reduced gray matter in the middle temporal gyrus of patients with bipolar disorder [49] and first episode schizophrenia [50] although there have also been inconsistencies in the results [51].

The observation of 2-way and 3-way interactions between G72, DAAO and diagnostic group is consistent with the idea that the two genes interact with each other at molecular level, as suggested by in vitro transcription [11]. As both these genes are thought to influence glutamate neurotransmission, the finding also provide indirect support for the hypothesis that glutamate dysfunction contributes to the pathophysiology of psychotic disorders [52]. The NMDA receptor is known to play an important
role in synaptic plasticity, neurodevelopment and excitotoxicity [53]. It is characterized by two distinct sub-units known as NR1 and NR2; NR1 is a binding site for co-agonists glycine and D-serine while NR2 is the agonist binding site for glutamate. NR1 must be occupied for glutamate to be able to open the channel; this therefore depends on availability of glycine and, to a greater extent, D-serine. Production and breakdown of D-serine is in part moderated by the DAAO enzyme, which in turn depends on its activator G72. Selective degradation of D-serine by the DAAO enzyme results in reduced NMDA neurotransmission. Furthermore, D-serine levels are decreased in the cerebrospinal fluid and serum of patients with schizophrenia [54], and administration of D-serine may reduce negative, positive and cognitive symptoms in schizophrenia [55]. It has therefore been proposed that increased activity of the DAAO enzyme may degrade D-serine, resulting in relative NMDA hypofunction in psychosis [11]. However, the exact mechanism by which G72 and DAAO may interact to moderate the availability of D-serine is not fully understood; furthermore it is still unclear how this mechanism becomes altered in schizophrenia and bipolar disorder [11].

There is preliminary evidence that glutamate regulation is altered not only in patients with full-blown psychosis but also in individuals with prodromal symptoms [17, 56]. It would therefore be of great interest to examine the effects of G72 and DAAO on cortical activation in individuals with prodromal signs of psychosis. The observation of effects of G72 and DAAO similar to those observed in patients who have developed full-blown psychosis, would provide support to the notion of altered glutamate regulation in the prodromal phase. Conversely the finding of effects of G72 and DAAO similar to those observed in healthy volunteers, would suggest that glutamate dysregulation may be a marker of transition to full-blown psychosis. It has also been hypothesized that glutamate hypofunction in cortico-striatal projections may lead to the changes in striatal dopamine concentration which are thought to underlie the emergence of psychotic symptoms [19]. Thus, it would interesting to examine epistatic interactions between genes implicated in glutamate and dopamine function on cortical activation in the prodromal phase of the disease, and whether these interactions are predictive of long-term clinical outcome.

It should be noted that, although a number of genetic studies have associated the G allele of G72 rs746187 and the G allele of DAAO rs2111902 with increased risk of schizophrenia and bipolar
disorder, the results have not always been consistent as to which of these alleles confers the higher risk [5-7]; this could be due to false positive results or reflect allelic heterogeneity (i.e. different alleles in the same marker being associated with disease); [57]. In the present investigation, we have therefore avoided reference to the terms low or high risk, and have referred to the alleles instead. Assuming that G72 rs746187 and DAAO rs2111902 do confer an increased risk of schizophrenia and bipolar disorder, it remains to be established whether or not the diagnosis-dependent epistatic effects identified in the present investigation lie upon the pathway between genes and clinical phenotype [58]. This is an important question, since neuroimaging endophenotypes may mediate the increased risk conferred by genes, but could also reflect gene effects which do not necessarily result in increased risk, consistent with the notion of pleiotropy [58].

The present investigation has a number of limitations. First, since all the patients with schizophrenia and some of those with bipolar disorder had been treated with antipsychotic medication, our results might have been affected by medication effects. However, within our patient samples, neither the dose, type nor duration of treatment differed between the genotype subgroups; furthermore, none of these variables was significantly correlated with brain activation in the right middle temporal gyrus, as revealed by a series of correlation analyses; finally, the modelling of these variables as covariates of no interest in the statistical analysis did not alter the peak foci of activation, or the Z scores. A second limitation of the present study is that the less frequent alleles for the two genes under investigation (i.e. G for G72 and G for DAAO) are found in a small fraction of the Caucasian population. Thus, in the present investigation, individuals with one or two copies of the less frequent alleles were combined within the same group; this means that our data cannot reveal whether the action of the risk allele on brain function is best described by a dominant or an additive model. A third limitation is that the size of some experimental groups was relatively small (Table 2). It is therefore important that the three-way interaction identified in the present study is replicated using a larger sample. The relatively small number of subjects in some experimental groups may have also limited our sensitivity and prevented us from detecting additional effects to the ones reported. A fourth limitation is that reaction times were not measured during scanning and could not be modelled in the statistical analysis; however we modelled correct and incorrect trials separately thereby minimizing the potential confounding impact of performance accuracy. A final limitation is that, within the DAAO and G72 genes, several SNPs have
been identified by genetic association, and there is no agreement as to which is the marker with the most significant effect on disease-risk; functional polymorphism with plausible causality has not been identified.

