

ORIGINAL RESEARCH ARTICLE

D1 receptor alleles predict PET metabolic correlates of clinical response to clozapine

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A goal of pharmacogenetics is to clarify associations between allelic variation and risk factors in psychiatric illness. We report changes in regional brain metabolism based on dopamine alleles. Treatment-resistant schizophrenic subjects were positron emission tomography scanned with 18F-fluorodeoxyglucose after 5 weeks each of placebo and clozapine treatment. Significant regional brain metabolic effects were found for the D1 receptor genotypes ($P < 0.05$), adjusted for multiple comparisons. Metabolic decreases for the 2,2 genotype but not the 1,2 genotype were observed in all major sectors of the brain, with the exception of the ventral parts of the caudate and putamen. Frontal, temporal, parietal, and occipital neocortices showed decreased metabolism as did the cingulate juxta-allocortex and the parahippocampal allocortex. Decreases were also observed in the thalamus, amygdala, and cerebellum bilaterally. No significant metabolic differences by genotype were observed for D3, 5HT_{2A}, and 5HT_{2C} polymorphisms. In terms of clinical response, the DRD1 2,2 genotype significantly improved with clozapine treatment, demonstrating a 30% decrease in the Brief Psychiatric Rating Scale positive symptoms in contrast to a 7% worsening for the 1,2 genotype ($P < 0.05$). In this preliminary study, brain metabolic and clinical response to clozapine are related to the D1 receptor genotype.

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Associations between allelic variation and liability to tardive dyskinesia, a side effect^{1,2} of neuroleptic treatment, have been demonstrated in schizophrenia. Despite the development of improved clinical scales and the establishment of relatively high inter-rater reliability, we still lack objective, quantifiable biological measures and predictors of response to treatment. Previous attempts to address the subjective nature of clinical response measures by objective biological measures have included the development of intermediate phenotypes or endophenotypes such as smooth-pursuit eye movements,³ P50,⁴ and ventricular brain ratio.⁵ An additional use of structural brain imaging as an intermediate phenotype includes

an association between parietal lobe, but not other major brain regions, volumes, and brain-derived neurotrophic factor (BDNF) alleles.⁶ Laruelle *et al*⁷ failed to find an association between D2 receptor binding and alleles at the *TaqI-A* RFLP site in the D2 receptor gene using single-photon emission computed tomography (SPECT) imaging. Despite their small sample sizes, these preliminary studies provide intriguing data regarding the ability of brain imaging to serve as an intermediate phenotype in pharmacogenetic studies. These studies, however, did not examine the relationship between allelic variation, clinical response to pharmacological treatment, and the intermediate phenotypes as revealed by brain imaging. The relationship between dopamine and serotonin receptor genotypes, regional brain metabolism, and clinical response to clozapine treatment was the focus of this study.

We report the changes in brain metabolism based on dopamine and serotonin alleles following treatment with clozapine. Positron emission tomography scanning using ¹⁸F-fluorodeoxyglucose (FDG PET) has the ability to measure regional brain metabolic response while the subject is performing an activation task. PET has the ability to resolve individual gyri and distinguish subcortical regions from each other. PET can measure brain work because of the close coupling between glucose utilization and neuronal activity.^{8,9} During the FDG uptake, the subject performed an attentional task, the degraded continuous performance task (CPT), activating brain systems related to essential elements of the illness. In this task, subjects view a series of blurred numbers presented a few seconds apart for 30 min. Target numbers appear at intervals and subjects must remain vigilant to detect these targets with a button press. Performance on this version of the CPT has been shown to be abnormal in schizophrenics and their relatives.¹⁰ Unlike fMRI (functional magnetic resonance imaging) and SPECT, FDG PET allows for absolute quantification of metabolic activity. Further, being able to separate FDG uptake from the scanning procedure allows for enhanced control of the experimental variables.

