DRD2/AKT1 interaction on D2 c-AMP independent signaling, attentional processing, and response to olanzapine treatment in schizophrenia

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Edited by Daniel R. Weinberger, National Institute of Mental Health, Intramural Research Program, National Institutes of Health, Bethesda, MD, and accepted by the Editorial Board November 23, 2010 (received for review September 10, 2010)

The D2/AKT1/GSK-3β signaling pathway has been involved in the downstream intracellular effects of dopamine, in the pathophysiology of cognitive deficits and related brain activity in schizophrenia, as well as in response to treatment with antipsychotics. Polymorphisms in the D2 (*DRD2* rs1076560) and AKT1 (*AKT1* rs1130233) genes have been associated with their respective protein expression and with higher-order cognition and brain function, including attention. Given the strong potential for their relationship, we investigated the interaction of these polymorphisms on multiple molecular and in vivo phenotypes associated with this signaling pathway. We measured AKT1 and GSK-3β proteins and phosphorylation in human peripheral blood mononuclear cells, functional MRI cingulate response during attentional control, behavioral accuracy during sustained attention, and response to 8 wk of treatment with olanzapine in a total of 190 healthy subjects and 66 patients with schizophrenia. In healthy subjects, we found that the interaction between the T allele of *DRD2* rs1076560 and the A allele of *AKT1* rs1130233 was associated with reduced AKT1 protein levels and reduced phosphorylation of GSK-3β, as well as with altered cingulate response and reduced behavioral accuracy during attentional processing. On the other hand, interaction of these two alleles was associated with greater improvement of Positive and Negative Syndrome Scale scores in patients with schizophrenia after treatment with olanzapine. The present results indicate that these functional polymorphisms are epistatically associated with multiple phenotypes of relevance to schizophrenia. Our results also lend support to further investigation of this downstream molecular pathway in the etiology and treatment of this disorder.

A large series of experimental data indicate that dopamine D2 receptors and schizophrenia are tightly related. First, these receptors are privileged targets of antipsychotic drugs, which antagonize their activity (1). Second, previous reports have suggested association between psychosis and relatively greater D2 density in striatum, even though change is moderate (2). Third, clinical symptoms and cognitive deficits have been associated with abnormal D2 signaling (3–12). The relationship between D2 receptors and cognitive deficits in schizophrenia is also supported by previous models postulating that relatively excessive D2 signaling may lead to lower cortical signal-to-noise ratio and reduced filtering of information, as well as blocking of distracting inputs (10–12). These brain processes contribute to different higher-order cognitive functions and are strongly involved in top-down modulation of attention (13, 14). Consistent with these models, previous data have suggested that attentional behavior is affected by D2 genetic variation (15). Furthermore, deficits in attentional processing are centrally implicated in schizophrenia (16, 17). In fact, patients with schizophrenia performing attentional tasks have abnormal activity in the cingulate cortex, a brain region tightly linked to attentional processing (14) and modulated by D2 receptors (18, 19), as well as by genetic variants possibly affecting D1/D2 ratio stimulation (10, 20).

Two isoforms of the D2 receptor are known. The D2 long isoform (D2L) is mainly postsynaptic and is a target for haloperidol, and the D2 short (D2S) isoform is mainly presynaptic and serves as an autoreceptor regulating dopamine synthesis and release (21). These two isoforms are coded by the D2 receptor gene (*DRD2*–11q23) with a mechanism of alternative splicing acting at exon 6. In a previous study (15), we have characterized a functional SNP within *DRD2* at intron 6 (rs1076560 – guanine > timine – G > T) associated with relative expression of the two isoforms in the frontal cortex. In particular, the T allele shifts splicing from D2S to D2L, decreasing the D2S/D2L ratio relative to the G allele. This SNP has also been associated with behavior and brain activity during cognitive and emotion processing in healthy humans and in patients with schizophrenia (15, 22, 23). More specifically, the T allele has been associated with less efficient prefronto-striatal activity during working memory (23) and with putatively greater levels of striatal dopamine (24). *DRD2* has also been weakly associated with diagnosis of schizophrenia (25).

Downstream of D2 receptors, different molecular pathways have been identified: the classic cAMP-PKA pathway and another cAMP-independent pathway that includes the serine/threonine protein kinase AKT1, which phosphorylates to inhibit another protein kinase, GSK-3β (reviewed in ref. 26). The specific relationship between D2 receptor signaling and AKT1 has been elucidated by data indicating that D2 stimulation by dopamine inhibits AKT1 signaling through dephosphorylation via the β-arrestin 2/phosphatase PP2A complex (27, 28) (for review, see refs. 26 and 29). Consistent with their preferential postsynaptic localization, another experiment has also indicated that knockout of D2L receptors is sufficient to reduce activity of this pathway (28). Moreover, other studies in mice have demonstrated that D2 but not D1 agonists impair performance at the T maze and prepulse inhibition of startle in AKT1-deficient mice (30, 31). Importantly, AKT1 levels in lymphoblasts and in prefrontal cortex of patients with schizophrenia are reduced (31, 32). Furthermore, clozapine, a D2 antagonist antipsychotic, increases AKT1 and

