BRAIN
A JOURNAL OF NEUROLOGY

Functional variants of the dopamine receptor D2 gene modulate prefronto-striatal phenotypes in schizophrenia

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Dopamine D2 receptor signalling is strongly implicated in the aetiology of schizophrenia. We have recently characterized the function of three *DRD2* SNPs: rs12364283 in the promoter affecting total D2 mRNA expression; rs2283265 and rs1076560, respectively in introns 5 and 6, shifting mRNA splicing to two functionally distinct isoforms, the short form of D2 (D2S) and the long form (D2L). These two isoforms differentially contribute to dopamine signalling in prefrontal cortex and in striatum. We performed a case–control study to determine association of these variants and of their main haplotypes with several schizophrenia-related phenotypes. We demonstrate that the minor allele in the intronic variants is associated with reduced expression of %D2S of total mRNA in post-mortem prefrontal cortex, and with impaired working memory behavioural performance, both in patients and controls. However, the fMRI results show opposite effects in patients compared with controls: enhanced engagement of prefronto-striatal pathways in controls and reduced activity in patients. Moreover, the promoter variant is also associated with working memory activity in prefrontal cortex and striatum of patients, and less robustly with negative symptoms scores. Main haplotypes formed by the three *DRD2* variants showed significant associations with these phenotypes consistent with those of the individual SNPs. Our results indicate that the three functional *DRD2* variants modulate schizophrenia phenotypes possibly by modifying D2S/D2L ratios in the context of different total D2 density.

Introduction

Dysregulation of dopamine is centrally implicated in the pathophysiology and treatment of schizophrenia. Phenotypic expression of this brain disorder includes 'negative symptoms' such as affective flattening and apathy and working memory (WM) deficits. These symptoms are modulated by dopamine (Weinberger, 1987; Bertolino *et al.*, 2004*b*). Furthermore, studies in animals have strongly suggested that these symptoms may be modulated by prefrontal D1 and D2 as well as by striatal D2 dopamine receptors (Seamans and Yang, 2004; Wang *et al.*, 2004; Kellendonk *et al.*, 2006; Drew *et al.*, 2007). Studies in healthy humans and in patients with schizophrenia have been consistent with this animal literature (Knable *et al.*, 1997; Okubo *et al.*, 1997; Abi-Dargham *et al.*, 2002; Aalto *et al.*, 2005; Mehta *et al.*, 2008). Moreover, brain imaging studies with radiotracers techniques in schizophrenia have also indicated increased pre-synaptic dopamine activity (Laruelle *et al.*, 1996; Abi-Dargham *et al.*, 2000), which is also weakly correlated with negative symptoms (McGowan *et al.*, 2004). Finally, a recent meta-analysis has suggested that schizophrenia is associated with relatively increased density of D2 receptors (Laruelle, 1998).

D2 receptors exist in two alternatively spliced isoforms, the D2 long (D2L) and the D2 short (D2S) (Khan *et al.*, 1998; Usiello *et al.*, 2000). Using an animal model of D2L knockout, the function of these two isoforms has been well characterized (Usiello *et al.*, 2000). Pre-synaptic D2S receptors are mainly found in meso-cortical projections serving to inhibit dopamine release (Rouge-Pont *et al.*, 2002); post-synaptic D2S receptors inhibit D1 receptor responses (Usiello *et al.*, 2000). D2L receptors are mainly post-synaptic, they are targeted by dopamine antagonists like haloperidol and work in synergy with D1 receptors (Usiello *et al.*, 2000).

Recently, we have characterized three novel SNPs within *DRD2*, the gene for D2 receptors, one in the promoter, rs12364283 (T > C), and two highly linked SNPs in intron 5, rs2283265 (G > T) and intron 6, rs1076560 (G > T) (Zhang *et al.*, 2007). The C allele of the promoter SNP is associated with enhanced total D2 mRNA expression, while the T allele of both intronic SNPs shifts splicing from D2S to D2L. The intronic T alleles are also associated with reduced WM and attention performance, together with greater (i.e. inefficient) WM cortical and subcortical activity in healthy humans (Zhang *et al.*, 2007).

DRD2 is a logical candidate for genetic studies in schizophrenia. As schizophrenia is a multigenic complex disease, we expect penetrance of genetic factors to be greater for relatively more simple and biologically based phenotypes (Meyer-Lindenberg and Weinberger, 2006). In a case–control study, we have evaluated association of these three functional *DRD2* variants and their main haplotypes with progressively more complex and distal phenotypes. Given that dopamine D2 receptor signalling modulates several phenotypes also commonly associated with schizophrenia and given our earlier data (Zhang *et al.*, 2007), we hypothesized that the three *DRD2* SNPs of proven functional relevance affect several schizophrenia-related phenotypes, including prefrontal mRNA measured in post-mortem human brain tissue, WM cortical and subcortical activity [measured with blood oxygenation level-dependent (BOLD)-functional MRI (fMRI)] and WM behavioural performance, negative symptoms and diagnosis of schizophrenia.

