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No Interaction of $GABA_A$ Alpha-1 Subunit and Dopamine Receptor D4 Exon 3 Genes in Symptomatology of Major Psychoses

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Previously, we reported on an association of the dopamine receptor D4 (DRD4) gene with delusional symptomatology of major psychoses. However, despite the strength of the association, it only accounted for 2% of the variance, indicating that contributions from other genes were probable. In the present study, we investigated the original cohort of subjects to evaluate the gene for the γ-aminobutyric acid type A ($GABA_A$) receptor alpha-1 subunit (GABRA1). The possible association of GABRA1 with the psychopathology of major psychoses was tested both alone and in interaction with DRD4. Four hundred and sixty-one inpatients affected by major psychoses were assessed by the operational criteria checklist for psychotic illness (OPCRIT) and were also typed for the DRD4 and GABRA1 variants using PCR techniques. Mania, depression, delusion, and disorganization were the four symptomatologic factors used as phenotype definitions. GABRA1 variants were not associated with these symptomatologic factors, and consideration of possible stratification effects such as sex and psychiatric diagnosis also did not reveal any association. GABRA1 variants did not significantly influence the association of DRD4 with delusional symptoms. No interaction was observed on the other symptom factors. The $GABA_A$ alpha-1 subunit gene does not, therefore, interact with DRD4 in the symptomatology of major psychoses. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 88:44–49, 1999.

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INTRODUCTION

A major limitation to psychiatric genetic research is the definition of the affected phenotype, and the accepted standard is the psychiatric diagnosis [Tsuang and Faraone, 1990; Cloninger, 1994]. However, it has been argued that a single gene could hardly be responsible for a major psychiatric disorder, whereas it might better account for single neuropsychological functions [Cloninger, 1994; Ginsburg et al., 1996; Grigorenko et al., 1997]. We have developed a phenotype definition that is based on psychotic symptomatology and is independent of psychiatric diagnoses [Serretti et al., 1996] and, pursuing this strategy, we have investigated potential associations of dopamine receptor D4 (DRD4) gene exon 1 and 3 variants [Van Tol et al., 1991; Catalano et al., 1993] with the four psychopathology factors, i.e., excitement, depression, delusion, and disorganization. Using this approach, we identified an association between DRD4*7 (exon 3) and delusional symptomatology in a sample of 461 subjects affected by major psychoses [Serretti et al., 1999]. However, despite the strength of the association, the variance accounted for was only about 2%. This is in accordance with current views on polygenic inheritance, where minor effect genes contribute only 1–10% of the total phenotypic variance [Comings, 1997; Risch, 1990], and it is probable that other genes contribute with additive, multiplicative, or epistatic effects [Frankel and Schork, 1996]. The aim of the present study was to investigate the original cohort of subjects to identify additional contributing genes.

Considerable evidence implicates the neurotransmitter γ-aminobutyric acid (GABA) in the biochemical pathophysiology of major psychoses. GABA is the major inhibitory neurotransmitter in the vertebrate brain, and GABAergic inhibitory interneurons are widely distributed throughout the central nervous system (CNS). GABA interacts with other systems which are involved

in the pathogenesis of mood disorders, such as the serotonergic, noradrenergic, and dopaminergic systems [Petty, 1995; Sabatino et al., 1994]. In particular, the interaction with the dopamine system was recently documented by Mrzljak et al. [1996] who, using subtype-specific antibodies, demonstrated the presence of DRD4 on GABAergic neurons in the cerebral cortex, hippocampus, thalamic reticular nucleus, globus pallidus, and substantia nigra (pars reticulata). The authors hypothesized that there is a DRD4-GABA interaction involving a disinhibition of excitatory transmission in intrinsic cortical, thalamocortical, and extrapyramidal pathways [Mrzljak et al., 1996]. In support of this view, Busatto et al. [1997] showed that positive symptom scores of schizophrenic subjects were negatively correlated to benzodiazepine receptor binding in the left medial temporal region, and negative symptoms were inversely related to receptor binding in the medial frontal region. This suggested that reduced inhibitory GABAergic tone in these areas may contribute to the appearance of psychotic symptoms. GABA effects are largely mediated by binding to the postsynaptic $GABA_A$ receptor, which is presumed to be a pentameric heteroligomer assembled from four classes of subunits with multiple members: α (1–6), β (1–3), γ (1–3), and δ (1). Fourteen genes encoding subunits of this receptor have been identified; they are scattered throughout the human genome, and mutations at subunit genes may alter the binding characteristics of the $GABA_A$ receptor [Pritchett and Seeburg, 1991; Wafford et al., 1991; Korpi et al., 1993]. The gene we have studied encoding the α1-subunit has been mapped to chromosome 5q34–35 [Johnson et al., 1992]. The human α1-subunit gene contains a highly polymorphic $(dCdA)_n$ repeat, varying from 1–14 dinucleotides [Walsh et al., 1992]. However, previous studies in schizophrenia [Byerley et al., 1995] and mood disorders [De Bruyn et al., 1996; Coon et al., 1994] failed to reveal any GABRA1 involvement. We then hypothesized that GABRA1 variants could be associated with the symptomatology of major psychoses and could influence the association of DRD4 with delusional symptoms.