Author contributions: G.B., G.U., A.U., and A.B. designed research; F.N., G.U., P.T., R.R., G.C., L.F., B.G., A.D.G., L.I., L.S., and T.P. performed research; G.B., F.N., G.U., P.T., R.R., A.U., and A.B. analyzed data; and G.B. and A.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. D.R.W. is a guest editor invited by the Editorial Board.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1013535108/-/DCSupplemental.

GSK-3β phosphorylation, as well as total cellular and intranuclear levels of β-catenin (33), a crucial factor for gene expression that is inhibited by GSK-3β activity (26).

The gene coding for *AKT1* (14q32.32) has also been associated with schizophrenia (31, 32, 34–37). Importantly, the A allele in a synonymous SNP in *AKT1* (rs1130233 – G > A) is associated with reduced AKT1 protein levels in lymphoblasts (38, 39), reduced cognitive performance, inefficient prefronto-striatal activity during cognition (39), as well as with diagnosis of schizophrenia (39).

Because genetic variation does not directly cause behavioral phenotypes but rather impacts on neuronal features that influence neural systems-level processing, we investigated the possible impact of *DRD2* rs1076560 and *AKT1* rs1130233 on a series of progressively more complex and distal phenotypes in healthy subjects and in patients with schizophrenia. In particular, given the known reciprocal relationship between D2 and AKT1 and their effects on GSK-3β activity (26), as the more proximal phenotype, we evaluated the interaction between *DRD2* and *AKT1* SNPs on AKT1 and GSK-3β protein levels and phosphorylation in human blood cells. Because this experiment suggested functional epistatic downstream effects of these two polymorphisms, we performed further specific analyses. Given the earlier involvement of anterior cingulate in the pathophysiology of schizophrenia, the expression of D2 receptors in anterior cingulate (40) and the involvement of D2 signaling in attentional processing (15), we evaluated cingulate physiology during attentional control as well as behavioral accuracy during sustained attention. Finally, given the strong involvement of D2 receptors and the recent implication of AKT1 (33) in determining response to antipsychotics, we also evaluated response to treatment with olanzapine in patients with schizophrenia.

Results

Association of DRD2 rs1076560 and AKT1 rs1130233 Genotypes with AKT1 and GSK-3β Protein Levels and Phosphorylation in Peripheral Blood Mononuclear Cells of Healthy Humans. Factorial ANOVA indicated a statistical trend for a main effect of *DRD2* rs1076560 ($F_{1,23} = 2.6$; $P = 0.1$) and of *AKT1* rs1130233 ($F_{1,23} = 2.0$; $P = 0.1$) on AKT1 protein, with *DRD2* GT subjects and *AKT1* A carriers having reduced mean levels. Furthermore, there was an interaction between *DRD2* and *AKT1* polymorphisms ($F_{1,23} = 8.9$; $P = 0.006$). Posthoc analysis revealed that *DRD2* GT/*AKT1* A carriers subjects had lower AKT1 protein levels relative to other genotypes groups (all $P < 0.04$) (Fig. 1*A*). On the other hand, no significant association between genotypes and AKT1 phosphorylation at Ser473 was found (all $P > 0.2$) other than a strong trend for an effect of *AKT1* rs1130233 ($F_{1,23} = 3.6$; $P = 0.07$), with reduced AKT1 phosphorylation in A carriers.

To examine potential downstream effects of these two polymorphisms, further analysis was performed to investigate association of *DRD2* and *AKT1* genetic variants on GSK-3β protein levels and phosphorylation. Factorial ANOVA revealed no genotype effects on GSK-3β protein levels (all $P > 0.4$). Analysis on GSK-3β phosphorylation at Ser9 indicated no effects of *DRD2* rs1076560 ($F_{1,23} = 1.3$; $P = 0.3$), a strong trend for a main effect of *AKT1* rs1130233 ($F_{1,23} = 3.5$; $P = 0.07$), and a significant interaction ($F_{1,23} = 12.4$; $P = 0.001$). Posthoc analysis indicated reduced phosphorylation of Ser9 GSK-3β in *DRD2* GT/*AKT1* A carrier subjects relative to all other genotypes groups (all $P < 0.04$) (Fig. 1*B*).

Association of DRD2 rs1076560 and AKT1 rs1130233 Genotypes with Cingulate Cortex Activity During Attentional Control in Healthy Subjects. In the functional MRI (fMRI) sample (Table 1), genotype groups were matched in terms of gender, age, handedness, and IQ (all $P > 0.1$). No genotype effects were present on variable attentional control (VAC) behavioral data (all $P > 0.05$) (Table S1), thus allowing us to compare brain responses without this potential confound.