Material and Methods

Post-mortem human brain tissues

Seventy DNA and RNA samples (35 controls and 35 patients with schizophrenia), extracted from prefrontal cortex autopsy tissues, were obtained from The Stanley Medical Research Institute's brain collection, courtesy of Drs M.B. Knable, E.F. Torrey, M.J. Webster and R.H. Yolken (Chevy Chase, MD, USA). DNA and RNA were extracted as described in (Zhang *et al.*, 2005). cDNA was synthesized with reverse transcriptase II (Invitrogen, Carlsbad, CA, USA) using both gene-specific primers and oligo(dT), as described in (Zhang *et al.*, 2005).

DRD2 mRNA levels by real-time RT–PCR

RT–PCR was performed with β-actin as control, using 50 ng of cDNA, 200 nM primers (as used for SNP rs6275), SYBR-Green, and AmpliTaq Gold and AmpErase UNG on an ABI 7000 (Applied Biosystems, University Park, IL, USA) (Pinsonneault *et al.*, 2006). Cycle thresholds of DRD2 mRNA were normalized to β-actin. Cycle thresholds were also measured for GADPH mRNA, yielding similar results compared with β-actin ($r^2 = 0.703$).

Quantitative detection of splice isoforms

D2L and D2S were measured after PCR amplification by using a Fam-labelled exon 5 forward primer and an exon 7 reverse primer on an ABI 3730 (Applied Biosciences (ABI), Foster City, CA, USA), as described in (Wang *et al.*, 2006). Standard curves were constructed by using varying mixtures of cloned D2L and D2S cDNA.

Subjects for genetic association with diagnosis and symptomatology

All subjects were unrelated, white Caucasians, resident in the province of Bari and provided written informed consent to participate in genetic studies. Protocols and procedures were approved by the local ethics committee and all subjects provided written informed consent to the study. Diagnoses of schizophrenia were made with the structured clinical interview for diagnosis for DSM-IV (First *et al.*, 1996). Healthy subjects also underwent SCID. This sample included 249 healthy subjects and 196 patients with schizophrenia (see Supplementary Table S1 for demographics) who had been on stable pharmacological treatment with anti-psychotics for at least 4 weeks. One hundred and fifty-two patients were also rated with the Positive and Negative Symptoms Scale (PANSS). For association analyses between genotypes and haplotypes with symptom ratings, patients were rank-ordered based on PANSS scores and divided in two groups of high and low symptoms. All patients were receiving treatment with anti-psychotics at the time of study. Forty-four patients did not provide consent to be rated with the PANSS or to the other procedures of the study. For further demographics of patients and controls, see Supplementary Tables S1 and S4.

Subjects for N-back WM behavioural studies

A subsample of 114 healthy subjects (age mean ± SD 28.4 ± 9.6; 51 males) and 91 patients with schizophrenia (age 28.1 ± 7.9; 74 males) provided informed consent and completed WM behavioural assessment. These two groups were matched for age, socio-economical status and handedness, but not for sex ($\chi^2 = 26.9$, df 1, $P < 0.0001$). All subsequent analyses in this sample were covaried for gender.

Subjects for WM fMRI studies

Of the above sample of patients, 46 (age 27.4 ± 6.9; 38 males) also underwent fMRI. Moreover, of the above group of healthy subjects, 90 (age 27.2 ± 7.6; 53 males) were selected so that they would be matched with patients for age, handedness and socio-cultural status [assessed with the Hollingshead Scale, (Hollingshead and Redlich, 1958)]. Healthy subjects were also selected so that the sample would be matched for behavioural performance across genotypes. This further matching was performed so that fMRI activation changes reflect the cortical tuning efficiency phenotype previously described and not task performance *per se* (Mattay *et al.*, 2003) across genotypes. In this sample, patients and controls were not matched for sex ($\chi^2 = 4.88$, df 1, $P = 0.02$). Therefore, all analyses in this sample were covaried for sex.

Genotype determination

Genotypes were determined as in (Zhang *et al.*, 2007). The SNP in the promoter region (rs12364283) of *DRD2* and the two intronic SNPs (rs1076560 and rs2283265) were genotyped in 70 prefrontal cortex samples and in all subjects recruited at the University of Bari. SNPs were analysed with allele-specific PCR primers as described in (Papp *et al.*, 2003) or SNaPshot [Applied Biosciences (ABI)] (Zhang *et al.*, 2005).

After genotype determination, the groups were divided based on *DRD2* genotype (Supplementary Table S1). The allelic distribution of all SNPs was in Hardy–Weinberg equilibrium (all $\chi^2 < 1.6$, all $P > 0.17$).

N-back WM paradigm for behavioural performance

Briefly, '*N*-back' refers to how far back in the sequence of stimuli the subject had to recall. The stimuli consisted of numbers (1–4) shown in random sequence and displayed at the points of a diamond-shaped box. There was a visually paced motor task which also served as a non-memory guided control condition (0-back) that simply required subjects to identify the stimulus currently seen. In the WM conditions, the task required recollection of a stimulus seen one (1-back) or two stimuli (2-back) previously while continuing to encode additionally incoming stimuli. Performance data were recorded as the number of correct responses (accuracy) and as reaction time.

