Title
Variations in Dysbindin-1 are associated with cognitive response to antipsychotic drug treatment

Permalink
https://escholarship.org/uc/item/3w10w6s7

Journal
NATURE COMMUNICATIONS, 9

ISSN
2041-1723

Authors
Scheggia, D
Mastrogiacomo, R
Mereu, M
et al.

Publication Date
2018-06-11

DOI
10.1038/s41467-018-04711-w

License
CC BY 4.0

Peer reviewed
Variations in Dysbindin-1 are associated with cognitive response to antipsychotic drug treatment

Diego Scheggia, Rosa Mastrogiacomo, Maddalena Mereu, Sara Sannino, Richard E. Straub, Marco Armando, Francesca Managò, Simone Guadagna, Fabrizio Piras, Fengyu Zhang, Joel E. Kleinman, Thomas M. Hyde, Sanne S. Kaalund, Maria Pontillo, Genny Orso, Carlo Caltagirone, Emiliana Borrelli, Maria A. De Luca, Stefano Vicari, Daniel R. Weinberger, Gianfranco Spalletta & Francesco Papaleo

Antipsychotics are the most widely used medications for the treatment of schizophrenia spectrum disorders. While such drugs generally ameliorate positive symptoms, clinical responses are highly variable in terms of negative symptoms and cognitive impairments. However, predictors of individual responses have been elusive. Here, we report a pharmacogenetic interaction related to a core cognitive dysfunction in patients with schizophrenia. We show that genetic variations reducing dysbindin-1 expression can identify individuals whose executive functions respond better to antipsychotic drugs, both in humans and in mice. Multilevel ex vivo and in vivo analyses in postmortem human brains and genetically modified mice demonstrate that such interaction between antipsychotics and dysbindin-1 is mediated by an imbalance between the short and long isoforms of dopamine D2 receptors, leading to enhanced presynaptic D2 function within the prefrontal cortex. These findings reveal one of the pharmacodynamic mechanisms underlying individual cognitive response to treatment in patients with schizophrenia, suggesting a potential approach for improving the use of antipsychotic drugs.
Antipsychotics are the first-line and most widely used medications for the management of schizophrenia spectrum and other psychotic disorders. In agreement with the heterogeneous nature of these disorders, clinical responses to antipsychotics drugs are highly variable. Thus, clinical guidelines strongly recommend adapting antipsychotic treatments to each individual case. However, to date, only very scarce biomarkers exist to implement more effective and personalized healthcare.

Efforts have been made to improve the assessment and definition of treatment-resistant schizophrenia. However, the identification of criteria for schizophrenia cognitive deficits is still incomplete, in part because of the lack of cognitive symptom domains in the most widely used clinical rating scales (e.g., the PANSS, BPRS, SANS, and SAPS). Nonetheless, the consensus is that clinical responses for negative symptoms and cognitive impairments are suboptimal and highly variable. Notably, cognitive deficits are considered one of the main sources of disability, having the most critical impact on public health and long-term outcomes. Treatments with first- and second-generation antipsychotics produce small neurocognitive improvements in both chronic and first-episode schizophrenia patients. Nevertheless, cognitive responses to antipsychotic drugs show marked interindividual variability, and the mechanism of this seemingly unpredictable variability is unknown.

All antipsychotic drugs interact with dopamine D2 receptors, with variable ranges of D2 occupancy suggested to be important for optimal clinical and cognitive responses. From a pharmacokinetic perspective, genetic variations such as those in CYP2D6, CYP3A4/5, and ABCB1 might impact the metabolism and distribution of antipsychotic drugs, potentially affecting the margin between the dosages that are required for efficacy and those associated with side effects. Shifting to a pharmacodynamic perspective, genetic variations influencing D2 receptors could in principle also influence the efficacy of antipsychotic drugs. Genetic variations in the dystrobrin binding protein 1 (DTNBP1) gene, encoding dysbindin-1 (Dys), a synaptic protein regulating synaptic vesicles and receptors recycling, can alter D2 receptor availability. Moreover, genetic variations in Dys might interfere with the dopaminergic system, altering cognitive functions in both mice and healthy humans.