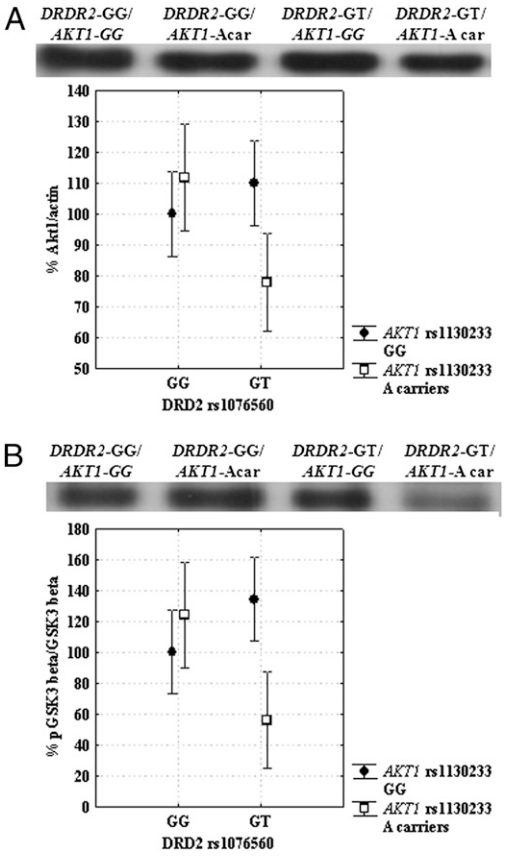

Fig. 1. Western blots and graphs showing interaction between *DRD2* rs1076560 and *AKT1* rs1130233 on AKT1 expression (*A*) and GSK-3β phosphorylation (*B*). *DRD2* GT/*AKT1* A carriers subjects had lower AKT1 expression and GSK-3β phosphorylation relative to all other genotypes groups. See text for statistics.

Imaging analysis revealed a main effect of load in a cingulate cluster extending bilaterally (local maxima in $x = 8$; $y = 30$; $z = 26$, BA32, $k = 112$, $Z = 7.43$) (Fig. 2), although no main effect of

Table 1. Demographics (± SD) of the samples included in the experiments performed

	fMRI	Cognitive behavior	Olanzapine study
n	73	176	66
Sex	45 F	108 F	14 F
Age	24.5 ± 4.5	25.6 ± 6.1	28.3 ± 7.2
Handedness	0.6 ± 0.5	0.7 ± 0.5	
IQ	110.1 ± 12.3	108.7 ± 3.3	
TIB			102.4 ± 8.3
PANSS at baseline			
Total			103.33 ± 21.3
Positive			25.11 ± 6.2
Negative			26.12 ± 10.1
General psychopathology			52.11 ± 12.2
N			
DRD2 GG/ *AKT1* GG	30	74	30
DRD2 GG/ *AKT1* A carriers	29	56	24
DRD2 T carriers/ *AKT1* GG	7	28	7
DRD2 T carriers/ *AKT1* A carriers	7	18	5

