Supporting Information

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SI Materials and Methods

Subjects. All subjects were evaluated with the Structured Clinical Interview (1) for the *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition* to exclude any psychiatric disorder or to confirm diagnosis of schizophrenia. Exclusion criteria were history of significant drug or alcohol abuse, active drug use in the past year, head trauma with loss of consciousness, and any significant medical condition.

The present experimental protocol was approved by the local institutional review board at the Policlinico of Bari. After complete description of the study to the subjects, written informed consent was obtained.

Genotyping. DNA was extracted from whole blood using standard procedures. *DRD2* rs1076560 was analyzed with allele-specific PCR primers as previously described (2). Amplification of the 574 bp DNA fragment containing the *AKT1* rs1130233 polymorphism (G > A; $Glu^{242}Glu$) was performed using forward 5′-CTACTCTCCATGGCACCAGA-3′ and reverse 5′-GCCAGGTACTCAGGTGTGC-3′ primers. PCR was executed on 100 ng genomic DNA in a standard 25-μL volume, containing 0.2-μM primers, 100 μM dNTP, 2.5 μL reaction Gold buffer (Applied Biosystems), 2 mM $MgCl_2$, and 2.5 U Ampli Taq Gold Polymerase (Applied Biosystems). Thermal conditions were 12 min initial denaturation at 94 °C, 35 cycles of 94 °C for 30 s, 62 °C for 30 s, 72 °C for 45 s, followed by 7 min of final elongation step at 72 °C. *AKT1* rs1130233 PCR products were sequenced in both directions using BigDye Terminator chemistry and run on an ABI Prism 3130 DNA sequencer (Applied Biosystems). Sequences were analyzed with SeqMan from Lasergene-DNASTAR package (DNASTAR Inc.). After genotype determination in all subjects, groups displayed Hardy-Weinberg equilibrium.

Given the low number of subjects homozygous for the minor alleles, we collapsed these individuals (when present) and heterozygous subjects within one group for further analyses. This procedure is consistent with a series of earlier studies evaluating polymorphisms with low minor allele frequencies, especially when codominance of the alleles is not known (i.e., ref. 3). The χ^2 analysis demonstrated equal distribution of *DRD2* genotypes in *AKT1* groups and vice versa (all $\chi^2 < 0.2$; all $P > 0.6$) indicating that the two genotypes are not correlated.

Protein Determination and Quantification. Peripheral human blood mononuclear cells (PBMCs) were isolated as previously described (4). Briefly, PBMCs were isolated from blood samples by Ficoll density gradient (ICN Biomedical, Inc.). Cells were rapidly rinsed in ice-cold PBS and solubilized in Triton X-100 lysis buffer (10 mM Tris HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10% glycerol, 1 mM phenylmethylsulphonyl fluoride, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 1 mM sodium orthovanadate, 50 mM sodium fluoride, and 10 mM β-glicerophosphate).

Aliquots (2 μL) of samples were used for the protein determination by Bio-Rad Protein Assay kit (Bio-Rad). Equal amounts of total proteins for each sample were loaded onto 10% polyacrylamide gels. Proteins were separated by SDS/PAGE and transferred overnight to membranes (PVDF) (Amersham Pharmacia Biotech). Membranes were immunoblotted overnight using selective antibodies against AKT1 (1:1,000; Cell Signaling Technology), P-Ser473-AKT1 (1:1,000; Cell Signaling Technology), GSK-3β (1:500; Cell Signaling Technology), P-Ser9-GSK-3β (1:500, Cell Signaling Technology). Selective antibodies against β-Actin were also used (1:1,000; Sigma). It is important to note that two sites of phosphorylation are present in AKT1: Thr-308 and Ser-473. However, we focused on the latter based on previous evidence of its specific association with GSK-3β activity (5–7). Blots were then incubated in horseradish peroxidase-conjugated secondary antibodies and target proteins visualized by ECL detection (Pierce), followed by quantification by Quantity One software (Bio-Rad). Optical density values of AKT1 and GSK-3β were normalized to β-Actin for variation in loading and transfer. The levels of P-Ser9-GSK-3β were normalized to total GSK-3β, and P-Ser473-AKT1 to total AKT1.

Functional MRI Task. Each stimulus of the variable attentional control (VAC) task was composed of arrows of three different sizes pointing either to the right or to the left; small arrows were embedded in medium-sized arrows that were in turn embedded in a large arrow. Subjects were instructed by a cue word (big, medium, or small) displayed above each stimulus to press a button corresponding to the direction of the large, medium or small arrows (right or left). To increase the level of attentional control required, the direction of the arrows was congruent or incongruent across all three sizes. This resulted in the following conditions:

Low level of attentional control. All three sized arrows were congruent in direction with each other. The cue was the word BIG.
Intermediate level of attentional control. Two types of stimuli were used: the big arrow was incongruent in direction to the small and the medium arrows in both; the cue was BIG in one of them, SMALL in the other.
High level of attentional control. Two types of stimuli were used: the medium-sized arrows were incongruent in direction to the big and the small arrows in both; the cue was SMALL in one of them, MEDIUM in the other.
A simple bold arrow pointing either to the left or right was used as a sensorimotor control condition.