Mechanistically, using lentiviral-vector-mediated microRNA (miR) silencing and neurochemical strategies for region-specific investigation in mice and postmortem human brain gene expression analyses, we demonstrate that this interaction between antipsychotics and Dys relies selectively on the function of dopamine D2 receptors within the prefrontal cortex (PFC). In particular, Dys genetic reduction is associated with an antipsychotic-dependent increase in the ratio between the D2Short (D2S) and D2Long (D2L) isoforms in the PFC of both humans and mice, which results in a potentiation of cortical presynaptic D2 signaling. A genetic approach in mice demonstrates that the D2S/D2L imbalance in favor of D2S is the cause of the cognitive improvements that occur in response to antipsychotic administration in the context of reduced Dys. Certainly, Dys is not expected to be the only relevant molecular probe involved in responses to antipsychotic drugs. Nevertheless, our findings provide another step toward personalized treatment for schizophrenia, suggesting Dys-related mechanisms as a tool for a more focused approach to alleviating cognitive disabilities based on a defined biological mechanism that moderates the response to antipsychotics treatment.

Results
Dys-antipsychotics interaction in human executive functions. We first investigated whether functional Dys genetic variants would differentiate cognitive abilities in patients with schizophrenia undergoing chronic antipsychotic drug treatment. We used a three-marker (rs2619538-rs3213207-pr047631) haplotype at the DTNBP1 gene locus (Dys Hap) previously associated with a pattern of cognitive-related PFC functional activation consistent with reduced Dys in mouse models. RNA sequencing from the dorsolateral PFC (DLPFC) of 594 human subjects demonstrated that carriers of the Dys Hap (T-A-A), hereinafter referred to as Dys Hap+/− and −/− (demographic details in Supplementary Table 1 and genotype frequencies in Supplementary Table 2), had reduced Dys expression (Fig. 1a) compared to Dys Hap++/− individuals. We recruited 259 patients receiving chronic treatment with antipsychotic medication, and we assessed their executive function abilities, as these are cognitive hallmarks of schizophrenia. Notably, executive function deficits in patients with schizophrenia are particularly evident in extradimensional set shifting (EDS) and in the analogous category shift of the Wisconsin Card Sorting Task (WCST). We found that Dys Hap++/− and −/− patients, with putatively reduced expression of Dys, performed better than Dys Hap+/− patients (Fig. 1b–d). In particular, Dys Hap−/− patients made fewer perseverative (Fig. 1b) and non-perseverative errors (Fig. 1c) and completed more WCST categories (Fig. 1d) than Dys Hap+/− patients did. Similarly, Dys Hap+−/− patients made fewer non-perseverative errors than Dys Hap++/− patients did. No Dys Hap-dependent differences were present in demographic characteristics, PANSS scores, or the duration or dosage of current antipsychotic treatments (Supplementary Table 1). All SNPs were in Hardy–Weinberg equilibrium (HWE; Supplementary Table 2).

To check for replicability in an independent cohort of subjects, we next investigated this association using the neurocognitive battery used in the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE), a double-blind multicenter trial sponsored by the National Institute of Mental Health (NIMH) to assess responses to antipsychotic drugs. Each patient was randomly assigned to treatment with a single antipsychotic drug, and any previous therapy was discontinued during the first 2 weeks of double-blind treatment. After a baseline assessment, patients were followed for up to 18 months. We used a sample of 359 patients who completed the study and for whom genetic data were available. We found that Dys Hap−/− and +/− patients, characterized by reduced Dys expression, displayed a better WCST performance compared to Dys Hap++/+ patients (Fig. 1e and Supplementary Table 3). Thus, the CATIE study confirmed that genetic variations associated with reduced Dys are associated with better executive function response to antipsychotic treatment in patients with schizophrenia.
Antipsychotics improves cognition based on Dys genetics. To reduce the possibility that multidrug treatments and the long duration of the illness could have confounded the results, we recruited another independent cohort of patients with schizophrenia in their first episode of psychosis. These patients were naive to medication when recruited and were treated for the first time with one antipsychotic (risperidone or aripiprazole) for 4 weeks, then evaluated with the WCST. Similar to chronic patients, first-episode patients with the Dys haplotype had better attentional set-shifting abilities than non-carriers (Dys Hap+/−) (Fig. 2a-c).