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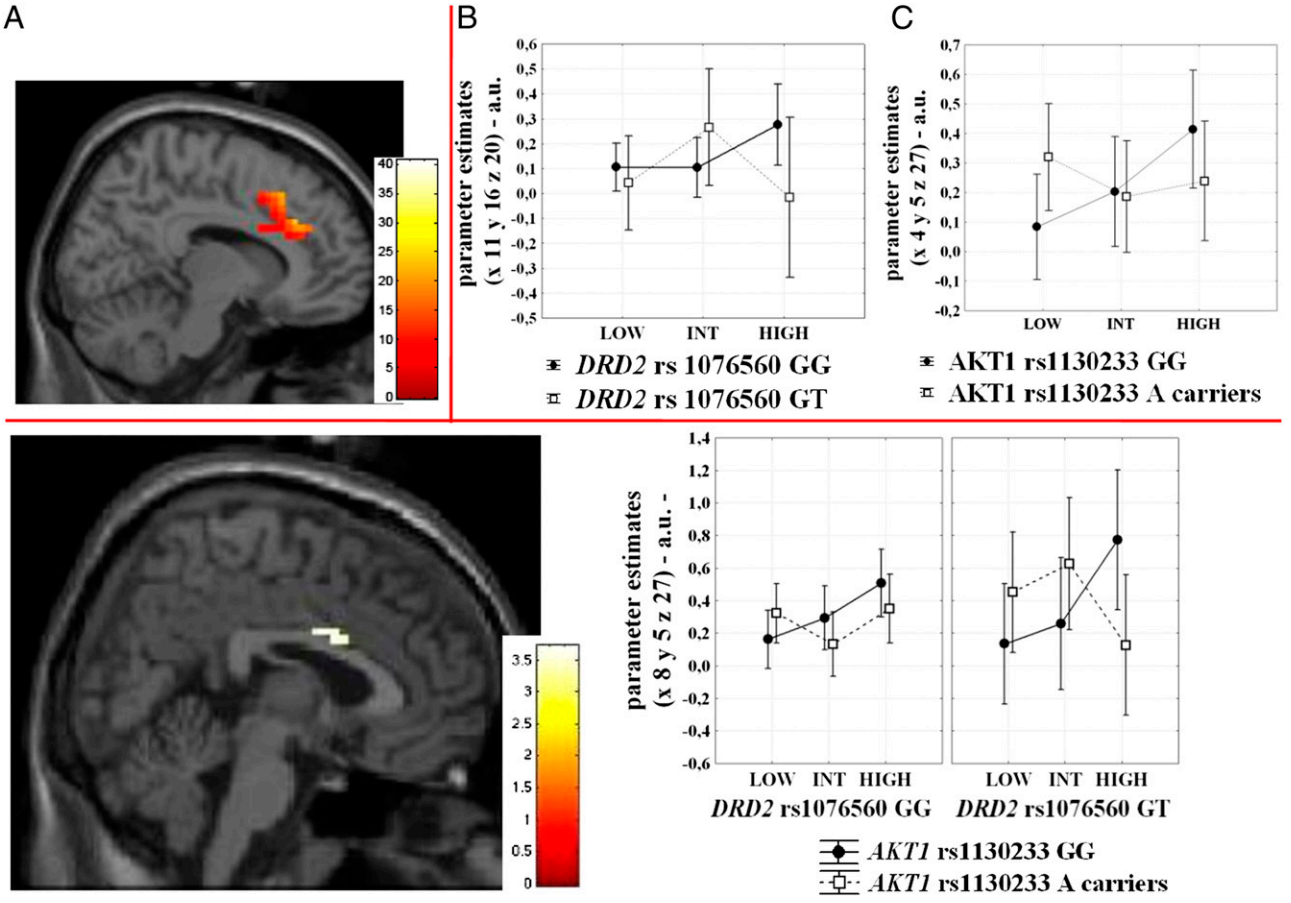

Fig. 2. (*Upper*) (*A*) Sagittal section of the brain illustrating the effect of load in the cingulate cortex during performance of the VAC (local maxima: $x = 8$; $y = 30$; $z = 26$). Color bar represents t values. (*B* and *C*) Graphs illustrating parameter estimates extracted from the clusters in the cingulate cortex showing the *DRD2* rs1076560 by load (local maxima: $x = 11$; $y = 16$; $z = 20$) (*B*) and the *AKT1* rs1130233 by load (local maxima: $x = 4$; $y = 5$; $z = 27$) (*C*) interaction. (*Lower*) Sagittal section of the brain showing the cingulate cluster associated with the *DRD2* rs1076560 by *AKT1* rs1130233 by load interaction during performance of the VAC task, and relative parameter estimates to illustrate load dependent differences between genotype groups. A drop in cingulate activity at the greater attentional load was present in *DRD2* GT/*AKT1* A carriers. See text for statistics. Color bar represents t values.

DRD2 rs1076560 or of *AKT1* rs1130233 was found. However, several interactions between genotypes and load were present. There was a significant interaction between *DRD2* genotype and load ($x = 11$; $y = 16$; $z = 20$, BA24, $k = 7$, $Z = 3.27$), and between *AKT1* genotype and load ($x = 4$; $y = 5$; $z = 27$, BA24, $k = 28$, $Z = 4.30$) on cingulate activity. Of note, an interaction between *DRD2* rs1076560, *AKT1* rs1130233, and load was also found in this brain area ($x = 8$; $y = 5$; $z = 27$, BA24, $k = 10$, $Z = 3.65$) (Fig. 2). Posthoc analysis on parameter estimates extracted from this cluster was performed to illustrate load-dependent differences among genotype groups. This investigation revealed that cingulate responses at the higher attentional control load were greater than those at the intermediate attentional level in all groups (all $P < 0.02$) but in *DRD2* GT/*AKT1* A carriers, who displayed reduced activity at the higher load ($P = 0.008$) (Fig. 2). Furthermore, between-group differences in cingulate activity were also evident at the higher attentional load when comparing DRD2 GT/AKT1 A carriers vs. *DRD2* GT/*AKT1* GG ($P = 0.02$) and *DRD2* GG/*AKT1* GG subjects ($P = 0.09$) (Fig. 2).