In conclusion, these data suggest that there is a non-additive interaction between the effects of variations in the genes implicated in glutamate regulation that affects cortical function. Also, the nature of this interaction is different in patients and healthy controls, providing support for altered glutamate function in psychosis. Future studies could explore the effects of DAAO and G72 in individuals with prodromal symptoms of psychosis, in order to elucidate glutamate dysfunction in this critical phase of the disorder.

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Supportive/Supplementary material

S1. Demographic data.
S2. Number of subjects, demographic and clinical characteristics within each genotype group.
S3. fMRI data acquisition and verbal fluency task.
References


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Table captions

Table 1. Degrees of freedom (df), F or $X^2$ test value and p-value are reported for comparisons between diagnostic groups that reached significance at $p<0.05$. C = controls; S = schizophrenic patients; BD = bipolar patients; SAPS = Scale for the Assessment of Positive Symptoms; SANS = Scale for the Assessment of Negative Symptoms; BDI = Beck Depression Inventory; ASRM = Altman Self-Rated Mania Scale; n.s. = not significant.

Table 2. C = controls; S = schizophrenic patients; BD = bipolar patients; n = number of subjects.

Figures legend

Figure 1. Significant G72 genotype by diagnosis interaction ($p<0.05$ after FWE correction) in the left precuneus. Parameter estimates refer to brain activation during performance of the verbal fluency task relative to baseline with negative values indicating deactivation during the task performance; error bars refer to standard error. C = controls; S = schizophrenic patients; BD = bipolar patients.

Figure 2. Significant interaction between G72, DAAO and diagnosis in the right middle temporal gyrus. Amongst individuals with the AA genotype for G72, the effect of DAAO genotype differed across diagnostic groups; however, such difference was not evident amongst individuals with one or two copies of the G allele for G72. Parameter estimates refer to the direction and size of the DAAO effect, with positive values indicating GG&GT>TT and negative values indicating TT>GG&GT; error bars refer to standard error. C = controls; S = schizophrenic patients; BD = bipolar patients.
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<td>BDI: mean (sd)</td>
<td>15.9 (12.8)</td>
<td>7.7 (6.5)</td>
<td>15.9 (12.8)</td>
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<tr>
<td>ASRM: mean (sd)</td>
<td>3.5 (2.4)</td>
<td>4.2 (2.8)</td>
<td>3.5 (2.4)</td>
</tr>
<tr>
<td>N. of errors: mean (sd)</td>
<td>9.4 (5.2)</td>
<td>9.4 (8.2)</td>
<td>14.7 (7.1)</td>
</tr>
<tr>
<td>N. of errors “easy”: mean (sd)</td>
<td>3.4 (2.7)</td>
<td>3.2 (3.9)</td>
<td>5.9 (4.0)</td>
</tr>
<tr>
<td>N. of errors “hard”: mean (sd)</td>
<td>6.0 (3.5)</td>
<td>6.2 (4.6)</td>
<td>8.8 (4.4)</td>
</tr>
</tbody>
</table>
Table 2. Number of subjects included in each experimental group after combining individuals with one or two copies of the less frequent alleles within the same group for both G72 and DAAO genotypes.