Ideally, it would have been interesting to have an untreated or placebo group of patients with schizophrenia never treated with antipsychotics. However, to recruit a sufficient number of these subjects for further genetic stratification would be challenging and would involve a number of ethical issues. Thus, we used mice to test the possibility that genetic variations selectively reducing Dys expression would confer executive function benefits following administration of antipsychotics. We tested mice with reduced levels of dysbindin-1 (Dys Hap+/−) and control littermates (Dys Hap+/+) using the Intra-/Extra-Dimensional Attentional Set-Shifting task (ID/ED Operon task)42, which is equivalent to the human WCST. Starting from 2 weeks before the test, mice were treated with risperidone, a commonly used antipsychotic, or with vehicle. Chronic treatment with risperidone not only rescued the attentional set-shifting impairment of Dys+/− mice but also improved their EDS performance compared to that of vehicle-and risperidone-treated Dys+/− mice (Fig. 2d-f and Supplementary Fig. 1a-c). Risperidone treatment had no effect on Dys+/+ mice (Fig. 2d-f and Supplementary Fig. 1a-c). We further tested whether any residual effect of injections and manipulations might have existed. Non-manipulated mice with genetically reduced Dys showed a selective deficit in EDS abilities compared to control wild-type littermates (Fig. 3a-c and Supplementary Fig. 2a-c). Furthermore, as predicted by these mouse data, healthy Dys Hap−/− humans (demographic characteristics in Supplementary Table 1) showed a cognitive disadvantage in attentional set-shifting on the WCST compared to matched healthy Dys Hap+/+ subjects (Fig. 3d-f). These results support the conclusion that genetic variations reducing Dys may improve the responsiveness of executive function to treatments with antipsychotic drugs.

Cortical D2 are necessary for Dys-antipsychotics effects. The mechanistic basis of the unpredictable variability in clinical responses to antipsychotics is still largely unexplored. Prompted by the behavioral effects, we sought to identify a mechanism for this pharmacogenetic interaction. We hypothesized that the antipsychotics-dysbindin-1 interaction might converge on dopamine D2 signaling within the PFC. Indeed, (1) D2 receptors are a common target of antipsychotic drugs45; (2) cortical D2 receptors, but not D1 receptors, are modulated by Dys genetic variations18,19; (3) down-regulation of Dys modules sensitivity to D2-like agonists20,44; (4) genetic variations of Dys interfere with the dopaminergic system, leading to altered cognition in both mice and healthy humans45; (5) as in humans, the selective alteration in EDS performance in mice points to involvement of the mPFC45; (6) no genotype, treatment and genotype-by-treatment effects were evident in Dys, D1, glutamatergic, and serotonergic receptors expression in the mPFC (Supplementary Fig. 3 and Supplementary Table 4). Nevertheless, antipsychotics target multiple receptors45, and D1 receptor signaling has been implicated more than D2 in PFC-dependent cognitive functions46. Thus, to assess the selective role of D2 signaling, we inactivated D2 receptors in Dys+/− mice by bilaterally injecting into the mPFC a lentivirus delivering a synthetic miR coupled to a GFP tag. Two weeks later, the virus-injected mice were chronically treated with risperidone or vehicle and then tested in the ID/ED task (Fig. 4a). Silencing D2 receptors in the mPFC of Dys+/− mice was sufficient to eliminate the beneficial effect of risperidone on attentional set-shifting abilities (Fig. 4b,c and Supplementary Fig. 4). This demonstrates that the dysbindin-antipsychotic interaction depends on D2 functioning in the mPFC.