Association of DRD2 rs1076560 and AKT1 rs1130233 Genotypes with Performance During Sustained Attention in Healthy Subjects. In the Continuous Performance Test (CPT) sample, there were no genotype effects on demographics (all $P > 0.1$). ANOVA on correct responses at the CPT revealed a main effect of *DRD2* rs1076560 ($F_{1,154} = 10.2$; $P = 0.001$), a main effect of *AKT1* rs1130233 ($F_{1,154} = 6.3$; $P = 0.01$), and an interaction between *DRD2* and *AKT1* genotypes ($F_{1,154} = 5.1$; $P = 0.02$). Posthoc analysis indicated that *DRD2* GT/*AKT1* A carriers have reduced number of correct responses relative to all other genotype groups (all $P < 0.007$) (Fig. 3). No significant effects were found on reaction time data (all $P > 0.2$).

Association of DRD2 rs1076560 and AKT1 rs1130233 Genotypes with Response to Treatment with Olanzapine in Patients with Schizophrenia. Genotype groups were matched in terms of demographics, mean olanzapine dose (mg 21.1 ± 7.5), and baseline Positive and Negative Syndrome Scale (PANSS) scores (all $P > 0.1$). ANOVA on the difference between PANSS total scores at 56 and 0 d of olanzapine treatment indicated a main effect of *DRD2* rs1076560 ($F_{1,62} = 3.99$; $P = 0.05$), a main effect of *AKT1* rs1130233 ($F_{1,62} = 3.94$; $P = 0.05$), and an interaction between *DRD2* and *AKT1* genotypes ($F_{1,62} = 5.42$; $P = 0.02$). Posthoc analysis revealed greater difference in symptom scores in *DRD2* GT/*AKT1* A carriers relative to all other groups (all $P < 0.02$) (Fig. 4). Exploratory ANOVAs were also performed on PANSS subscales. Negative symptoms scores revealed a main effect of *DRD2* rs1076560 ($F_{1,62} = 7.39$; $P = 0.008$) and a *DRD2* by *AKT1* genotype interaction ($F_{1,62} = 3.76$; $P = 0.05$). Posthoc analysis indicated greater improvement in *DRD2* GT/*AKT1* A carriers relative to all other groups (all $P < 0.05$). General psychopathology symptoms scores revealed an interaction between *DRD2*

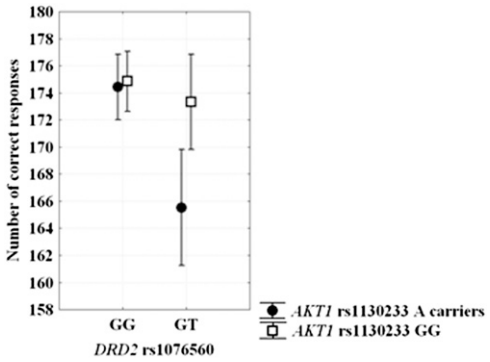

Fig. 3. Graph showing *DRD2* rs1076560 by *AKT1* rs1130233 interaction on the number of correct responses at the CPT. *DRD2* GT/*AKT1* A carriers had lower accuracy relative to all other genotypes groups. See text for statistics.

and *AKT1* genotypes ($F_{1,62} = 4.38$; $P = 0.04$), with greater improvement in *DRD2* GT/*AKT1* A carriers relative to other genotype groups ($P < 0.04$). No other statistically significant effects were found on PANSS scores.

CPT scores were available for 61 patients with schizophrenia (*DRD2* GG/*AKT1* GG $n = 29$; 23 *DRD2* GG/*AKT1* A carriers $n = 23$; *DRD2* GT/*AKT1* GG $n = 5$; *DRD2* GT/AKT1 A carriers $n = 4$). ANOVA indicated a trend for an effect of *DRD2* rs1076560 ($F_{1,57} = 2.3$; $P = 0.1$), a main effect of *AKT1* rs1130233 ($F_{1,57} = 4.8$; $P = 0.03$), and an interaction between *DRD2* and *AKT1* genotypes approaching significance ($F_{1,57} = 3.5$; $P = 0.06$). Further exploratory posthoc analysis showed that *DRD2* GT *AKT1* A carrier subjects have greater improvement in the number of CPT correct responses relative to other genotype groups (all $P < 0.05$) (Fig S1).

Discussion

The present results consistently suggest epistatic effects of *DRD2* rs1076560 and *AKT1* rs1130233 genotypes on multiple, progressively more distant and complex phenotypes. In particular, we found in peripheral blood mononuclear cells (PBMCs) of healthy humans that these genetic variants interact in conferring individual variability in AKT1 protein levels and phosphorylation of GSK-3β. Furthermore, interaction of these two genotypes is associated with cingulate activity and behavior during attentional tasks in healthy subjects, as well as with response to 8 wk of olanzapine treatment in patients with schizophrenia in terms of symptoms scores and, to a limited extent, attentional behavior.