Subjects were instructed to respond to task stimuli with the right hand using a button box (right button for "right" response, left button for "left" response), and to press the response button as fast and accurately as possible. Furthermore, they were asked to move their thumb to a small plastic knob placed between buttons after each response. All subjects were trained on the task before the fMRI session. Each stimulus was presented for 800 ms, and the order of the stimuli was randomly distributed across the session. The total number of stimuli was 241: 50 HIGH (25 stimuli of each of the two stimulus types that subtended this level of conflict), 68 INT (34 stimuli of each of the two stimulus types that subtended this level of conflict), 57 LOW, and 66 simple bold arrows (sensorimotor control condition); the total duration of the task was 10 min, 8 s. A fixation cross-hair was presented during the interstimulus interval, which ranged from 2,000 to 6,000 ms. Stimuli were presented via a back-projection system and responses were recorded through a fiber-optic response box that allowed measurement of accuracy and reaction time for each trial.

Functional MRI Acquisition. Functional MRI was performed with a gradient echo-planar imaging sequence (TR/TE = 2,000/30; 26 interleaved slices, thickness = 4 mm, gap = 1 mm; voxel size 3.75 × 3.75 × 5 mm; scans = 300; flip angle = 90°; field of view = 24 cm; matrix = 64 × 64) while subjects performed the VAC task. The first four scans were discarded to allow for signal saturation.

Functional MRI analysis. Images for each subject were realigned, spatially normalized into the Montreal Neurological Institute (MNI)

template (12-parameter affine model), and spatially smoothed (10-mm Gaussian filter). After realignment, datasets were also checked for small-motion correction (<2 mm in translation, <1.5° in rotation). The fMRI responses were modeled using a canonical hemodynamic response function and temporally filtered using a high-pass filter of 128 Hz and a hrf-shape low-pass filter. Vectors were created for each condition using the timing of correct responses. To account for differences in head movement between groups, residual movement was also modeled as a regressor of no interest. A *t* statistic was then used to produce a statistical image for blood-oxygen level-dependent responses relative to brain processing of stimuli for each level of attentional control (HIGH, INT, and LOW).

Functional MRI Clusters Localization. All analyses were constrained by a mask obtained by combining the HIGH, INT, and LOW group-activation maps of both groups of subjects ($P < 0.05$). Brodmann's areas were assigned to activated clusters using the Talairach Daemon (http://www.talairach.org/daemon.html) after converting the MNI coordinates of the local maxima in the activated clusters to Talairach coordinates (http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml).

Continuous Performance Test. In brief, cue-target sequences (A–X, 80% of frequency), randomly chosen noncue-target sequences (e.g., B–X, 10% of frequency), and the cue followed by a distractor (e.g., A–Y, 10% of frequency) were randomly presented (interstimulus interval: 750 ms). Total number of stimuli was 450. Subjects were instructed to respond by pressing a key only whenever the letter X followed the letter A. Each letter appeared for 200 ms and subjects had 1 s to respond.

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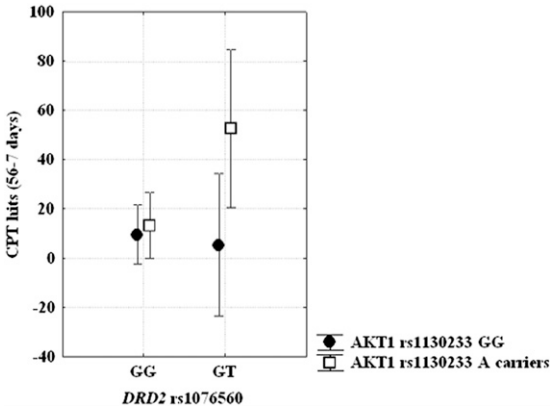

Fig. S1. Effect of *DRD2* rs1076560 and *AKT1* rs1130233 on improvement of Continuous Performance Test hits after olanzapine treatment in patients with schizophrenia. See text for statistics.

Table S1. Behavioral data (±SD) at the VAC task for each genotype group

	High load		Intermediate load		Low load	
	% Correct responses	Reaction time (ms)	% Correct responses	Reaction time (ms)	% Correct responses	Reaction time (ms)
DRD2 GG/ *AKT1*GG	87.13 ± 8.8	1108.6 ± 168.4	95.1 ± 5.3	955.7 ± 146.4	99.6 ± 1.1	795.7 ± 134.0
DRD2 GG/ *AKT1*A carriers	89.5 ± 7.6	1147.7 ± 158.1	93.8 ± 4.8	976.8 ± 154.0	99.8 ± 0.7	818.6 ± 148.9
DRD2 T carriers/ *AKT1*GG	86.9 ± 11	1071.8 ± 169.4	91.2 ± 9.1	934.1 ± 126.9	99.8 ± 0.6	787.3 ± 112.1
DRD2 T carriers/ *AKT1*A carriers	83.7 ± 7.7	1001.7 ± 114.5	89.3 ± 6.9	872.9 ± 69.6	100 ± 0.0	722.1 ± 56.9

See text for statistics.