The aim of the present study [Serretti et al., 1999] was to investigate the original cohort of subjects to evaluate a possible interaction between GABRA1 and DRD4 polymorphisms in the symptomatology of major psychoses.

MATERIALS AND METHODS

Sample

Four hundred and sixty-one psychiatric inpatients (244/217 female/male, mean age = 42.93 years, SD = 14.41, age of onset = 29.48 years, SD = 13.25), consecutively admitted to the Department of Neuropsychiatry at the Institute H. San Raffaele (DSNP-HSR), were included in this study. This sample was exactly the original cohort of subjects in which the DRD4-delusion association had been detected [Serretti et al., 1999].

All patients were evaluated using the operational criteria checklist for psychotic illness (OPCRIT) [McGuffin et al., 1991]. Lifetime diagnoses were assigned by two independent psychiatrists on the basis of interviews and medical records, according to DSM-III-R criteria [American Psychiatric Association, 1987]. We included all subjects affected by major psychoses, whereas the presence of concomitant diagnoses of mental retardation or drug dependence, together with somatic or neurological illnesses that impaired psychiatric evaluation (e.g., hypothyroidism mimicking a depressive state), represented exclusion criteria. The subjects included were affected by schizophrenia, including all subtypes (n = 162), major depressive disorder (n = 83, including 26 obsessive-compulsive comorbid subjects), bipolar disorder (n = 152), delusional disorder (n = 56), and psychotic disorder not otherwise specified (NOS) (n = 8).

Written informed consent was obtained from all probands, who were unrelated and of Italian descent, with antecedents from all parts of the country.

DNA Analysis

Genomic DNA was extracted from anticoagulated thawed blood according to the method of Lahiri and Nurnberger [1991]. For GABRA1, a polymerase chain reaction (PCR) was performed with the following primers: 5′-TGA TAG CTA GAA AGC TAG CAA G-3′ (1) and 5′-GCT CAT TAA ACA CTG TGT TCC T-3′ (2). One hundred nanograms of genomic DNA were diluted to 12.5 μl using water, and heated to 99°C for 3 min. A reaction mixture was then added which contained 10 pmol of each primer, 200 μmol/l deoxynucleoside triphospate (dNTPs), 2 μmol/l [R110] dUTP (Perkin Elmer Italia, Monza, Italy), 10 mmol/l Tris-HCl (pH 8.3), 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, and 1.5 U Taq-Polymerase (Perkin Elmer Italia, Monza, Italy) in a total volume of 25 μl. Twenty-five cycles were performed with a profile of 95°C for 20 min, 56°C for 20 min, and 72°C for 20 min. This profile was followed by a 72°C chase for 4 min. One microliter of PCR product was combined with 12 μl of deionized formamide and with 0.5 μl of Gene Scan-500 size standard (Perkin Elmer Italia, Monza, Italy), and then heated for 2 min at 95°C and chilled on ice. Capillary electrophoresis was performed on an ABI PRISM™ 310 Genetic Analyzer (Perkin Elmer Italia, Monza, Italy), and fragments were analyzed using GeneScan™ analysis and Genotyper DNA fragment analysis software. Depending on the number of repeats present, alleles of 206, 204, 202, 200, 198, 196, 194, 192, 190, 188, 184, 182, and 180 bp were produced (GABRA1*1–GABRA1*13). A total of 429 subjects was typed for GABRA1 variants; the remaining 32 could not be typed because their DNA was refractory to amplification. The 32 untyped subjects did not differ in clinical and demographic features from the whole sample. For D4 exon 3 typing, a polymerase chain reaction (PCR) was carried out with primers and conditions as described elsewhere [Macciardi et al., 1994]. This PCR polymorphism detects a seven-allele system (DRD4*2 = 0.1, DRD4*3 = 0.03, DRD4*4 = 0.7, DRD4*5 = 0.01, DRD4*6 = 0.01, DRD4*7 = 0.15, and DRD4*8 = 0.01).