Our molecular results suggest specific effects of *DRD2* rs1076560 and *AKT1* rs1130233 genotypes on the cAMP-independent D2 signaling cascade, which is a crucial pathway for intraneuronal transduction of dopamine signaling (41). More specifically, consistent with knowledge that phosphorylation of AKT1 is a process concerted between D2 receptors with the β-arrestin 2/phosphatase PP2A complex, we did not find association between *DRD2* and *AKT1* genotypes and AKT1 phosphorylation at Ser473. On the other hand, our data indicate that *DRD2* GT/*AKT1* A carrier individuals have reduced AKT1 protein levels. Other studies (39) have also demonstrated that the A allele of *AKT1* rs1130233 is associated with reduced expression of AKT1 relative to the G allele. Furthermore, previous reports have indicated greater relative measures of D2L ratio in *DRD2* rs1076560 T allele subjects (15), suggesting relative greater D2L signaling in these individuals compared with GG. Therefore, genetically determined increase in D2L signaling in *DRD2* rs1076560 T allele subjects may determine molecular mechanisms leading to a further relative decrease in AKT1 protein within a genetic context already favoring lower expression of this protein (the *AKT1* rs1130233 A allele). Importantly, we have demonstrated these molecular effects in PBMC rather than in neurons, and this is a limitation of the present results. However, even considering the physiological difference between lymphocytes and neurons, some speculative inferences are possible based on previous studies and on the present findings. In particular, the previously demonstrated relative greater expression of D2L in brain tissue of *DRD2* rs1076560 T carriers (15), together with data indicating that D2L knock-out mice display greater AKT1 activity in the brain (28), support relevance of the genetic interaction for D2 signaling transduction in the neuron, where D2L is the main mediator of D2 signaling at the postsynaptic level (21).

Our results on genetic interaction between D2 and AKT1 are in line with and further substantiated by the effect of phosphorylation of GSK-3β. GSK-3β is an important molecular target downstream of AKT1 and is involved in a series on mechanisms of gene expression, including inactivation of β-catenin (26, 42). Previous studies have indicated that AKT1 signaling inhibits GSK-3β activity via phosphorylation (43). Indeed, we found that *DRD2* GT/*AKT1* A carrier individuals also have reduced phosphorylation of GSK-3β, although no difference was evident on GSK-3β protein levels. Therefore, all these results together further suggest downstream functional effects of the interaction between *DRD2* rs1076560 and *AKT1* rs1130233 genotypes on GSK-3β within the cAMP independent pathway. GSK-3β phosphorylation may in turn affect regulation of gene-expression mechanisms of neurodevelopment and synaptic growth, which may be altered in schizophrenia (44). Consistently, previous studies have found reduced AKT1 levels and phosphorilated GSK-3β (31) in patients with schizophrenia. Our results suggest that these earlier findings may be also because of the interaction between genetic variation in *DRD2* and *AKT1* genes, previously associated with schizophrenia phenotypes (23, 39). Further studies addressing these effects at the neuronal level are needed to confirm these speculations.

Our fMRI data in healthy subjects also indicate a specific interaction between *DRD2* rs1076560, *AKT1* rs1130233, and load on cingulate activity during attentional control processing. The relationship between increasing load of attentional control and activity in anterior cingulate is linear, increasing from lower to higher loads (14, 17). This relationship was not evident in *DRD2* GT/*AKT1* A carrier individuals whose activity in anterior cingulate dropped off from the intermediate to the higher level of attentional control. This pattern of response is strongly reminiscent of the results we have recently reported in patients with schizophrenia, in whom a similar drop of cingulate responses was present at the high attentional control load (17).

Behavioral results during attentional processes as elicited by the CPT were consistent with these physiological data. Here, *DRD2* GT/*AKT1* A carrier subjects had reduced accuracy relative

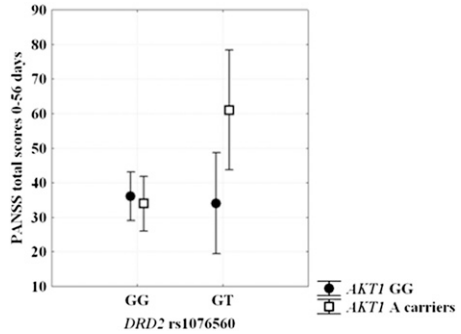

Fig. 4. Modulation by *DRD2* rs1076560 and *AKT1* rs1130233 of response to 8 wk of olanzapine treatment. *DRD2* GT/*AKT1* A carriers showed greater PANSS total scores improvement relative to all other genotypes groups. See text for statistics.

to all other genotype configurations, further supporting the detrimental role of the interaction between these genetic variants for attentional processes in healthy subjects. Previous data have indicated that relatively greater D2 signaling is associated with less filtering of information flow and blocking of distracting inputs (10). Thus, greater relative D2 postsynaptic signaling possibly associated with lower D2S/L ratio in *DRD2* rs1076560 T carriers may interact with genetically determined lower expression of *AKT1* rs1130233, also previously associated with cognitive inefficiency (39), in determining altered processing of attentional inputs, which indeed characterizes schizophrenia (16).