Statistical analyses

Statistical associations of alleles and genotypes with diagnosis and symptomatology were evaluated with χ^2. Associations with WM behavioural performance (accuracy and reaction time at 0-back, 1-back, and 2-back) were evaluated with repeated measures factorial analysis of covariance (ANCOVA; covarying for gender) using genotypes and diagnosis as independent variables. Associations with *DRD2* expression were evaluated with factorial ANOVA using genotype and diagnosis as independent variables for mRNA levels. All *post hoc* analyses were performed with Fisher LSD.

All haplotype analyses were performed with Helixtree (http://golden helix.com). Haplotypes were estimated with an Expectation/Maximization algorithm. Association of haplotypes with phenotypes were calculated with step-wise logistic regression (>3% frequency) using a window with fixed size of 3. For association of haplotypes with WM performance, patients and controls were separately rank-ordered in high and low performers and diagnosis was used as a non-genetic covariate. Similarly, for association with negative symptoms scores, patients were rank-ordered in high and low scores.

Acquisition of the N-back WM fMRI data

Only the 0-back and the 2-back conditions were acquired during fMRI. Each subject was scanned using the same 3.0 Tesla MR scanner with a gradient-echo echo planar imaging sequence using the following parameters: 20 contiguous slices echo time = 30 ms, repetition time = 2000 ms; field of view 24 cm; matrix 64 × 64 (Bertolino *et al.*, 2004*a*, *b*). We used a simple block design in which each block consisted of eight alternating 0-back and 2-back conditions (each lasting 30 s), obtained in 4 min and 8 s, 120 whole-brain fMRI volumes. The first four scans at the beginning of each time series were acquired to allow the signal to reach a steady state and were not included in the final analysis.

Pre-processing and statistical analyses of the fMRI data

Analysis of the fMRI data was completed using Statistical Parametric Mapping 5 (SPM5; http://www.fil.ion.ucl.ac.uk/spm). Images for each subject were realigned to the first volume in the time series to correct for head motion (<2.5 mm of translation, <1.5° rotation), spatially normalized into a standard stereotactic space (Montreal Neurological Institute, MNI, template) using a 12 parameter affine model and smoothed to minimize noise and residual differences in gyral anatomy with a Gaussian filter, set at 10 mm full-width at half-maximum. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean. For each experimental condition, a box car model convolved with the haemodynamic response function (HRF) at each voxel was modelled. Pre-determined condition effects at each voxel were calculated using a *t*-statistic, producing a statistical image for the contrast of 2-back versus 0-back. All these individual contrast images were then used in second-level random effects models to determine task-specific regional responses at the group level for the entire sample. To detect the association between *DRD2* genotypes and diagnosis with fMRI activation, the contrast images of all subjects were included in a factorial ANCOVA (covarying for gender) with SPM5. To detect association of haplotypes with fMRI activation, we performed separate multiple regression analyses for patients and controls within SPM5 using as covariate of interests the probabilities of haplotypes (rs12364283, rs2283265 and rs1076560) with frequency >3% and gender as a covariate of no interest (Meyer-Lindenberg *et al.*, 2006). Because of our strong *a priori* hypothesis based on earlier fMRI studies of WM as well as on known projections of dopamine neurons (Grace, 1991; Callicott *et al.*, 1999, 2000; Bertolino *et al.*, 2004*b*, 2006*a*, *b*), a statistical threshold of $P < 0.005$, $k = 6$, with a further Family Wise Error (FWE) small volume correction for multiple comparisons (using a 12 mm radius sphere centered around the coordinates in DLPFC and in striatum published in previous studies [left middle frontal gyrus *x* −22, *y* 44, *z* 3 (Callicott *et al.*, 2003); right middle frontal gyrus *x* 30, *y* 45, *z* 9 (Callicott *et al.*, 2000); left striatum *x* −11, *y* 1, *z* 14 (Simon *et al.*, 2002); right striatum *x* 20, *y* −4, *z* 18 (Tan *et al.*, 2007)]) $P = 0.05$ was used to identify significant responses for all comparisons in these anatomical regions (Meyer-Lindenberg *et al.*, 2008). Because we did not have *a priori* hypotheses regarding the activity of brain regions outside of DLPFC and striatum,

we used a statistical threshold of $P = 0.05$, corrected for multiple comparisons across all voxels, for these whole-brain comparisons. All fMRI results are reported in Talairach coordinates system. Uncorrected results from non *a priori* regions that did not survive correction for multiple comparisons are reported in Supplementary Table S3.

Results

Association with prefrontal mRNA

In a post-mortem sample of prefrontal cortex we found no association between total D2 mRNA and diagnosis or genotype (all

Fig. 1 Mean ± 0.95 confidence intervals (CIs) of prefrontal %D2S mRNA in patients and controls; S = short.