Statistical Analysis

We previously described the factor analysis of the OPCRIT checklist in a sample of patients affected by

major psychoses [Serretti et al., 1996]. We identified four factors: excitement, depression, delusion, and disorganization. Standardized factor scores were derived from these factors and are considered to be dependent variables when investigating their distribution across GABRA1 and DRD4 alleles and genotypes.

Differences were assessed using one-way ANOVA, with the Newmann-Keuls test evaluating post hoc comparisons. Our sample is composed of mixed mood and schizophrenic spectrum disorders, and this could introduce a bias into the analysis of genetic effect. The diagnostic composition rates of our sample were not established a priori, and our example must be considered as randomly extracted from all possible sets of samples. To control for this bias, we repeated the analysis using a mixed-effect, two-factor ANOVA, where GABRA1 typing was considered the fixed effect and diagnostic status a random effect. For this calculation we used the BMDP 3V program with the restricted maximum likelihood method [Dixon, 1990].

To investigate the interaction, we used a set of strategies: as a first approach we stratified GABRA1 typings across DRD4 variants. While stratification is the standard means of evaluating interactions, it has a major limitation in that a large sample is needed to obtain stable results, particularly when highly polymorphic markers are considered. A regression approach was proposed and developed to partially avoid this bias [Morabito and Macciardi, 1988; Comings et al., 1996]. Moreover, this approach gives insight into the nature of the interactive model, and it may differentiate between additive and multiplicative or epistatic effects. Then we tested both polymorphisms conjunct through a multiple regression, including each allelic variant as dummy variable (e.g., a subject with genotypes DRD4*2/4 and GABRA1*13/13 is defined as: DRD4*2 = 1, DRD4*3 = 0, DRD4*4 = 1, DRD4*5 = 0, DRD4*6 = 0, DRD4*7 = 0, DRD4*8 = 0, GABRA1*1–GABRA1*12 = 0, and GABRA1*13 = 2).

However, the multiple regression model may furnish biased estimates of each allele coefficient due to the lack of independence among alleles. The regression approach has therefore only been used for the evaluation of interaction effects; in this case we used the following model:

$$y = a + bDRD4{*}i + cGABRA1{*}j + dDRD4{*}i{*}GABRA1{*}j$$
$$(P \text{ value}) \quad (P \text{ value}) \quad (P \text{ value})$$

where y is the symptom score, a is the intercept, b and c are the coefficients indicating main effects, and d is the interaction coefficient. In parentheses, under the coefficients, the probability of beta = 0 is reported. GABRA1 is a highly polymorphic marker, but the majority of alleles (98%) and genotypes (97%) cluster in the ranges GABRA1*4–8 and GABRA1*12–13. The GABRA1 variants contained in this range were considered for the regression analysis (main variants). Alpha values were conservatively considered significant when lower than 0.01.

RESULTS

The association of GABRA1 variants with excitement, depressive, disorganized, and delusional symptomatology was estimated using one-way ANOVA. GABRA1 variants were not associated with these four symptomatologic factors, even when possible stratification biases like sex or diagnosis were included in the model (Table I).

Diagnostic status may be considered a random effect, since we did not decide how many schizophrenic or mood disorder patients we would include in our sample a priori, so we repeated the ANOVA with a mixed model including diagnosis as a random effect, but once again, no significant association emerged (data not shown).

DRD4 – GABRA1 Interaction

DRD4 – GABRA1 interaction was evaluated initially using a traditional stratification technique, but given the elevated polymorphism of GABRA1, DRD4 association with the factors was repeated only considering GABRA1*4–8 and GABRA1*12–13 subjects, in turn (Table II).

However, traditional stratification analysis should be considered with caution, as small cell numbers do not allow meaningful statistics to be obtained. To avoid this bias, we applied the regression technique. A number of possible interactions can be tested (3 DRD4 variants multiplied by 7 GABRA1 main variants multiplied by 4 factors = 84 interactions). The aim of the present paper was to consider the possible influence of GABRA1 variants on the DRD4*7 association with delusional scores, so we considered those interactions first, including the DRD4*7 variant and the delusional factor:

$$\begin{aligned} DEL = &- .01 + .04\ GABRA1{*}4 + .20\ DRD4{*}7 \\ &\quad (\text{n.s.}) \qquad\quad (0.03) \\ &- .08\ GABRA1{*}4{*}DRD4{*}7 \\ &\quad (\text{n.s.}) \end{aligned}$$