Clozapine is a pre-eminent atypical antipsychotic agent,¹¹ and is effective in at least one-third of patients who have failed to respond to conventional antipsychotic treatment.^{11,12} It is an effective antipsychotic agent without causing the extrapyramidal motor side effects that are observed with conventional antipsychotic agents. The mechanism of action of clozapine's unique clinical efficacy is unknown. The major hypotheses put forth to explain clozapine's mechanism of action are the balance between 5HT₂ and D₂ receptors^{13,14} and the D₁/D₂ receptor balance.¹⁵ PET scan receptor occupancy studies demonstrate that clozapine has the least D₂ binding and the most D₁ binding of the antipsychotic compounds.¹⁶

In our study, significant results were found for the D₁ receptor genotypes and all these differences were metabolic decreases following clozapine. Figure 1 demonstrates the average metabolic difference before and after clozapine treatment by genotype. Each image is the average of seven individuals with 2,2 genotypes, or eight with 1,2 genotypes. Statistically

significant metabolic changes are demonstrated on a representative brain slice for each level. For the 2,2 genotype group, significant metabolic decreases were observed bilaterally and in all lobules of the neocortices and allocortices in the frontal, temporal, occipital, parietal lobes, cingulate cortex, amygdala, and parahippocampal gyrus. Some decreases were also seen in the thalamus, cerebellum, as well as the dorsal caudate and putamen; however, the ventral sectors of the striatum did not exhibit any metabolic changes. In contrast, the 1,2 genotype showed a few significant metabolic decreases limited to the left dorsolateral prefrontal cortex, and temporal tip and parietal sensory/ideational speech areas bilaterally following treatment with clozapine. A single metabolic increase in the right inferior temporal cortex was also observed. A separate analysis contrasting the 2,2 and 1,2 genotypes following clozapine was conducted. The patterns of differences observed in this analysis (data not shown) are in the same areas and metabolic direction as the analysis in Figure 1,