<table>
<thead>
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<th></th>
<th>C</th>
<th>S</th>
<th>BD</th>
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<tr>
<td><strong>G72 rs746187</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>AA</td>
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<tr>
<td>AG&amp;GG</td>
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<tr>
<td><strong>DAAO rs2111902</strong></td>
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<td>TG&amp;GG</td>
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<td>TG&amp;GG</td>
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<tr>
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</tbody>
</table>
Figure 2

G72 x DAAO x Diagnosis Interaction

x = 60  y = -12  z = -12

DAAO effect for AA G72  DAAO effect for AG/GG G72

C  S  BD  C  S  BD
Supplementary Material S1

There were no significant differences across the 3 diagnostic groups with respect to age, ethnicity, handedness and year of education. Full-scale IQ was assessed using the WAIS-III (Wechsler Adult Intelligence Scale-III) [1], the WAIS-R (Wechsler Adult Intelligence Scale-R) [2], the WASI-FSIQ-4 (Wechsler Abbreviated Scale of Intelligence) [3], or the Quick Test [4], a passive response picture-vocabulary test. Previous studies have shown that the WAIS-III correlates highly with both the WAIS-R (93.9%) [1] and the WASI-FSIQ-4 (92%) [3]. The Quick test has also been shown to yield comparable results to the WAIS-R (91%) [5]. The proportion of subjects assessed with each method was comparable across diagnostic as well as genotypic groups. The 3 diagnostic groups differed in terms of IQ (F=18.570; df=2; p<0.001) and male:female ratio (X² =17.060; df=2; p<0.001). Post hoc t-tests revealed that the group of healthy volunteers had a higher IQ than both groups of patients with schizophrenia and bipolar disorder and that the group of patients with schizophrenia had a higher male:female ratio than both healthy volunteers and patients with bipolar disorder; the difference in IQ is consistent with previous studies [6-8].

References


Within the control group, there were 22 subjects with the AA G72 genotype including 12, 7 and 3 with the TT, TG and GG DAAO genotype respectively; 15 subjects with the AG G72 genotype including 9, 6 and 0 with the TT, TG and GG DAAO genotype respectively; 10 subjects with the GG genotype including 6, 4 and 0 with the TT, TG and GG DAAO genotype respectively. Within the schizophrenic group, there were 17 subjects with the AA G72 genotype including 7, 7 and 3 with the TT, TG and GG DAAO genotype respectively; 20 subjects with the AG G72 genotype including 9, 9 and 2 with the TT, TG and GG DAAO genotype; 3 subjects with the GG G72 genotype including 2, 1 and 0 with the TT, TG and GG DAAO genotype. Within the bipolar group, there were 10 subjects with the AA G72 genotype including 5, 4 and 1 with the TT, TG and GG DAAO genotype respectively; 18 subjects with the AG G72 genotype including 7, 10 and 1 with the TT, TG and GG DAAO genotype; 5 subjects with the GG G72 genotype including 4, 1 and 0 with the TT, TG and GG DAAO genotype. Age, IQ, gender, ethnicity, handedness, and years of education did not differ significantly as a function of either G72 or DAAO genotype within each diagnostic group (p>0.05). Medication variables including dose of antipsychotic medication (in chlorpromazine equivalent), duration of illness and duration of medication did not differ as a function of G72 or DAAO genotype within the schizophrenia or bipolar groups (p>0.05). In the schizophrenia group, symptom profile was assessed using the Scale for the Assessment of Positive Symptoms (SAPS) and the Scale for the Assessment of Negative Symptoms (SANS) which measure symptoms experienced within the month prior to the interview; in the bipolar group, symptom profile was examined using the Beck Depression Inventory (BDI) and the Altman Self-Rated Mania Scale (ASRM). Within the schizophrenia sample, SAPS and SANS scores did not differ as a function of G72 or DAAO genotype. Within the bipolar sample, scores on the BDI were associated with both G72 (F=5.176; df=1; p=0.031) and DAAO (F=4.94; df=1; p=0.034) genotypes (see Table 1); in contrast, scores on the ASRM were not associated with either G72 or DAAO genotype.
Supplementary Material S3