$$\begin{aligned} DEL = &- .18 + .03\ GABRA1{*}5 + .21\ DRD4{*}7 \\ &\quad (\text{n.s.}) \qquad\quad (0.04) \\ &- .03\ GABRA1{*}5{*}DRD4{*}7 \\ &\quad (\text{n.s.}) \end{aligned}$$

$$\begin{aligned} DEL = &- .01 + .00\ GABRA1{*}6 + .16\ DRD4{*}7 \\ &\quad (\text{n.s.}) \qquad\quad (0.11) \\ &+ .14\ GABRA1{*}6{*}DRD4{*}7 \\ &\quad (\text{n.s.}) \end{aligned}$$

$$\begin{aligned} DEL = &- .01 + .07\ GABRA1{*}7 + .22\ DRD4{*}7 \\ &\quad (\text{n.s.}) \qquad\quad (0.01) \\ &- .47\ GABRA1{*}7{*}DRD4{*}7 \\ &\quad (\text{n.s.}) \end{aligned}$$

$$\begin{aligned} DEL = &- .01 + .01\ GABRA1{*}8 + .23\ DRD4{*}7 \\ &\quad (\text{n.s.}) \qquad\quad (0.02) \\ &+ .13\ GABRA1{*}8{*}DRD4{*}7 \\ &\quad (\text{n.s.}) \end{aligned}$$

$$\begin{aligned} DEL = &- .01 - .01\ GABRA1{*}12 + .25\ DRD4{*}7 \\ &\quad (\text{n.s.}) \qquad\quad (0.07) \\ &- .08\ GABRA1{*}12{*}DRD4{*}7 \\ &\quad (\text{n.s.}) \end{aligned}$$

TABLE I. Symptom Factor Scores and GABRA1 Variants*

	Excitement		Depression		Delusion		Disorganization		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	N
GABRA1 alleles									
1	−0.746	0.000	0.858	0.000	−1.231	0.000	−1.030	0.000	1
2	2.401	0.000	1.551	0.000	−0.268	0.000	−0.985	0.000	1
3	−0.436	0.209	−0.547	0.958	−0.006	1.561	0.474	0.921	2
4	−0.087	0.934	0.242	1.049	0.129	1.070	−0.304	0.956	59
5	−0.017	0.996	0.068	0.979	0.035	1.038	−0.116	1.038	160
6	0.000	1.054	−0.032	1.015	0.124	1.053	0.047	1.011	118
7	0.029	1.043	0.142	0.959	0.044	0.867	0.122	1.277	32
8	−0.017	1.099	0.016	1.047	0.232	1.009	0.046	1.102	72
9	−0.864	0.000	−1.152	0.000	−1.247	0.000	−0.834	0.000	1
10	−0.841	0.000	−1.189	0.000	0.276	0.000	−1.101	0.000	1
11	0.468	1.329	0.790	1.137	−0.290	0.859	−0.204	1.021	5
12	−0.007	1.045	0.124	1.027	0.053	1.067	−0.120	1.014	291
13	−0.058	0.959	−0.074	0.892	0.059	0.930	0.095	1.089	115
Total	−0.018	1.023	0.067	1.003	0.075	1.027	−0.060	1.044	858
GABRA1 genotypes									
5/4	0.352	1.157	0.031	0.834	0.290	0.964	−0.398	0.886	14
5/5	−0.164	0.864	−0.197	0.931	0.336	0.970	0.050	1.159	13
6/4	−0.475	0.486	−0.013	0.898	0.431	1.144	0.112	0.984	12
6/5	−0.170	0.957	0.177	1.249	−0.100	1.055	−0.324	1.020	18
6/6	0.253	1.035	−0.103	0.961	0.536	1.326	0.724	1.267	7
7/4	−0.687	0.202	1.153	0.670	−0.111	1.224	−0.753	0.045	3
7/5	0.353	1.212	0.350	1.092	−0.295	0.656	−0.034	1.181	8
7/6	0.378	1.280	−0.424	0.728	0.057	1.018	−0.372	0.967	6
8/4	0.784	1.173	0.454	1.435	−0.062	0.904	−0.951	0.332	5
8/5	−0.148	1.045	0.047	1.027	0.031	1.007	0.105	1.511	10
8/6	0.247	1.328	0.180	1.061	0.337	1.054	0.078	0.964	13
8/7	−0.487	0.318	−0.217	1.142	−0.080	0.816	0.299	1.401	5
8/8	−0.749	0.114	0.980	0.932	1.137	0.316	0.286	2.034	2
12/4	−0.347	0.752	0.559	1.150	0.214	1.167	−0.280	1.152	18
12/5	0.014	1.054	0.165	0.994	−0.031	1.124	−0.145	0.969	57
12/6	0.127	1.158	−0.084	1.021	−0.045	0.961	−0.069	0.845	40
12/7	−0.005	1.145	−0.048	0.948	0.367	1.302	1.103	1.750	4
12/8	−0.102	1.089	−0.188	0.985	0.305	1.127	0.086	0.930	26
13/4	0.168	1.035	0.084	1.061	−0.705	0.930	−0.609	0.246	5
13/5	−0.062	0.907	−0.091	0.850	0.034	1.063	0.033	0.987	25
13/6	−0.332	0.821	−0.216	0.990	0.146	0.993	0.339	1.105	14
13/7	0.466	1.282	0.430	0.678	0.366	0.535	0.234	1.426	6
13/8	0.137	1.182	−0.210	1.030	0.027	0.914	0.128	1.153	9
12/12	−0.038	1.021	0.206	1.064	0.017	1.129	−0.236	1.017	51
13/12	0.032	1.015	0.043	0.882	0.089	0.896	0.036	1.104	41
13/13	−0.413	0.673	−0.357	0.881	0.184	0.887	0.375	1.311	7
Total	−0.018	1.023	0.067	1.003	0.075	1.028	−0.060	1.045	419