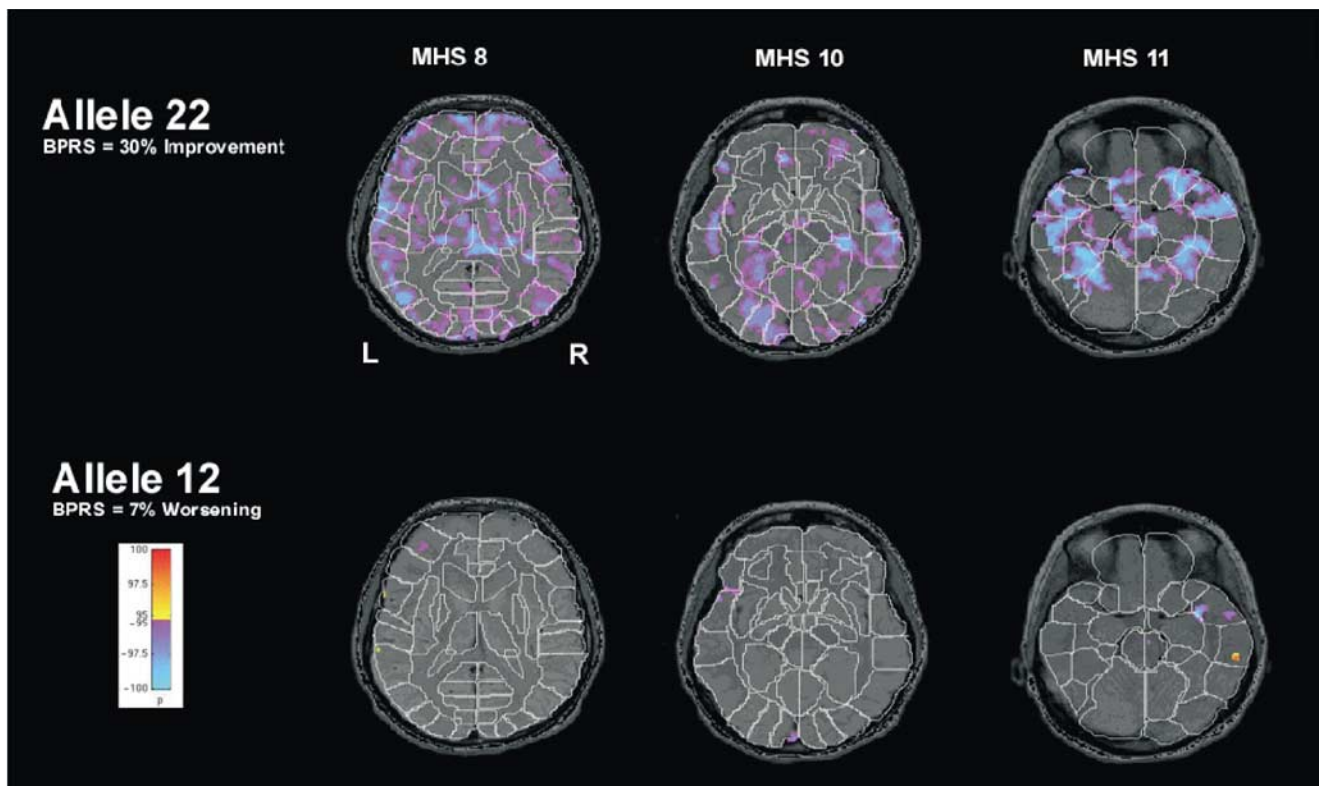


Figure 1 Brain metabolic changes following clozapine treatment by DRD1 genotype. The top row represents the metabolic changes following clozapine for the 2,2 DRD1 homozygotes and the second row represents the corresponding metabolic changes for the 1,2 DRD1 subjects. The columns represent the levels corresponding to the Matsui and Hirano stereotaxic atlas. Each figure represents the statistically significant changes following clozapine corrected for multiple comparisons by the Monte Carlo procedure superimposed on an average MRI. The flame scale is a p map in which red colors indicate areas that have significantly higher metabolism following clozapine treatment compared to baseline while blue colors indicate areas that have significantly lower metabolism compared to baseline. The 2,2 DRD1 homozygote patients have statistically significant metabolic decreases in frontal, temporal, parietal, and occipital lobes. Decreases are also observed in the cingulate gyrus, parahippocampal gyrus, amygdala, thalamus, and cerebellum. The 1,2 heterozygotes have metabolic changes confined to the left dorsolateral prefrontal, bilateral temporal tip and sensory/ideational speech areas, and right inferior temporal cortices after clozapine treatment. The 2,2 DRD1 homozygotes have responded to clozapine with a 30% BPRS symptom score improvement compared to a 7% worsening for the 1,2 DRD2 subjects ($P < 0.05$).

with a slightly smaller area of activation. The allele frequencies for all four loci were consistent with those previously published.¹⁷ No significant metabolic differences by genotype were observed for the D3 (Ser9Gy), 5HT_{2A} (T102C), and 5HT_{2C} (Cys23Ser) polymorphisms.

The ventral striatum, which is relatively enriched in D3 receptors, showed no metabolic changes following clozapine. In contrast, the cortex, rich in D1 receptors, absolute and relative to D2, D4, and D5 receptors, showed the greatest metabolic decrease. This is consistent with the finding that D1 receptors are excitatory to pyramidal neurons in the cortex.¹⁸ In addition, D1 receptors are excitatory to medium spiny neurons of the striatum. Clozapine blocks these D1 receptors more than other antipsychotics and, therefore, should cause less cortico-striatal and nigro-striatal excitation in these dorsal striatal regions than other antipsychotic compounds, which is consistent with our observed metabolic decreases. It may be that treatment-responsive patients have a greater number of D1 receptors or altered D1/D2 synergy compared to treatment-unresponsive patients. This possibility has yet to be investigated. An alternative explanation emphasizing the relatively greater metabolic changes observed in the D1 2,2 homozygotes in the cerebellum and thalamus, areas rich in serotonergic but not D1 receptors, could implicate the D1-serotonergic balance as an important differentiating characteristic.

In terms of clinical response, the DRD1 2,2 genotype schizophrenic patients significantly improved with clozapine treatment, demonstrating a 30% decrease in Brief Psychiatric Rating Scale (BPRS) positive symptoms. In contrast, the 1,2 genotype showed a worsening of 7% for the BPRS positive symptoms ($P < 0.05$). There were no statistically significant pre-clozapine treatment baseline differences in total BPRS score for the 2,2 and 1,2 groups ($t = 0.061$, NS) or CGI ($t = -0.087$, NS). The ages for the two groups were similar (36.1 ± 8.3 for the 2,2 group and 33.4 ± 5.2 for the 1,2 group; $t = -0.79$, NS). The 2,2 group consisted of six males and two females, all right-handed; the 1,2 group consisted of seven males, all right-handed. There was no significant difference in CPT performance during FDG uptake ($D' = 2.70 \pm 0.65$ for the 2,2 group and 2.53 ± 0.49 for the 1,2 group; $t = -0.48$, NS).