Antipsychotic-Dys interaction potentiates presynaptic D2. We then examined the effect of antipsychotic treatment on cortical D2 signaling in subjects with reduced levels of Dys. In mice, Dys reduction, risperidone treatment and their interaction all failed to affect the expression of D2 (Fig. 5a) in the mPFC. However,
because D2 receptors exist in two different splicing isoforms, we also assessed whether the dysbindin-antipsychotics interaction might differently impact the D2L and D2S isoforms. Chronic treatment with risperidone increased the D2S/D2L ratio in the mPFC of Dys+/− but not Dys+/+ mice (Fig. 5b). To investigate whether a similar effect could be observed in the human brain, we analyzed the gene expression of D2 receptor isoforms in a sample (n = 101) of DLPFC tissue from patients with schizophrenia. As in mice, we found that the D2S/D2L ratio was increased in Dys Hap+/− patients if and only if antipsychotic treatments could be...
The D2S isoform is the predominant D2 presynaptic autoreceptor, providing negative feedback control of dopamine synthesis and release. Moreover, synaptic secretion of accumulated antipsychotics exerts an autoinhibitory effect on vesicular exocytosis, affecting neurotransmitter release. Thus, to assess the functional and dynamic consequences of the dysbindin-by-antipsychotics interaction for the cortical D2S/D2L ratio, we performed in vivo microdialysis in the mPFC of freely moving Dys+/- and +/+ mice after chronic risperidone or vehicle treatments (Fig. 6a). Risperidone treatment in Dys+/- mice restored basal dopamine to wild-type levels while having no effect in Dys+()+ Fig. 6b). Notably, infusion of the D2-prefering agonist quinpirole in the mPFC by reverse dialysis unraveled a larger reduction of dopamine release in Dys+/- than in +/+ following risperidone treatment (Fig. 6c), but not in vehicle-treated mice (Fig. 6d). This enhanced presynaptic D2 response in Dys+/- mice was selectively dependent on the effects of risperidone on mPFC D2 receptors, as mPFC lentiviral D2 silencing abolished it (Fig. 6e). These results provide in vivo evidence supporting the increased D2S/D2L ratio and demonstrate that risperidone treatment enhanced cortical presynaptic D2 functioning exclusively when Dys was reduced. These results add important insights into previous evidence showing that potentiation of D2 pathways in the PFC of humans, monkeys, and rodents might facilitate executive functions.

Increased D2S/D2L ratio in Dys improves executive functions.