*Factor scores and GABRA1 alleles and genotypes. Rare genotypes are not displayed. Means and standard deviations of factor scores are reported. Factor scores were not associated with GABRA1 variants.

$$\begin{aligned} DEL = .01 &- .06\ GABRA1{*}13 + .15\ DRD4{*}7 \\ &\text{(n.s.)} \qquad\quad (0.14) \\ &+ .20\ GABRA1{*}13{*}DRD4{*}7 \\ &\text{(n.s.)} \end{aligned}$$

None of the main GABRA1 variants influenced the observed association between DRD4*7 and delusional score. Finally, we considered all other possible interactions on factor scores. Due to the possibility of false-positive results, we set the alpha value to a conservative level of 0.01. No significant interaction was observed.

DISCUSSION

The aim of the present study was to evaluate the possible association of the $GABA_A$ receptor alpha-1 subunit gene with psychopathology, independent of psychiatric diagnoses, and to analyze the possible influence of GABRA1 variants on the DRD4-delusion association.

GABRA1 polymorphism was not associated with psychopathology in our sample. The results do not exclude an influence of the GABA system on the symptomatology of major psychoses, as other $GABA_A$ receptor subunits (alpha-2–6, beta-1–3, gamma-1–3, and delta) were not tested. Moreover, regulation of receptor protein expression may influence the overall activity of the system, and that is not detected when studying associations at the level of subunit genes.

The use of psychiatric diagnoses to define the affected phenotype for association studies has been criticized for its low specificity, and the study of basic psy-

TABLE II. Symptom Factor Scores and GABRA1/DRD4 Variants*

		Excitement		Depression		Delusion		Disorganization		
G1	D4	Mean	SD	Mean	SD	Mean	SD	Mean	SD	N
4	2	−0.553	0.374	−0.716	0.719	−1.171	0.086	−0.489	0.441	2
4	4	−0.024	0.980	0.312	1.072	0.144	1.114	−0.263	0.966	44
4	7	−0.355	0.696	0.104	0.970	0.470	0.919	−0.770	0.555	9
5	2	0.138	1.009	−0.044	0.897	−0.031	0.885	0.056	1.205	16
5	4	−0.123	0.964	0.106	0.962	−0.026	1.078	−0.070	1.082	105
5	7	0.236	1.043	0.000	1.036	0.248	1.023	−0.251	0.875	31
6	2	−0.309	0.919	0.379	1.331	−0.163	1.300	−0.319	0.890	9
6	4	−0.071	1.028	−0.051	1.004	0.065	1.051	0.077	0.999	84
6	7	0.430	1.175	0.004	0.977	0.401	0.923	−0.092	0.995	20
7	2	0.503	1.305	0.234	1.415	0.452	0.917	−0.035	0.844	6
7	4	−0.075	1.023	0.146	0.826	−0.104	0.865	0.062	1.459	19
7	7	−0.104	0.453	−0.361	0.719	0.286	0.652	0.747	1.610	3
8	2	0.376	1.255	0.524	1.181	−0.138	1.052	−0.169	0.984	9
8	4	−0.162	1.001	−0.059	1.000	0.310	1.065	0.044	1.129	45
8	7	0.329	1.290	0.143	1.087	0.228	0.910	0.229	1.034	15
12	2	0.007	1.056	0.197	1.131	−0.169	1.071	−0.197	0.933	58
12	4	0.013	1.054	0.152	1.026	0.099	1.056	−0.123	1.011	184
12	7	−0.151	0.960	−0.094	0.843	0.237	0.999	−0.127	1.066	32
13	2	−0.027	0.970	0.093	0.939	0.093	0.992	−0.007	1.052	33
13	4	−0.058	0.950	−0.093	0.872	0.009	0.908	0.182	1.119	77
13	7	0.192	1.480	−0.773	0.366	0.926	0.168	−0.999	0.038	3
Total		−0.017	1.019	0.075	0.998	0.081	1.029	−0.065	1.040	804