We have previously demonstrated that clinically effective doses of clozapine decrease brain metabolism, especially in the frontal and temporal lobes.¹⁹ Furthermore, we have shown that the phenotype of brain metabolic response to clozapine is stable over 12 weeks (Potkin *et al*, unpublished data). In the present study, we have demonstrated that brain metabolic response and clinical response to clozapine are related to DRD1 receptor genotype. The limitations in this study are related to small sample size, especially for genetic studies. This precluded us from analysing deviation from Hardy-Weinberg equilibrium. The sample was limited to treatment-resistant schizophrenic patients, and, therefore, may not

generalize to other subgroups of schizophrenic patients. There were two African Americans who may have influenced particularly the D3 analysis by introducing more genetic heterogeneity. There do not appear to be significant differences in allele frequencies across ethnic groups for the DRD1 polymorphism (Kennedy *et al*, unpublished data). We were able to detect metabolic and clinical differences between the 1,2 and 2,2 DRD1 genotypes. The rarer DRD1 1,1 homozygote genotype did not occur in our sample.

Future studies could include direct measurement of DRD1 receptor number and affinity. D1 receptors were reduced in the prefrontal cortex of drug-naive and drug-free schizophrenic patients,²⁰ but were not measured in treatment-resistant patients nor evaluated with regard to treatment response. Future D1 receptor PET studies might help to explain the failure of the 1,2 genotype to respond to clozapine. However, failure to respond to treatment is likely to be more complex than receptor number and involve multiple brain systems' responses to pharmacological intervention. Nevertheless, the approach of combining pharmacogenetics with brain imaging offers the potential for understanding clinical response to treatment and, importantly, helps define intermediate phenotypes for future genetic studies. It is hoped that using brain imaging to define intermediate phenotypes will help clarify the heterogeneity in the illness(es) we call schizophrenia.

Materials and methods

Sample

Fifteen treatment-resistant subjects (13 men, two women) meeting DSM III-R criteria for schizophrenia participated in this study. A retrospective review of their charts confirmed that these subjects would have also met DSM-IV criteria. The subjects were 33.7 ± 7.5 years of age, and two were African American. All were recruited from Metropolitan State Hospital (Norwalk, CA, USA). Informed consent was obtained from the subjects or their conservators.

Study design

Subjects received 5 weeks of daily placebo and 5 weeks of daily clozapine. The treatment was double-blind and the order randomly assigned. Subjects were blindly rated weekly by experienced research psychiatrists using the BPRS. The average number of days from the start of clozapine treatment to PET scan was 39.2 ± 10.1 . The mean dose of clozapine per day was 460 ± 11 mg. The average number of days of placebo treatment was 32.2 ± 9.9 . The mean BPRS score following placebo treatment was 43.2 ± 11.7 and 35.5 ± 9.8 following clozapine treatment ($t = 3.38$, $P < 0.003$).

Positron emission tomography

Changes in regional brain activity were imaged as glucose metabolic rate using FDG that had been

prepared in the University of California, Irvine, cyclotron as described elsewhere.^{21,22} Nine planes (Computer Technology Inc. NeuroECAT, Boston, MA, USA) at 10-mm increments and parallel to the canthomeatal line were scanned between 45 and 100 min after FDG injection. The measured resolution of the scanner was 7.6 mm in plane and 10.9 mm in the z-dimension (axial). A calculated attenuation correction and smoothing filter were used. Each subject's PET data were spatially normalized into a standard brain so that voxel-by-voxel comparisons could be made between subjects following methods similar to those of Friston *et al.*²³ Results were displayed on standardized MRI based on the Matsui and Hirano²⁴ stereotaxic brain atlas. Voxels with statistically significant *t*-test differences at $P < 0.05$ are displayed. Anatomical localization was accomplished through the concordance of Talairach and Tournoux coordinates and regions of interest (ROI) determined by an in-house probabilistic brain atlas following confirmation by a neuroanatomist (JF) who was blind to the group assignments. A resampling-based image cluster analysis (discussed in greater detail in Wu *et al.*²⁵) was used to estimate the probability for a given profile of contiguous connected clusters exceeding the threshold of $P < 0.05$ (37 voxels). The probabilities of a given size contiguous cluster were assessed using this distribution. Monte Carlo simulations using sample sizes corresponding to the *n*'s in our comparisons were run using our normal control pool with 100 random drawings to determine empirically the distribution.^{26,27} The Monte Carlo simulation protected against Type I errors due to multiple comparisons. All significant voxels whose cluster sizes were less than the threshold cluster size were thus eliminated.