As suggested by previous models (10), the relationship between genetic modulation of D2 signaling and cognitive processing may also relate to clinical symptoms of schizophrenia. In particular, the net effect of relatively predominant D2 vs. D1 stimulation is of reducing inhibition of neuronal network activity, which may lead to easier access of inputs into cognitive buffers. More specifically, greater D2 signaling may be associated with reduced filtering of information, with reduced blocking of distracting inputs and with multiple network representations, thus overloading cortical processing with too much information. This physiological state may lead to less optimal cognitive processing as well as to possible coexistence of multiple cognitive representations, both internally generated or driven by environmental stimuli (10). Consistently, other models have also attributed a role to dopamine in conferring salience to internal representations or external stimuli (45). Altered dopamine signaling may drive to altered attribution of salience to these stimuli or representations; antipsychotic treatment targeting D2 receptors may dampen such aberrant physiology associated with dopamine signaling (45).

Relevance of the impact of *DRD2* rs1076560 and *AKT1* rs1130233 variants for schizophrenia is further suggested by our data on their interaction on response to antipsychotic treatment. In this case, *DRD2* GT/*AKT1* A carrier individuals with schizophrenia had better response after 8 wk of olanzapine monotherapy. This effect was statistically significant in term of PANSS total, negative symptoms, and general psychopathology scores. These results are in line with the notion that olanzapine blocks D2 signaling and with data showing that second generation antipsychotics activate AKT1 (33) or mimic AKT1 activity increasing GSK-3β phosphorylation (46). These results are also consistent with a similar, albeit weaker, effect found on the CPT number of correct responses.

Interestingly, patients with schizophrenia carrying the two "risk" alleles (*DRD2* T and *AKT1* A) had better response to treatment with olanzapine, a beneficial effect that would seem at odds with the effects of these two alleles in healthy subjects. This finding can be interpreted in two ways, which are not mutually exclusive. First, patients with this genotype configuration may respond better because their dopamine cAMP-independent pathway is more profoundly altered in terms of dopamine D2 signaling (24), and thus there is more "room" for improvement by treatment with a drug which specifically acts on it (33). Second, it is possible that genetic variants interact with dysregulated levels of dopamine in patients, determining an effect which is not immediately derived by studying healthy subjects only (47, 48). Both these explanations are speculative and have to be treated with caution. Nonetheless, to our knowledge, this a unique demonstration in humans of the involvement of the D2-AKT1 signaling pathway in modulating the effect of antipsychotic treatment.

Previous theories have hypothesized that dopamine dysregulation may characterize the pathophysiology of schizophrenia. More specifically, dopamine levels may be reduced in the cortex (especially prefrontal) but they may be increased in the striatum (44). In an earlier longitudinal study we have reported that treatment with olanzapine in patients with schizophrenia is associated with attenuated improvement in subjects carrying the *COMT* Valine allele (49, 50). In the present study, we report that the same treatment protocol is associated with greater improvement in patients with schizophrenia carrying the two risk alleles in *DRD2* and *AKT1*. In other words, the risk allele is associated with poorer response when evaluating a gene controlling cortical dopamine (*COMT*), although it is associated with better response when the genes in question are more expressed in the striatum (*DRD2*, *AKT1*). These apparently incongruent findings may be reconciled if examined in the context of the hypothesized dysregulation/imbalance between cortical and subcortical dopamine.

In conclusion, the present results suggest that the interaction between genetic factors conferring risk for impairment in the D2-AKT1 signaling pathway may be relevant for the understanding of correlates of the pathophysiology of schizophrenia at the molecular, neuronal networks, and behavioral level. These aspects should also be taken into account to disambiguate mechanisms associated with individual response to antipsychotic treatment in schizophrenia.

Materials and Methods

A total of 190 healthy subjects and 66 patients with schizophrenia were included in this study (see *SI Materials and Methods* for inclusion and exclusion criteria). All subjects underwent one or more of the below described procedures. Furthermore, the subjects were genotyped for *DRD2* rs1076560 and *AKT1* rs1130233, as specified in *SI Materials and Methods*.

Association of DRD2 rs1076560 and AKT1 rs1130233 Genotypes with AKT1 and GSK-3β Protein Levels and Phosphorylation in PBMC of Healthy Humans. Based on previous literature indicating D2 expression in T cells and on relevance of D2 receptor signaling for T-cell normal metabolism and function (51, 52), we explored the potential impact of *DRD2* and *AKT1* genetic variants on cAMP independent D2 signaling cascade in human PBMC. Blood samples were drawn from 29 healthy individuals (18 females, mean age ± SD 26.8 ± 4.8; *DRD2* GG/*AKT1* A carriers $n = 7$; *DRD2* GG/*AKT1* GG $n = 8$; *DRD2* GT/*AKT1* A carriers $n = 6$; *DRD2* GT/*AKT1* GG $n = 8$) from the larger group enrolled in this study. AKT1, P-Ser473-AKT1, GSK-3β, P-Ser9-GSK-3β were quantified as specified in *SI Materials and Methods*. Factorial ANOVA was then used for statistical analysis on averaged and normalized proteins values.