*Factor scores and DRD4 alleles stratified for GABRA1 alleles. Means and standard deviations of factor scores are reported. No significant interaction was observed.

chopathologic traits has been proposed as an alternative [Cloninger, 1994; Ginsburg et al., 1996]. We have developed a phenotype definition that is based on a lifetime score of excitement, depressive, delusional, and disorganized symptoms [Serretti et al., 1996]. We previously investigated this cohort of subjects using DRD4 exon 1 and 3 markers [Van Tol et al., 1991; Catalano et al., 1993], and DRD4*7 (exon 3) proved to be associated with delusional symptomatology [Serretti et al., 1999]. The DRD4*7-delusion association was not influenced by GABRA1 variants, so we may hypothesize that GABRA1 variants do not have any effect on the DRD4*7 liability for delusional symptoms. Evidence for a possible DRD4-GABA interaction was documented by Mrzljak et al. [1996], who reported the presence of DRD4 on GABAergic neurons in the cerebral cortex, hippocampus, thalamic reticular nucleus, globus pallidus, and substantia nigra. Our data do not support a direct interaction as evidenced by the DRD4 and GABRA1 markers; other $GABA_A$ receptor subunits remain to be evaluated.

A limitation of our study is inherent in the marker we used. GABRA1 is a highly polymorphic marker, and the 13 variants scatter subjects into many small groups. On the other hand, no difference in pharmacologic activity of short and long variants was revealed, and thus there is no a priori reason for collapsing GABRA1 variants. In our sample, the distribution of GABRA1 allele frequencies was similar to that in published samples [Johnson et al., 1992].

The power of our sample was enough to detect a standardized difference (effect size) as small as 0.25 (depending on the frequency of the GABRA1 risk allele, considering a power of 0.8 and alpha of 0.05 two-tailed); thus it is possible that smaller differences were missed. Nevertheless, a minimum detectable standardized difference of 0.2–0.3 is usually considered a small effect.

In the present study we adopted an innovative technique to analyze gene interaction. It is most probable that complex traits are under polygenic control, with each gene contributing 1–10% of the total variance. However, the cumulative effect may not simply be additive. A wide range of nonlinear gene interactions have been proposed, such as epistatic effects, where a gene contribution is under the control of a second gene, or multiplicative effects, where the presence of both genes produces an effect that is greater than the sum of the effects of the two genes alone [Frankel and Schork, 1996]. To the best of our knowledge, the interaction has only been included in linkage analysis but limited to two-locus analyses and with heavy a priori parametrization [Neuman and Rice, 1992; Macciardi and Cavallini, 1993]. For association studies, stratification is the most common technique [Ebstein et al., 1997], but it is exposed to bias related to the sample size and to the lack of probability estimators. Those biases are partially overcome by the regression technique, which permits a reliable estimation of the degree of gene interaction, including explained variance and the probability value [Comings et al., 1996; Cheverud and Routman, 1995]. Regression techniques have been applied both in animals for tumor susceptibility [Fijneman et al., 1996; Van Wezel et al., 1996] and in humans for Tourette syndrome [Comings et al., 1996], allowing researchers to detect interactions even when the main effect was negligible. The use of regression techniques therefore constitutes a suitable tool for the analysis of polygenic disorders.

In conclusion, GABRA1 was not associated with psychopathology when defined as excited, depressive, de-

lusional, and disorganized symptoms in major psychoses; GABRA1 did not interact with DRD4 in the psychopathology of major psychoses.

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