$P > 0.4$). However, intronic SNP rs1076560 (or rs2283265, being in complete LD) was strongly associated with D2S/D2L ratios, with GG subjects showing higher % D2S mRNA in prefrontal cortex than GT subjects ($P = 0.004$, Fig. 1), consistent with our earlier independent results (Zhang *et al*., 2007). No other significant association was found (all $P > 0.1$). Therefore, GG genotype at the intronic SNPs is associated with greater prefrontal expression of D2S regardless of diagnosis.

Association with WM brain activity measured with fMRI

Next, we evaluated associations with brain activity during WM using the N-back task in patients and controls (2-back, all $P < 0.05$, Family Wise Error small volume correction for multiple comparisons). In this sample, WM behavioural performance was matched across genotypes ($P > 0.5$) but patients performed worse than controls ($P < 0.001$). ANCOVA (covarying for gender) demonstrated that patients had reduced activity in the WM network (Supplementary Tables S2 and S3), no effect of rs1076560 genotype, and an interaction between diagnosis and genotype. GT healthy subjects had greater activity in prefrontal cortex and the caudate head, while the opposite effect was evident in patients (Supplementary Table S2, Fig. 2A and B). A similar analysis with rs2283265 did not demonstrate statistically significant effects. Analysis with rs1236428 revealed an interaction between genotype and diagnosis, showing that TC patients have significantly less BOLD activity than TT patients (Fig. 3A and B).

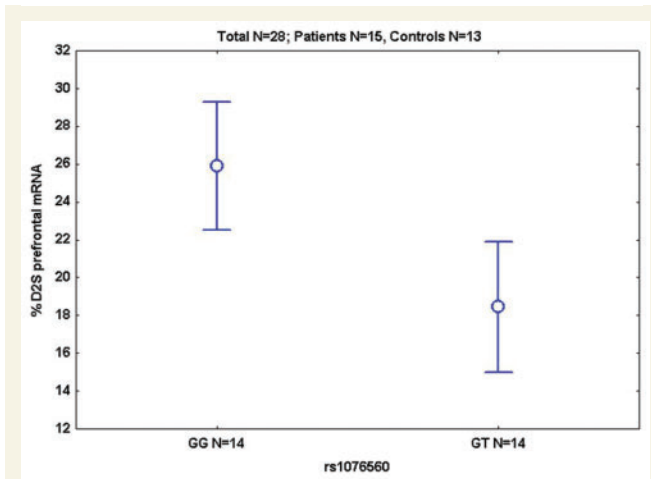The *DRD2* SNPs (rs12364283, rs2283265, rs1076560) form three haplotypes with frequency >5%: *TGG*, *CTT*, and *CGG*
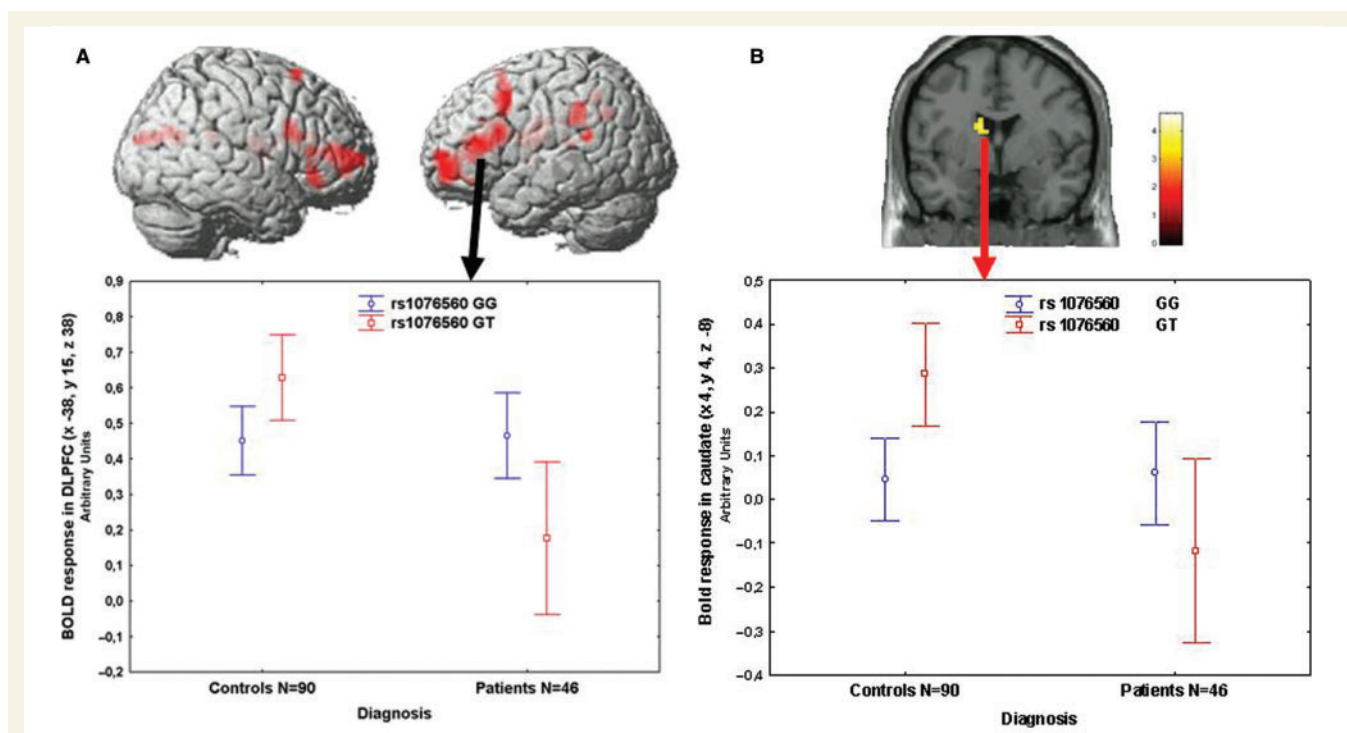