**Verbal fluency task.** During fMRI scanning, subjects performed an overt verbal fluency task involving two main conditions: generation and baseline [1]. In the generation condition, subjects were presented with a series of letters on a computer screen; the task required them to respond to each letter by generating a word that started with that letter. Letter cues were presented in blocks of seven with a stimulus onset asynchrony of 4000 ms; all cues in a given block were of the same letter but each block involved a different letter. The paradigm thus resembles the classical version of the task used in neuropsychological studies except that each response is cued at regular intervals, rather than the subject responding freely as many times as they can following a single cue. A paced paradigm is more compatible with an fMRI study, as it reduces variation in the timing of overt verbal responses within and between subjects, and reduces the risk that only a small proportion of the block is associated with performance of the task, as would occur if a subject rapidly articulated all their responses in the initial part of the block and then disengaged from the paradigm. A further benefit of using a paced task with a relatively long inter-stimulus interval was that there was less risk of large between-subject and between-group variation in task performance, which could confound interpretation of differences in activation, particularly between the control and patient groups. In the baseline condition, subjects were presented with the visual word “rest” and were required to say “rest” out loud; “rest” cues were also presented in blocks of seven with a stimulus onset asynchrony of 4000 ms. Functional MRI data were acquired during two separate acquisition runs, each including 5 blocks of letters alternating with five blocks of “rest” trials. This resulted in a total of 70 letter stimuli and 70 “rest” trials for each subject. Verbal responses were recorded by means of a microphone that was compatible with the MRI apparatus; this allowed us to identify "incorrect" trials in which the subject did not generate any response or generated repetitions, derivatives or grammatical variations of the previous word.

**fMRI data acquisition.** The T2*-weighted gradient-echo single-shot echo-planar images were acquired on a 1.5-T, neuro-optimized IGE LX System (General Electric, Milwaukee) at the Maudsley Hospital, London, U.K. Twelve noncontiguous axial planes (7-mm thickness, slice skip: 1 mm) parallel to the anterior commissure–posterior commissure line were collected. A "clustered" acquisition (TE=40 ms, flip angle=70°) was used in order to minimize the impact of head movement during verbalization [2, 3]. A clustered acquisition sequence capitalizes on the delay of the haemodynamic
response, which reaches its peak about 3–5 s after stimulus onset [4]. A letter cue was presented for 750 ms and an overt verbal response could be made over a silent period of 2900 ms; an image was then acquired over 1100 ms resulting in a total repetition time (TR) of 4000 ms.

References


Supplementary Material S4

To minimize movement-related artifacts, all volumes from each subject were realigned and unwarped using the first as reference [1], normalized to a standard MNI-305 template, and spatially smoothed with an 8-mm FWHM isotropic Gaussian kernel. First, the statistical analysis of regional responses was performed in a subject-specific fashion by convolving each onset time with a synthetic haemodynamic response function (HRF). To minimize performance confounds, we modeled correct and incorrect trials separately by using an event-related model. This design resulted in a total of 4 experimental conditions: (i) easy generation, (ii) hard generation, (iii) repetition, and (iv) incorrect responses. The latter condition was excluded from the group analysis to control for effects of group differences in task performance on brain activation. To remove low-frequency drifts, the data were high-pass filtered by using a set of discrete cosine basis functions with a cut-off period of 128 s. The parameter estimates were calculated for all brain voxels by using the general linear model, and contrast images for “easy generation > repetition” and “hard generation > repetition” were computed in a subject-specific fashion. Second, to permit inferences at the population level [2], the subject-specific contrast images were entered into a second level analysis using the general linear model. The less frequent alleles for the two genes under investigation (i.e. G for G72 and G for DAAO) are found in a small fraction of the Caucasian population; thus, in the present investigation, individuals with one or two copies of the less frequent alleles for G72 and DAAO were combined within the same group. We avoided using a 3 x 2 x 2 ANOVA with diagnostic group, G72 genotype and DAAO genotype as factors, as this would have resulted in some cells with as few as 5 subjects each. Instead we used an ANCOVA model in which diagnostic group (controls, schizophrenic patients, bipolar patients) and G72 genotype (AA, AG/GG) were modelled as between-subject factors, and DAAO genotype (TT, TG/GG) was modelled as an interactive covariate. Modelling DAAO genotype as an interactive covariate involved entering 6 regressors made of −1 (for individuals who were TT homozygotes) and 1 (for individuals with one or two copies of the G allele), one for each of the 6 experimental groups that resulted from modelling diagnostic group and G72 genotype as factors. This statistical model, which has previously been used to examine three-way interactions [3], allowed us to test for the main effect of the task, the main effect of diagnostic group, the main effects of G72 and DAAO genotypes, and any non-additive interactions between the two genes, either diagnosis-dependent or diagnosis-independent. Task load (easy, hard) was also modelled in same the statistical model as a within-subject factor to minimize error variance;
however this manipulation was irrelevant to the hypotheses of the present study, and we therefore report results for the hard and easy conditions combined. Estimation of the model included correction for non-sphericity to account for possible unequal variance between experimental groups [4]. The t-images for each contrast at the second level were transformed into statistical parametric maps of the Z-statistic.

References