Association of DRD2 rs1076560 and AKT1 rs1130233 Genotypes with Cingulate Cortex Activity During VAC in Healthy Subjects. Seventy-three healthy subjects (Table 1) were enrolled to evaluate the association of *DRD2* rs1076560 and *AKT1* rs1130233 with brain activity during VAC processing. All subjects underwent fMRI while performing the VAC task, which elicits increasing demand for attentional control and which was identical to that published in previous studies (14, 15, 17, 20). This task allows investigation of brain activity during three levels of attentional control (low, intermediate, high), which were obtained manipulating both the relative directions of arrows with different sizes and the related cue words (*SI Materials and Methods*).

Functional MRI was performed on a GE Sigma 3T scanner (*SI Materials and Methods*). Analysis was completed using Statistical Parametric Mapping 5 (SPM5 -http://www.fil.ion.ucl.ac.uk/spm). After single-subject processing (*SI Materials and Methods*), a random-effects ANOVA was performed to investigate the main effect of increasing level of attentional control, of *DRD2* rs1076560 genotype, of *AKT1* rs1130233 genotype, and their interaction. Based on previous data demonstrating association of cingulate activity with other dopamine genes using the present task (20), as well as on findings of abnormal activity in this brain region in patients with schizophrenia during attentional processes (17), we focused our analyses on cingulate blood-oxygen level-dependent (BOLD) responses (see *SI Materials and Methods* for cluster localization). We used a statistical threshold of $P < 0.001$, minimum cluster size (k) = 5, with further family-wise error small-volume correction at $P < 0.05$ applied on the activated clusters, using the cingulate as the volume of interest as defined by the WFU_PickAtlas (http://fmri.wfubmc.edu/cms/software#PickAtlas) (see *SI Materials and Methods* for clusters localization). To further explore load dependent differences between genotype groups, posthoc analysis with Fisher's test outside of SPM was also used on BOLD responses extracted from the cluster showing significant genotypes by load interaction using MarsBar (http://marsbar.sourceforge.net/). ANOVAs and χ^2 were used to compare demographics and behavioral data. Fisher's test was used for posthoc analyses.

Association of DRD2 rs1076560 and AKT1 rs1130233 Genotypes with Performance During Sustained Attention in Healthy Subjects. One-hundred seventy-six healthy subjects (Table 1), partially overlapping ($n = 59$) with those included in the fMRI study, performed the A-X version of the CPT, which is a measure of selective attention and context processing (53) (*SI Materials and Methods*).

Performance data were recorded as the number of correct responses and reaction time. ANOVAs and χ^2 were used to compare demographics and behavioral data. Fisher's test was used for posthoc analyses.

Association of DRD2 rs1076560 and AKT1 rs1130233 Genotypes with Response to Treatment with Olanzapine in Patients with Schizophrenia. Sixty-six patients with schizophrenia with current exacerbation of symptoms requiring hospitalization (Table 1) and who had been drug-free for at least 1 wk or 1 mo if under depot medication, were treated for 8 wk with olanzapine monotherapy (50). Titration was allowed for the first 4 wk. Then, the dose was kept constant until 8 wk of treatment. Symptoms were assessed at study entry (day 0) and at day 56 (8 wk) with the PANSS by a trained psychiatrist, who was blind to genotype. The CPT (see above) was also administered after 7 and 56 d of treatment to investigate the effect of olanzapine treatment on behavior associated with sustained attention.

ANOVA and χ^2 were used as appropriate to compare demographics and mean dose of olanzapine. The difference between PANSS total scores at 56 and 0 d as well as between CPT scores at 56 and 7 d of olanzapine treatment was entered into factorial ANOVA with *DRD2* and *AKT1* genotypes as predictors. Further exploratory factorial ANOVAs were performed on PANSS subscales. Fisher's test was used for all posthoc analyses.

ACKNOWLEDGMENTS. We thank Riccarda Lomuscio and Rita Masellis for help in data acquisition, all the people who participated to this study, and Paolo Stratta and Alessandro Rossi for making the computerized version of the Continuous Performance Test available to us. This study was supported in part by a National Alliance for Research on Schizophrenia and Depression Young Investigator award (to G.B.) and a research award (to A.B.) by Fondazione Cassa di Risparmio di Puglia. A.U. represents the Mariano Scippacercola Foundation.

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