Fig. 2 (**A**) 3D rendering (above, image thresholded at $P < 0.005$, uncorrected) and mean ± 0.95 CIs of BOLD response (below) of the interaction between diagnosis and rs1076560 genotype on WM prefrontal activity. (**B**) Coronal section (above) and mean ± 0.95 CIs of BOLD response (below) of the interaction between diagnosis and rs1076560 genotype on WM activity in the left head of the caudate.

(Supplementary Table S1). Multiple regression analyses in patients demonstrated that the *TGG* haplotype predicted greater activity bilaterally in the caudate (Fig. 4A, Supplementary Table S2). In contrast, *TGG* predicted lower activity in the WM network in healthy subjects (more efficient, Fig. 4B, Supplementary Table S2). The other haplotypes had no significant effect in either group. These results reveal opposite effects of rs1076560 on brain activity in patients versus healthy subjects and additionally show an effect of promoter rs12364283 on WM pathways in patients. Consistently, the TGG haplotype had opposite effects in patients and controls.

Association with WM behavioural performance

Next, we evaluated associations with WM performance using the N-back task with increasing difficulty (0-back as control, 1-back and 2-back). ANCOVA indicated an interaction between rs1076560 genotype and WM difficulty suggesting that GG subjects perform significantly better than GT but only at the more difficult 2-back task (*post hoc* $P = 0.05$, Fig. 5). No genotype by diagnosis by WM load interaction was found. rs2283265 behaved similarly [interaction between genotype and WM load: $F(2,402) = 6.5$, $P = 0.001$], while no effect was evident with rs12364283 (all $P > 0.2$). Moreover, the *CTT* haplotype was

associated with low WM performance (frequency 0.06 in both groups, $\chi^2 = 8.9$, coefficient $= 2.72$, $P = 0.002$, odds ratio 15.2). These results reveal consistent effects of rs1076560 and of the *CTT* haplotype on WM behavioural performance at higher cognitive load.

Association with symptoms and diagnosis

Negative symptoms (rated with PANSS) were weakly associated with promoter rs1236428 (Supplementary Table S1, $P = 0.07$). This association was more pronounced for haplotype *TGG* ($P = 0.03$), showing that the T allele of rs1236428 was more frequent in patients with low negative symptoms (Supplementary Table S1). No association was found with the other symptom scores. In 249 controls and 196 patients with schizophrenia (Supplementary Table S1) genotyped for these three SNPs we did not find significant association between diagnosis and alleles, genotypes or haplotypes.