Genetic analyses

DNA was extracted from blood samples using the high-salt method and polymerase chain reaction (PCR) was performed to obtain genotypes in a standard fashion. The coding region of the DRD1 receptor gene has been scanned for polymorphic sites, but none so far has provided enough polymorphism information content to be useful in genetic studies. The DRD1 polymorphism that we used is recognized by the restriction enzyme DdeI and is located about one kilobase upstream of the initiation codon. PCR amplification was done according to Sunahara *et al.*²⁸ The DdeI restriction fragments were visualized using agarose gel electrophoresis. No studies have yet been carried out to determine whether this site alters promoter function. The other polymorphisms examined were D3 receptor MscI Ser9Gly polymorphism,²⁹ and the serotonin 2A and 2C receptors. The serotonin 2A polymorphism that was typed is a T/C variant located at nucleotide position 102 (using an adapted protocol³⁰). The serotonin 2C receptor polymorphism was the cysteine-to-serine substitution at amino acid position 23 (using an adapted protocol³¹).

Statistical analyses

For each receptor, the subjects were divided on the basis of genotypes. Infrequent genotypes such as the DRD1 1,1 homozygote were not included in the analysis. Additionally, PCR amplification was not successful for some samples or sufficient subject DNA was not available. In total, there were 15 samples available for DRD1, 15 for D2, 12 for D3, and 11 for DAT. The percentage change in clinical symptoms as assessed by the BPRS between genotypes was computed by *t*-tests. The absolute regional metabolism before and following clozapine treatment was calculated for each genotype using ANOVA with appropriate constraints for repeated measures and Type I error.

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References

- 1 Basile VS, Masellis M, Badri F, Paterson AD, Meltzer HY, Lieberman JA *et al.* Association of the *MscI* polymorphism of the dopamine D₃ receptor gene with tardive dyskinesia in schizophrenia. *Neuropsychopharmacology* 1999; **21**: 17–27.
- 2 Basile VS, Ozdemir V, Masellis M, Walker ML, Meltzer HY, Lieberman JA *et al.* A functional polymorphism of the cytochrome P450 1A2 (CYP1A2) gene: association with tardive dyskinesia in schizophrenia. *Mol Psychiatry* 2000; **5**: 410–417.
- 3 Holzman PS. Eye movements and the search for the essence of schizophrenia. *Brain Res* 2000; **31**: 350–356.
- 4 Freedman R, Adler LE, Leonard S. Alternative phenotypes for the complex genetics of schizophrenia. *Biol Psychiatry* 1999; **45**: 551–558.
- 5 Jeste DV, Kleinman JE, Potkin SG, Luchins DJ, Weinberger DR. Ex uno multi: subtyping the schizophrenic syndrome. *Biol Psychiatry* 1982; **17**: 199–222.
- 6 Wassink TH, Nelson JJ, Crowe RR, Andreasen NC. Heritability of BDNF alleles and their effect on brain morphology in schizophrenia. *Am J Med Genet* 1999; **88**: 724–728.
- 7 Laruelle M, Gelernter J, Innis RB. D2 receptors binding potential is not affected by Taq1 polymorphism at the D2 receptor gene. *Mol Psychiatry* 1998; **3**: 261–265.
- 8 Phelps ME, Huang SC, Hoffman EJ, Selin CJ, Sokoloff L, Kuhl DE. Tomographic measurement of local cerebral glucose metabolic rate in humans with [¹⁸F]2-fluoro-2-deoxy-D-glucose: validation of method. *Ann Neurol* 1979; **6**: 371–388.
- 9 Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD *et al.* The (¹⁴C)deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J Neurochem* 1977; **28**: 897–916.
- 10 Nuechterlein KH. Signal detection in vigilance tasks and behavioral attributes among offspring of schizophrenic mothers and among hyperactive children. *J Abnorm Psychol* 1983; **92**: 4–28.
- 11 Kane J, Honigfeld G, Singer J, Meltzer H. Clozapine for the treatment-resistant schizophrenic. *Arch Gen Psychiatry* 1988; **45**: 789–796.