To demonstrate selectively that a D2S/D2L imbalance in favor of D2S is causally linked to the improved executive functions found in the context of reduced Dys, we backcrossed Dys+/- mutants with D2L+/- mice (Fig. 7a). D2L+/- mice have genetically reduced expression of the D2L isoform and upregulation of D2S. In agreement with this expression pattern, we confirmed detected after postmortem analysis (Fig. 5c). The expression of D2 receptor isoforms was not altered by the Dys Hap in medication-naive healthy subjects (n = 199; Fig. 5d). Thus, we uncovered an interaction between antipsychotic drug treatments and Dys genetic variations in the PFC that alters the expression of D2 receptor isoforms in favor of D2S.
Injections of Veh or Ris for 28 days (to parallel behavioral experiment in Figs. 2, 4) and then have been implanted with a dialysis probe. On the following day in vivo microdialysis quinpirole-induced dopamine release was measured. In Dys−/− mice, the administration of risperidone (red dots) alters the balance between short and long isoforms of D2 receptors, resulting in the potentiation of D2 presynaptic signaling. Antipsychotic drugs accumulate in synaptic vesicles and are secreted from upon exocytosis. Moreover, antipsychotic drugs preferentially bind D2L postsynaptic receptors, which might cause D2L/D2S imbalance in favor of D2S autoreceptors. 6 Genetic variations reducing dysbindin-1 confer unique potentiation of cortical D2 autoreceptor activity following antipsychotics. a Localization of probe dialyzing portion within the mPFC and timeline of the experiment. Dys+/+ and Dys−/− mice received chronic vehicle or risperidone treatment and, after 4 weeks, were implanted with a dialysis probe for measurement of basal extracellular dopamine levels and quinpirole-induced dopamine release. b Increased basal extracellular dopamine levels in the mPFC of Dys−/− are restored by risperidone (two-way ANOVA, genotype × treatment, F(2, 27) = 4.61, p < 0.05; n = 6–7/group). p = 0.39 vs. Dys+/+. c Quinpirole infusion (gray area) equally decreased extracellular dopamine release in the mPFC of Dys+/+ (white circles) and Dys−/− (blue circles) mice after chronic treatment with Veh (two-way RM ANOVA, time, F(6, 60) = 2.83; p < 0.05), *p < 0.05 vs. baseline. d Dys−/− (blue circles) mice following chronic treatment with Ris (in red) showed higher efficacy of mPFC quinpirole infusion (gray area) in reducing extracellular dopamine release (two-way RM ANOVA, time × genotype, F(6, 60) = 5.76, p < 0.0005). Quinpirole had no effect on risperidone-treated +/+ mice (white circles; p = 0.38 vs. baseline). **p < 0.005 vs. Dys−/+. e Dys−/− mice received a synthetic microRNA (miR) to inactivate D2 receptors (LV-miR-D2, green circles) or a control miR (LV-miR-control, purple circles) in the mPFC. After 2 weeks, animals received daily injections of Veh or Ris for 28 days (to parallel behavioral experiment in Figs. 2, 4) and then have been implanted with a dialysis probe. On the following day in vivo microdialysis quinpirole-induced dopamine release was measured. In Dys+/− treated with Ris, D2 silencing even increased dopamine release. Injection of a control miR in Dys+/− Ris-treated mice further confirmed a decrease of quinpirole-mediated dopamine release (two-way RM ANOVA, time × group effect, F(12, 78) = 4.05, p < 0.0005; n = 5–7 each group). Error bars represent S.E.M. *p < 0.05, **p < 0.005. f Figure model. In basal drug-naïve conditions, genetic variations resulting in reduced dysbindin-1 expression increases tonic extracellular dopamine levels (black dots). Long-term administration of risperidone (red dots) alters the balance between short and long isoforms of D2 receptors, resulting in the potentiation of D2 presynaptic signaling. Antipsychotic drugs accumulate in synaptic vesicles and are secreted from upon exocytosis. Moreover, antipsychotic drugs preferentially bind D2L postsynaptic receptors, which might cause D2L/D2S imbalance in favor of D2S autoreceptors.

Discussion

The main finding of this study is that genetic variations associated with reduced Dys expression provide a background for a more favorable cognitive executive functions response to antipsychotic drugs. We show that this pharmacogenetic interaction mechanistically relies on an enhancement of presynaptic cortical dopamine/D2 signaling through an imbalance of the D2S and D2L isoforms.