Discussion

The results of the present study indicate that *DRD2* variants are associated with several schizophrenia phenotypes known to be
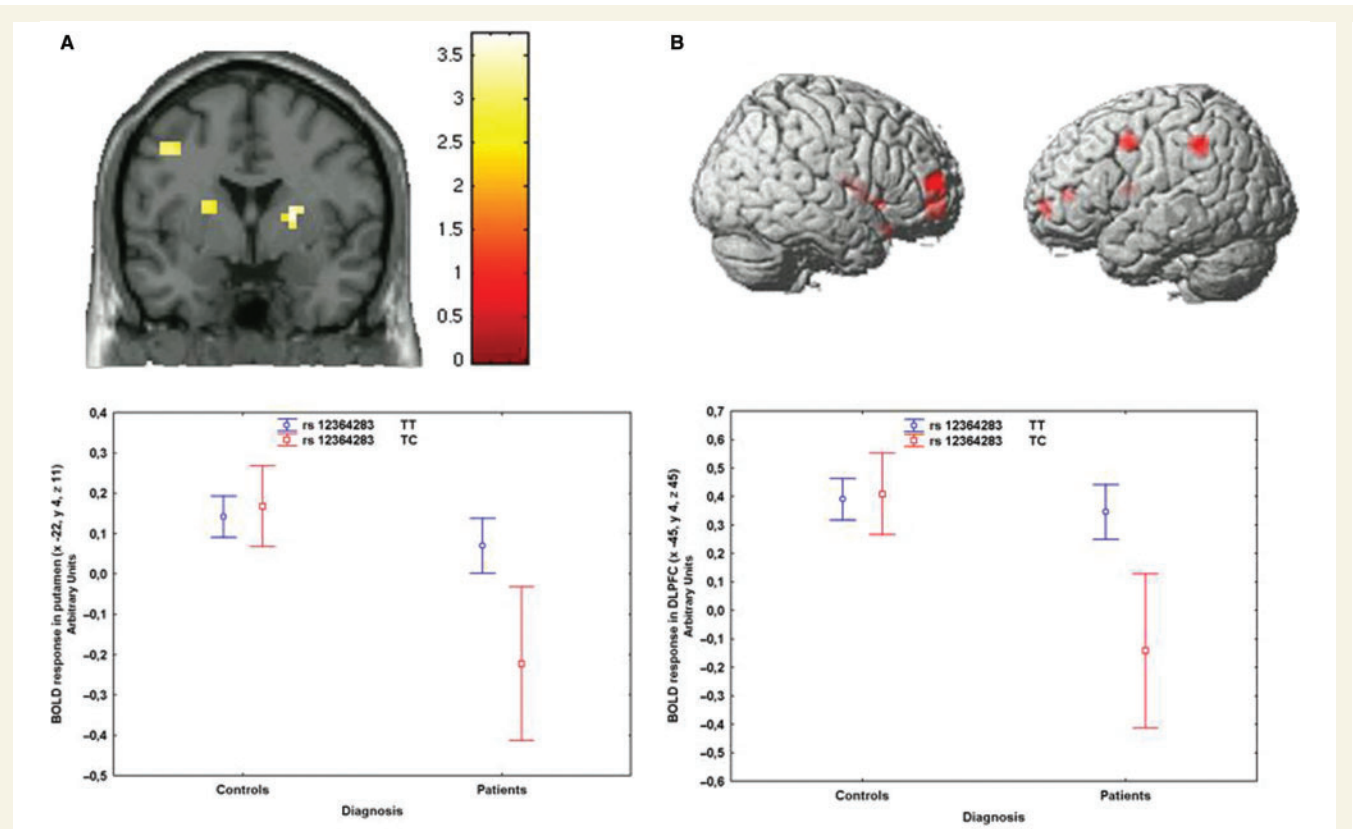

Fig. 3 (**A**) Coronal section (image thresholded at $P < 0.005$, uncorrected) of the BOLD response of the interaction between diagnosis and rs12364283 genotype on WM activity in the striatum (above). Mean ± 0.95 CIs of the BOLD response in left putamen in patients and controls separated for rs12364283 genotype (below). (**B**) 3D rendering (image thresholded at $P < 0.005$, uncorrected) of BOLD response of the interaction between diagnosis and rs12364283 genotype on WM prefrontal activity (above). Mean ± 0.95 CIs of the BOLD response in left DLPFC in patients and controls separated for rs12364283 genotype (below).
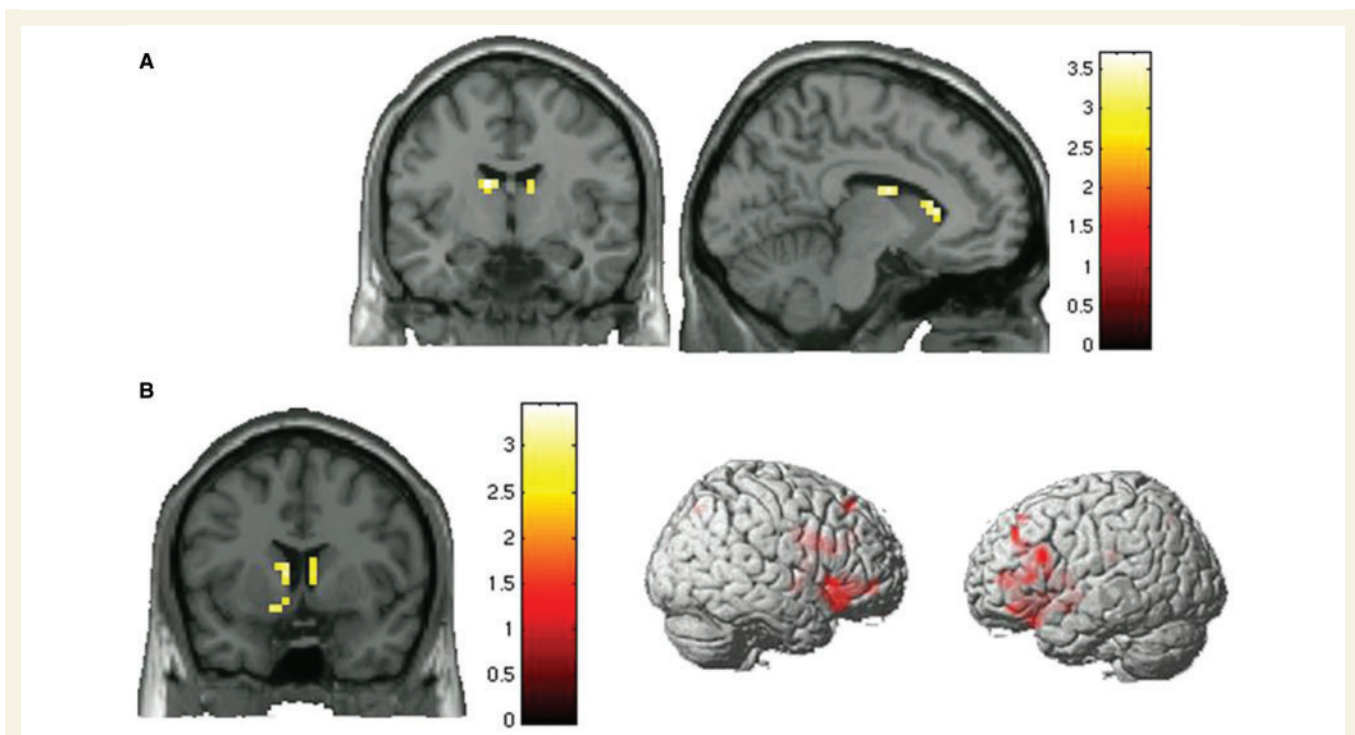
Fig. 4 (**A**) Coronal and sagittal sections (images thresholded at $P<0.005$, uncorrected) of the BOLD response of the multiple regression in patients with schizophrenia. The TGG (rs12364283, rs2283265 and rs1076560) haplotype has a positive relationship with BOLD activity in caudate during WM. (**B**) Coronal section and 3D rendering (images thresholded at $P<0.005$, uncorrected) of the BOLD response of the multiple regression in controls. The TGG (rs12364283, rs2283265 and rs1076560) haplotype has a negative relationship with BOLD activity in caudate and prefrontal cortex during WM.
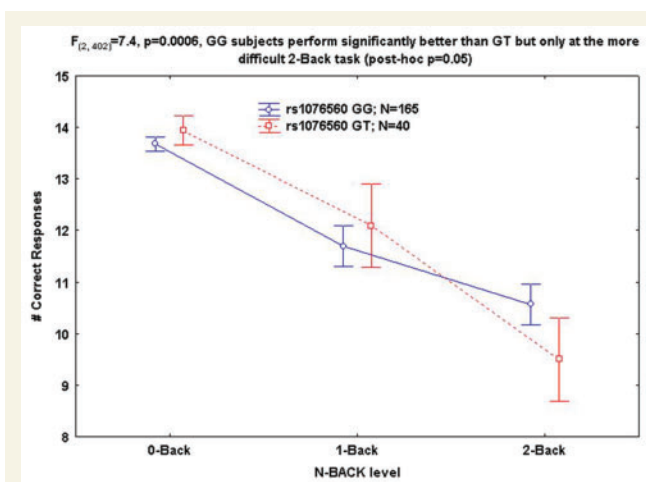