- 12 Meltzer HY, Pringuey D. Treatment-resistant schizophrenia: the importance of early detection and treatment. Introduction. *J Clin Psychopharmacol* 1998; **18**: 1S.
- 13 Ichikawa J, Meltzer HY. Relationship between dopaminergic and serotonergic neuronal activity in the frontal cortex and the action of typical and atypical antipsychotic drugs. *Eur Arch Psychiatry Clin Neurosci* 1999; **249**: 90–98.
- 14 Meltzer HY. The role of serotonin in schizophrenia and the place of serotonin–dopamine antagonist antipsychotics. *J Clin Psychopharmacol* 1995; **15**: 2S–3S.
- 15 Seeman P, Van Tol HH. Dopamine receptor pharmacology. *Trends Pharmacol Sci* 1994; **15**: 264–270.
- 16 Nordstrom AL, Farde L, Nyberg S, Karlsson P, Halldin C, Sedvall G. D1, D2, and 5-HT2 receptor occupancy in relation to clozapine serum concentration: a PET study of schizophrenic patients. *Am J Psychiatry* 1995; **152**: 1444–1449.
- 17 Jonsson E, Sedvall G, Brene S, Gustavsson JP, Geijer T, Terenius L *et al*. Dopamine-related genes and their relationships to monoamine metabolites in CSF. *Biol Psychiatry* 1996; **40**: 1032–1043.
- 18 Wang J, O'Donnel P. D(1) dopamine receptors potentiate nmda-mediated excitability increase in layer V prefrontal cortical pyramidal neurons. *Cerebral Cortex* 2001; **11**: 452–462.
- 19 Potkin SG, Buchsbaum MS, Jin Y, Tang C, Telford J, Friedman G *et al*. Clozapine effects on glucose metabolic rate in striatum and frontal cortex. *J Clin Psychiatry* 1994; **55**: 63–66.
- 20 Okubo Y, Suhara T, Suzuki K, Kobayashi K, Inoue O, Terasaki O *et al*. Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature* 1997; **385**: 634–636.
- 21 Buchsbaum MS, Potkin SG, Marshall J, Lottenberg S, Heh CW, Tafalla R *et al*. Effects of clozapine and thiothixene on glucose metabolic rate in schizophrenia. *Neuropsychopharmacology* 1992; **6**: 155–163.
- 22 Buchsbaum MS, Potkin SG, Siegel BV, Lohr J, Katz M, Gottschalk LA *et al*. Striatal metabolic rate and clinical response to neuroleptics in schizophrenia. *Arch Gen Psychiatry* 1992; **49**: 966–974.
- 23 Friston K, Frith C, Liddle P, Frackowiak R. Comparing functional (PET) images: the assessment of significant change. *J Cerebr Blood Flow Metab* 1991; **11**: 690–699.
- 24 Matsui T, Hirano A. *An Atlas of the Human Brain for Computerized Tomography*. Lagaku-Shoin: Tokyo, 1978.
- 25 Wu JC, Bell K, Najafi A, Widmark C, Keator D, Tang C *et al*. Decreasing striatal 6-FDOPA uptake with increasing duration of cocaine withdrawal. *Neuropsychopharmacology* 1997; **17**: 402–409.
- 26 Good P. *Permutation Test: A Practical Guide to Resampling Methods for Testing Hypothesis* 1st edn. Springer-Verlag: Berlin, 1994.
- 27 Pollack S, Bruce P, Borenstein M, Liberman J. The resampling method of statistical analysis. *Psychopharmacol Bull* 1994; **30**: 227–234.
- 28 Sunahara RK, Guan HC, O'Dowd BF, Seeman P, Laurier LG, Ng G *et al*. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature* 1991; **350**: 614–619.
- 29 Lannfelt L, Sokoloff P, Martres MP, Pilon C, Giros B, Jonsson E *et al*. Amino acid substitution in the dopamine D3 receptor as a useful polymorphism for investigating psychiatric disorders. *Psychiatr Genet* 1992; **2**: 249–256.
- 30 Warren Jr JT, Peacock ML, Rodriguez LC, Fink JK. An MspI polymorphism in the human serotonin receptor gene (HTR2): detection by DGGE and RFLP analysis. *Hum Mol Genet* 1993; **2**: 338.
- 31 Lappalainen J, Zhang L, Dean M, Oz M, Ozaki N, Yu D-H *et al*. Identification, expression, and pharmacology of a cys23–ser23 substitution in the human 5HT2C receptor gene (HTR2C). *Genomics* 1995; **27**: 274–279.

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