The NIMH reports that the cost of treating patients with schizophrenia in the US is nearly $19 billion a year (one-fourth of all mental health costs). On the European continent, the annual mean cost per patient is estimated to range from approximately €7000 to €40,000, depending on the country. Antipsychotics are the frontline drugs for the management of schizophrenia and are effective in treating acute and chronic symptoms of schizophrenia, as well as reducing the risk of psychotic relapses, suicidal behavior, and hospitalization. However, the percentage of patients able to integrate in the community and experience stable remission is only 30%, especially due to cognitive deficits, which are especially resistant to treatment. Indeed, the effect of antipsychotic medications on cognitive function is less clear, despite the imbalance of the D2 isoforms towards elevated expression of D2S in the PFC of both D2L+/− mutant and Dys+/− × D2L+/− double mutant mice (Fig. 7b). We then tested the executive functions of wild-type, Dys+/−, D2L+/−, and double Dys+/− × D2L+/− littermates in the ID/ED Operon task. Double Dys+/− × D2L+/− mutant mice outperformed wild-type controls and Dys+/− animals exclusively in the EDS stage (Fig. 7c, d). By contrast, D2L+/− single mutants showed the same EDS performance as wild-type littermates (Fig. 7c, d). These findings demonstrate that the imbalanced D2S/D2L ratio produced by treatment with antipsychotics in mice with a genetic reduction of Dys leads to improvements in attentional set shifting.
the fact that cognitive impairments in schizophrenia are strong predictors of poor function and outcome.\textsuperscript{5,33} Moreover, the capacity for self-care is related to cognitive performance, and once hospitalization has occurred, the presence of cognitive impairment slows the rate of the overall clinical improvement, lengthening the hospital stay.\textsuperscript{36} Considering the overall highly heterogeneous population of patients with schizophrenia, it has been reported that treatment with first-generation and second-generation antipsychotics produces small but consistent neurocognitive improvements.\textsuperscript{6,7} Further investigations have highlighted that cognitive functions in patients with chronic schizophrenia show marked heterogeneity\textsuperscript{1,12}, spanning from normal to impaired. Many genetic and environmental factors likely underlie these variable responses to schizophrenia treatments. Our current findings highlight one of the mechanisms that could be used to identify a subset of patients with schizophrenia whose executive functions are likely to respond better to antipsychotics, based on Dys mechanisms. This could affect a significant number of patients, as the Dys haplotype is a common genotype (Supplementary Table 2). Ultimately, these results might be potentially applied to increase the effectiveness of antipsychotics and reduce the duration of the empirical testing often required to select appropriate medication or doses of antipsychotic drugs. Our findings are in keeping with the idea of improving the use of existing drugs,\textsuperscript{37} with the final goal of implementing a precision medicine approach.\textsuperscript{37} This study supports the idea that focusing on the genetics of individual patients rather than on the overall population is applicable to psychiatric disorders, analogous to what has been achieved with chemotherapeutic strategies in cancer genomics.

Our findings complement previous evidence that points to genetic variations influencing pharmacokinetic factors as a source of variability in the responses to antipsychotic drugs. In particular, risperidone is transformed primarily by hepatic metabolism via the cytochrome drug-metabolizing gene \textit{CYP2D6}\textsuperscript{38} to its major active metabolite, 9-hydroxyrisperidone or paliperidone. The \textit{CYP2D6} gene is highly polymorphic, resulting in large individual differences in \textit{CYP2D6} enzymatic activity.\textsuperscript{39,60} Similarly, genetic variants in \textit{CYP3A}, \textit{CYP1A2}, and the \textit{ABCB1} membrane transporter, P-glycoprotein, have also been suggested to regulate the bioavailability of antipsychotic drugs.\textsuperscript{61} Moreover, a recent study highlighted an interaction between pharmacogenetic factors and antipsychotic influences on potassium channel occupancy, with lower or higher binding being potentially detrimental to neurocognitive function.\textsuperscript{13,17} Moreover, it has been suggested that antipsychotic drugs preferentially bind D2L receptors.\textsuperscript{37,63} Thus, chronic preferential antagonism of D2L receptors might shift the D2L/D2S balance toward the D2S isoform. The elevated propensity toward this process brought by reduced Dys might be related to its impact on intracellular trafficking at the synaptic level. Indeed, antipsychotic drugs accumulate in synaptic vesicles and are secreted upon exocytosis.\textsuperscript{49} Moreover, Dys has an established role in rapid presynaptic modulation in synaptic homeostasis.\textsuperscript{64} Indeed, in vivo activity-dependent D2 functionality was enhanced in chronically treated Dys mutant mice, whereas it was dampened in wild-type animals. Finally, D2S receptors are internalized and desensitized more readily than D2L receptors.\textsuperscript{65–67} Overall, the unique enhanced functionality of presynaptic D2 receptors in the mPFC of subjects with Dys hypofunction only after treatment with antipsychotics adds to previous evidence showing that (1) potentiation of D2 pathways in the PFC of humans, monkeys and rodents might facilitate executive functions\textsuperscript{50–52} and that (2) the specific genetic deletion of presynaptic D2 receptors results in cognitive deficits...
and deficits of LTD expression, essential for spatial memory consolidation, novel spatial learning, and behavioral flexibility. Thus, presynaptic D2 could be a key element to consider in the long-term effects of antipsyychotic treatments and could provide insights into the failure of antipsychotics to ameliorate cognitive deficits in a large part of the population.