Fig. 5 Mean ± 0.95 CI of the interaction between WM behavioural performance and rs1076560 genotype.

modulated by dopamine, while no association with diagnosis was evident in this relatively small sample. GG subjects for rs1076560 and for rs2283265 have greater relative D2S mRNA in prefrontal cortex than GT subjects, regardless of diagnosis. The fMRI results demonstrate association of rs1076560 (which is strongly linked to rs2283265) with prefrontal and striatal activity during WM, with opposite effects in patients and controls. In controls, subjects heterozygous for rs1076560 have reduced performance and greater brain activity, i.e. they are inefficient. In patients, who perform worse than controls and have reduced brain activity, heterozygote subjects have reduced performance and reduced activity, i.e. they do not fully engage prefrontal–striatal resources. Thus the GG genotype is advantageous in both controls and patients. Similar analysis with rs1236428 revealed that TT patients have greater BOLD activity than TC patients. Consistent with the single SNP results, the TGG haplotype (rs12364283, rs2283265 and rs1076560) had opposite effects on brain activity in patients and controls. The single SNP and haplotype associations with WM behaviour are consistent with the fMRI data. Finally, the promoter SNP weakly predicted negative symptoms by itself and more strongly when part of a haplotype including the two intronic SNPs (again TGG). These results may further suggest that the T allele in the promoter SNP is advantageous in patients especially when in combination with the G allele from both intronic SNPs. All these results together indicate that genetically determined D2 receptor signalling modulates manifestation of several phenotypes in schizophrenia.

Dopamine modulation of WM is very well known. While a role for D1 receptors has long been recognized, more recent studies have demonstrated that D2 receptors are also involved. Behavioural and electrophysiological experiments in non-human primates indicate that D2 agonists and antagonists may, respectively, increase and decrease tuning of prefrontal neurons to the task at hand, especially during the response phase

