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ORIGINAL ARTICLE

Association study of Cannabinoid receptor 1 (*CNR1*) gene in tardive dyskinesia

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Tardive dyskinesia (TD) is a severe, debilitating movement disorder observed in 25–30% of the patients treated with typical antipsychotics. Cannabinoid receptor 1 (CNR1) activators tend to inhibit movement, an effect prevented by rimonabant and other selective CNR1 antagonists. Furthermore, CNR1 receptor is downregulated in Huntington's disease and upregulated in Parkinson's disease. Twenty tagSNPs spanning the *CNR1* gene were analyzed in schizophrenia patients of European ancestry ($n=191$; 74 with TD). Significant genotypic ($P=0.012$) and allelic ($P=0.012$) association was observed with rs806374 (T>C). Carriers of the CC genotype were more likely to be TD positive (CC vs TT + TC, odds ratio = 3.4 (1.5–7.8), $P=0.003$) and had more severe TD (CC vs TT + TC; 9.52 ± 9.2 vs 5.62 ± 6.9, $P=0.046$). These results indicate a possible role of CNR1 in the development of TD in our patient population. However, these observations are marginal after correcting for multiple testing and need to be replicated in a larger patient population.
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Keywords: *CNR1*; association; movement; tardive dyskinesia; schizophrenia; genetics

Introduction

Antipsychotic drugs continue to represent the cornerstone of treatment in schizophrenia. The older 'typical' antipsychotics revolutionized care but were compromised by a notable liability for drug-induced movement disorders, including acute extrapyramidal symptoms and tardive dyskinesia (TD).[1] Although the new generation of 'atypical' antipsychotics drugs have become the current standard of care, it is not entirely clear how much more beneficial they are in terms of extrapyramidal symptoms[2,3] and they continue to harbor a risk for TD, albeit somewhat diminished.[4,5] TD can be debilitating and potentially irreversible; moreover, it has been linked with non-compliance to treatment, reduced quality of life and social discrimination.[6,7] TD develops in a subset of patients (~30%) and shows familial occurrence indicating a genetic basis.[8] Other factors that influence development of TD are age, female gender, affective disorders and organic brain dysfunction (for details see[9,10]). The pathophysiology of TD is not well understood and there are no predictive tools available to estimate an individual's risk profile. Therefore, identification of genetic and non-genetic factors influencing TD development could potentially lead to drugs that would avoid this risk, or at the very least effective treatments for TD.

The cannabinoid receptor 1 (*CNR1* or CB1), an integral part of the endocannabinoid system, is a target for endogenous cannabinoids (N-arachido-

noylethanolamine and 2-arachidonoylglycerol) as well as exogenous cannabinoid such as Δ9-tetrahydrocannabinol. *CNR1* is a G-protein-coupled receptor expressed presynaptically in both glutamatergic (excitatory) and GABAergic (inhibitory) synapses and primarily acts as a synaptic circuit breaker, attenuating neurotransmitter release.[11,12] *CNR1* is the most abundant G-protein coupled receptor in the brain and is relatively abundant in the basal ganglia structures.[13–15] The CB1 receptor is expressed presynaptically in the GABAergic neurons of both the 'direct' and 'indirect' pathway as well as in the glutamatergic afferents, from cortical sites to the striatum.[15–18] CB1 knockout mice exhibit hypolocomotion[19] and administration of CB1 receptor agonists is generally associated with inhibition of movement, an effect prevented by antagonists such as rimonabant (SR141716A).[15] In rats, colocalization of the CB1 receptors with both dopamine D1 and D2 receptors has been observed.[20] Exogenous as well as endogenous cannabinoids generally inhibit dopamine-mediated behavior such as D1 receptor-mediated grooming and D2 receptor-mediated stereotypical oral movements and horizontal locomotion. This effect is reversed by the CB1 receptor antagonist SR141716A.[20–22] A role of the endocannabinoid system in the development of movement disorders (Parkinson's and Huntington's disease, HD), as well as treatment side effects such as levodopa-induced dyskinesia, has been demonstrated (for details see[15,18]). A lack of long-term depression in the indirect pathway neurons is observed in Parkinson disease.[23,24] The indirect pathway long-term depression is mediated by dopamine stimulation of D2 receptors, causing release of endocannabinnoids, which bind to the presynaptic CB1 receptors. D2 agonists and endocannabinnoid degradation inhibitors restore indirect pathway long-term depression and locomotor activity.[18,23] In Huntington's disease, glutamate excitotoxicity-mediated loss of the indirect pathway neurons in particular reduces inhibitory control of unwanted movements.[18,23] Thus, a dysfunctional indirect pathway appears to be a contributor to aberrant movements, and endocannabinnoids acting via CB1 receptors have an important role in these disorders.