In conclusion, by translating our findings from mice and back again, we provide evidence for a biologically supported approach to antipsychotic treatment response. This might open insights into the failure of antipsychotics to ameliorate cognitive technologies.

Methods

Animals. All procedures were approved by the Italian Ministry of Health (permits n. 230/2009-B and 107/2015-PB) and local Animal Use Committee and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the European Community Council Directives. Three to 6-month-old male Dys heterozygous mutant mice were used for each experiment. Dys × D2L double mutant mice were used as a Taqman endogenous control assay for 8.

Mice quantitative real-time PCR

Mice samples were homogenized and total RNA was subsequently isolated with a PureLink RNA extraction Kit (Ambion, Life Technologies), used as a housekeeping (normalizing) gene. TaqMan assay kits included optimized concentrations of primers and probes to detect the target gene expression. Quantification of gene expression of D2L and D2L forms was performed with custom made primers (DR2 Short Forward primer: 5′-ACGGCCTCCATGCTGACCC-3′; DR2 Short Reverse primer: 5′-CGGAGCACCTTGATTGAGG-3′; DR2 Long Forward primer: 5′-ACGGGCTTTGCATGGA-3′; DR2 Long Reverse primer: 5′-GGTTGACAAGCTGCTGGATTG-3′; GAPDH Forward primer: 5′-AGTCGGTGT-GAAGGAATGG-3′ Reverse primer: 5′-TGTAAGACCATGTGATGGTGCA-3′). Total volume reaction was 25 µl using SyBr Green Master Mix reagent (Applied Biosystems); 1–5 µl of cDNA were used as template for the reaction, with 10 µM of primer sets and reverse primers. Relative gene expression was quantified with the ∆∆CT Comparative method.

Stereotaxic viral injection. A lentiviral vector (Lvp PKG eGFP-miR-D2, 10^11 TU/ml, ICM—Plaforme de Vectoriologie, Paris), coexpressing under the drive of the ubiquitous PKG promoter the eGFP and a miR, specifically directed against the mRNA of D2 (LV-miR-D2), was used to downregulate D2 receptors in the mPFC. Mice were infused bilaterally (0.4 µl/each site, rate 0.3 µl/min) into the mPFC, at two different antero-posterior sites and two dorso-ventral sites according to the Paxinos and Franklin mouse brain atlas (AP: 1.8 and 2.0, ML: ±0.3; DV: −2.4 and −2.9 from Bregma).

In vivo microdialysis. Concentric dialysis probe, with a dialysis portion of 2.0 mm, were prepared with AN69 fibers (Hospit Daco, Rologna, Italy). Mice were anesthetized with isoflurane and then placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA) for the probe implantation. The probe was implanted into the mPFC, according to the Paxinos and Franklin mouse brain atlas (AP: ±1.9; ML: ±0.1; DV: −3.0 from Bregma). Microdialysis sessions started 24 h after the surgical procedure. Two microdialysis probes were perfused with a Tyrode’s solution (12.2 mM NaCl, 4.0 mM KCl) at a constant flow rate of 1 µl/min. Collection of basal dialysate samples (20 µl) started 30 min after. After 60 min of basal sampling, a solution of Quinpirole 25 nM (Sigma, Dorset, UK) was administered through the probe for another hour of sampling collection. Dialysate samples (20 µl) were observed via HPLC equipment equipped with an HPLC equipment and an HPLC equipment, and subsequently analyzed for cAMP by radioimmunoassay.