(Arnsten *et al*., 1995; Williams and Goldman-Rakic, 1995; Wang *et al*., 2004). Several studies have demonstrated that D2 agonists and antagonists, respectively, improve and deteriorate WM performance also in humans (Mehta and Riedel, 2006). Consistently, D2 agonists and antagonists, respectively, reduce and increase activity in prefrontal cortex and striatum during WM (Kimberg *et al*., 2001; Mehta *et al*., 2003), suggesting that D2 receptor modulation modifies tuning of neuronal resources. D2 receptors are found with different density and localization in prefrontal cortex and in striatum. While D2S receptors are found mainly, but not exclusively, in the pre-synaptic environment with relatively greater abundance in prefrontal cortex; D2L receptors are mainly found postsynaptically, being relatively more abundant in striatum. The effects we have measured *in vivo* may have resulted from prefrontal and/or striatal modulation of dopamine signalling. Earlier studies have indicated that post-synaptic D1 receptors are negatively modulated by post-synaptic D2S, while they act in synergy with post-synaptic D2L (Usiello *et al*., 2000). Thus, we propose that subjects homozygous for the G allele at the two intronic SNPs have a more favourable modulation of prefrontal D1 receptors based on greater D2S/D2L ratios. This interpretation is consistent with our mRNA data in prefrontal cortex suggesting that a post-synaptic mechanism may be relevant. A prefrontal pre-synaptic mechanism may also be involved with the known modulation by pre-synaptic D2 receptors of NMDA and of GABA A receptors of pyramidal neurons and interneurons (Tseng and O'Donnell, 2007; Tseng *et al*., 2007). Moreover, our results may also be explained by modulation of dopamine signalling in the striatum within the cortico-striato-thalamo-cortical network. Whereas the overall D2 receptor number modulates GABA-mediated inhibition of striatal neurons, inhibition of glutamate release preferentially involves the D2S variant (Centonze *et al*., 2003, 2004). Therefore, reduced D2S expression is expected to increase excitability of striatal medium spiny neurons. Future studies are needed to resolve the molecular/neuronal mechanisms accounting for our effects. On the other hand, the opposite effects manifested by the intronic SNPs in patients and controls, the interaction between the promoter SNP and diagnosis of schizophrenia, the opposite effects of haplotypes with brain activity during WM are consistent with the assumption that dopamine transmission in schizophrenia is disrupted (Carlsson *et al*., 1999). Thus, these interactions may arise from different dopamine activity between patients and controls.

The weak association with negative symptoms of the promoter SNP also may suggest that genetically determined total number of D2 receptors may be clinically relevant, especially in the context of homozygosity for the G allele in the two intronic SNPs. Once again, the T allele of the promoter SNP and the G allele of the intronic SNPs seem to be beneficial in that they are associated with lower negative symptoms. These data are consistent with some evidence in earlier cross-sectional and longitudinal studies (Himei *et al*., 2002; Lane *et al*., 2004) indicating association between *DRD2* polymorphisms and negative symptoms. On the other hand, in this case–control study we did not find any evidence for association of the three variants with diagnosis of schizophrenia. A recent meta-analysis of all case–control studies investigating the *DRD2* Ser311Cys (rs1801028) variant has indicated association with schizophrenia with an odds ratio of 1.3 for the Cys allele (Glatt *et al*., 2003). As rs1801028 is in strong LD with the intron 5/6 SNPs, we propose that the main functional effects may be exerted by their effect on splicing, rather than the imputed amino acid change caused by the non-synonymous SNP. This meta-analysis also suggested that the effect of this *DRD2* polymorphism on schizophrenia risk is reliable and uniform across populations, although the magnitude of its effect is small. Similarly, another recent meta-analysis also suggested that rs1801028 may be implicated in risk for schizophrenia (Allen *et al*., 2008). Moreover, another recent family-based sample in Han-Chinese has indicated association of *DRD2* SNPs and haplotypes with schizophrenia involving the intron 5 SNP rs2283265 by itself and as part of a haplotype block as well as a haplotype block including rs1801028 (Glatt *et al*., 2008). Of note, these two SNPs are in high LD. Finally, *DRD2* has also been implicated in linkage areas suggested in the genome-wide linkage meta-analysis (Lewis *et al*., 2003). Thus, these previous studies and the LD structure of the gene suggest that *DRD2* may be implicated in risk for schizophrenia and that our inability to demonstrate statistically significant association with diagnosis is related to the relatively small number of cases and controls.

A limitation of the present study has to be acknowledged. Since all our patients were treated with anti-psychotic drugs, we cannot definitively exclude an effect of treatment on the measured phenotypes. However, several factors indicate that the effect of treatment is not a confounder of our results. First, genotype groups did not differ in terms of chlorpromazine equivalents (all $P > 0.1$). Second, all patients had been on stable treatment for at least 1 month. Third, there was no correlation between WM performance or brain activity with chlorpromazine equivalents or between lifetime exposure to anti-psychotics and %D2S mRNA (data not shown, all $P > 0.3$). Fourth, our prefrontal post-mortem data demonstrate unchanged total D2 mRNA. Previous studies have indicated that treatment with anti-psychotics increases total D2 mRNA thus suggesting that pharmacological treatment is unlikely to account for our results (Martres *et al*., 1992; D'Souza *et al*., 1997; Lidow *et al*., 1997).

In conclusion, our results indicate associations of three *DRD2* variants with prefronto-striatal phenotypes of relevance to schizophrenia, showing that endo-phenotypes may help identify genetic variants affecting disease presentation (Hall *et al*., 2006). The potential utility of these genetic variants as biomarkers in schizophrenia therapy requires further study.

Supplementary material

Supplementary material is available at *Brain* online.

Acknowledgements

participated to the study as well as to our colleagues who referred them to us.

Funding

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