Human subjects. The study protocol for healthy controls and patients with schizophrenia was approved by the local ethics committee of the IRCCS Fondazione Santa Lucia of Roma. The study on patients with first episode of psychosis was approved by the Ethics Committee of the Children Hospital Bambino Gesù of Roma. All participants provided written informed assent and their parents/legal guardians, when informed consent. Three-hundred and forty-one healthy adults and 259 outpatients with schizophrenia who met DSM-IV-TR criteria (using the Structured Clinical Interview for mental disorders, SCID-I/UP) were recruited and assessed at the Santa Lucia Foundation in Rome. Forty-five first-episode psychosis patients with schizophrenia who met DSM-IV-TR criteria were recruited and assessed at the Bambino Gesù Hospital in Rome (see Supplementary Tables 1 and 2 for genotypic/demographic data). To reduce the possibility of environmental variables, only individuals born and educated in Italy and of Caucasian ethnicity were included. Healthy individuals were screened for a current or lifetime history of DSM-IV-TR Axis I and II disorders using the SCID-I/NP and SCID-II; they were interviewed to confirm the first-degree relatives of any major psychiatric (e.g. mood disorders, schizophrenia spectrum disorders, substance abuse or dependence) or neurological disorders; and they had normal or corrected to normal vision. In patients under 18 years old, mental disorders were assessed using the schedule for affective disorders and Schizophrenia for school age children and adolescents (K-SADS-PL). To implement the specificity of the first-episode psychosis diagnosis, positive, negative, disinhibition, and general symptoms were assessed with the Structured Interview for Psychosis-Risk Syndromes (SIPS). Alcohol and drug use sections by sections and the Composite International Diagnostic Interview (CIDI). Functioning was rated globally on the Childhood Global Assessment Scale (CGAS) and differentially on the Global Functioning: Social (GF-Social) and the Global Functioning: Role (GF-Role) scales. All patients were screened for autism-spectrum disorder using the Autism Spectrum Quotient (AQ) and the Autism Diagnostic Observation Schedule—generic (ADOS-g). All patients met criteria for autism-spectrum disorder. Demographic and clinical details included age, sex, age of illness onset, illness duration, medical history including alcohol and drug use, admission and...
The PCR data were acquired from the Sequence Detector Software (SDS version 2.1) using Custom Taqman SNP Genotyping Assays for rs2619538 (C/G), rs3213207, and rs1047631 or here, the ‘Dys haplotype’
. DNA samples were submitted to genetic analysis for the SNPs on the ABI7900 real-time PCR instrument (Applied Biosystems) using Custom Taqman SNP Genotyping Assays for rs2619538 (C/G), rs3213207, and rs1047631 or here, the ‘Dys haplotype’
. Heterozygous
. DNA samples were submitted to genetic analysis for the SNPs on the ABI7900 real-time PCR instrument (Applied Biosystems) using Custom Taqman SNP Genotyping Assays for rs2619538 (C/G), rs3213207, and rs1047631 or here, the ‘Dys haplotype’
designed using PRIMER
system with 384-well format (Applied Biosystems, Foster City, CA, USA) using Commercial software (STATISTICA-StaSoft, 12). Results are expressed as mean ± standard error of the mean (S.E.M.) throughout. For analysis of variance in mice behavioral task we used ANOVA to examine the number of trials necessary to reach the criteria, time needed to complete each stage and the latency to respond. One-way ANOVAs for each single genotype were also performed to evaluate the variance of performance across different stages. Moreover, as in previous studies
planned comparisons were performed to test the source of significant interactions in the different stages. For human data, a one-way ANOVA was carried out to examine demographic variables, mRNA expression data, the number of perseverative and non-perseverative errors and the number of categories passed on the WCST across genotypes (Dys Hap=−/−, +/− and +/+) Post hoc analyses were conducted using Newman–Keuls test with multiple comparisons corrections, when statistical significance emerged in the main effects or interactions. The accepted value for significance was p<0.05. Result sheets of statistical tests from Stasoft detailing (wherever applicable) estimates of variance within each group, confidence intervals, effectiveness of pairing, comparison of variances across groups, are available upon request.

Data availability. The data that support the current study are available from the corresponding author upon reasonable request.

Received: 5 February 2018 Accepted: 15 May 2018 Published online: 11 June 2018

References

5. Kahn, R. S. & Keefe, R. S. E. Schizophrenia is a cognitive illness: time for a change in focus. JAMA Psychiatry 70, 1107–1112 (2013).
Author contributions

Additional information
Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-018-04711-w.

Competing interests: The authors declare no competing interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018