Based on the above evidence, in this study, we investigated the association of 20 tagging single nucleotide polymorphisms (SNPs) in the *CNR1* gene locus for association with TD in chronic schizophrenia patients.

Materials and Methods

Subjects

The clinical and demographic characteristics of the sample ($n = 191$) used in the present study have been described in detail in previous publications.[25–28] The subjects were recruited from three clinical sites in the United States: Case Western Reserve University in Cleveland, Ohio (Dr HY Meltzer, $N = 50$); Hillside Hospital in Glen Oaks, New York (Dr JA Lieberman, $N = 43$) and University of California at Irvine, California (Dr SG Potkin, $N = 10$). The fourth clinical site was the Centre for Addiction and Mental Health in Toronto, Ontario, Canada (Dr G Remington, $N = 88$). Schizophrenia ($n = 178$) or schizoaffective disorder ($n = 13$) patients diagnosed according to DSM-III-R or IV (APA, 2000) were included. Patients suffering from type 2 diabetes, head injury with loss of consciousness and seizure disorder were excluded from the study. A detailed medication history for all patients was not available; however, patients recruited in the USA (HYM, JAL, SGP) were all DSM-IIIR diagnosed patients with chronic schizophrenia and without exposure to atypical antipsychotic medication before TD assessment. The chronic patients recruited in Canada (GR) were on combinations of atypical and typical antipsychotics at the time of TD assessment. Nonetheless, all patients have undergone at least 1 year of treatment with typical antipsychotics before TD assessment. The presence of TD was assessed using the Abnormal Involuntary Movement Scale (AIMS) or the modified Hillside Simpson Dyskinesia Scale for 43 patients recruited from Hillside Hospital.[29–31] The modified Hillside Simpson Dyskinesia Scale is comprised of 28 individual symptom items, seven-body area global items (face, lips, jaw, tongue, upper extremities, neck and trunk, lower extremities) that correspond to the AIMS and an overall global score. The latter two duplicate the items of the AIMS, therefore we could extract the AIMS score for the patients scored using Hillside Simpson Dyskinesia Scale.[31–33] TD was diagnosed to be present if the patient had at least 3 months of total cumulative neuroleptic exposure, presence of at least moderate abnormal involuntary movements in one or more body areas or at least mild movements in two or more body areas and absence of conditions that might cause abnormal involuntary movements.[30] All the patients are of self-reported European ancestry based on the family details of over last three generations. In all, 191 European ancestry schizophrenia patients were studied, of which 74 were positive for the diagnosis of TD. AIMS scores were available for a subset of patients ($n = 162$). The demographic characteristics are presented in Table 1.

Genetic analysis

10 ml of venous blood was obtained from study participants and DNA was extracted using the high salt method[34] at the Centre for Addiction and Mental Health, Toronto. All the genotyping was carried out using TaqMan SNP genotyping

Table 1 Demographic characteristic of the study population

	TD-Y (n = *74*)	*TD-N* (n = *117*)	P-*value*
Age (mean ± s.d.)	40.5 ± 10.8 (74)	36.1 ± 9.3 (117)	0.003
Gender			
Male n (%)	43 (58.1)	84 (72.4)	0.041
Female n (%)	31 (41.9)	32 (27.6)	—
AIMS (mean ± s.d.)	12.9 ± 7.6 (65)	1.5 ± 2.0 (97)	0.000[a]

Abbreviations: AIMS, Abnormal Involuntary Movement Scale; TD-N, tardive dyskinesia absent; TD-Y, tardive dyskinesia present.
[a]Kruskal–Wallis test.

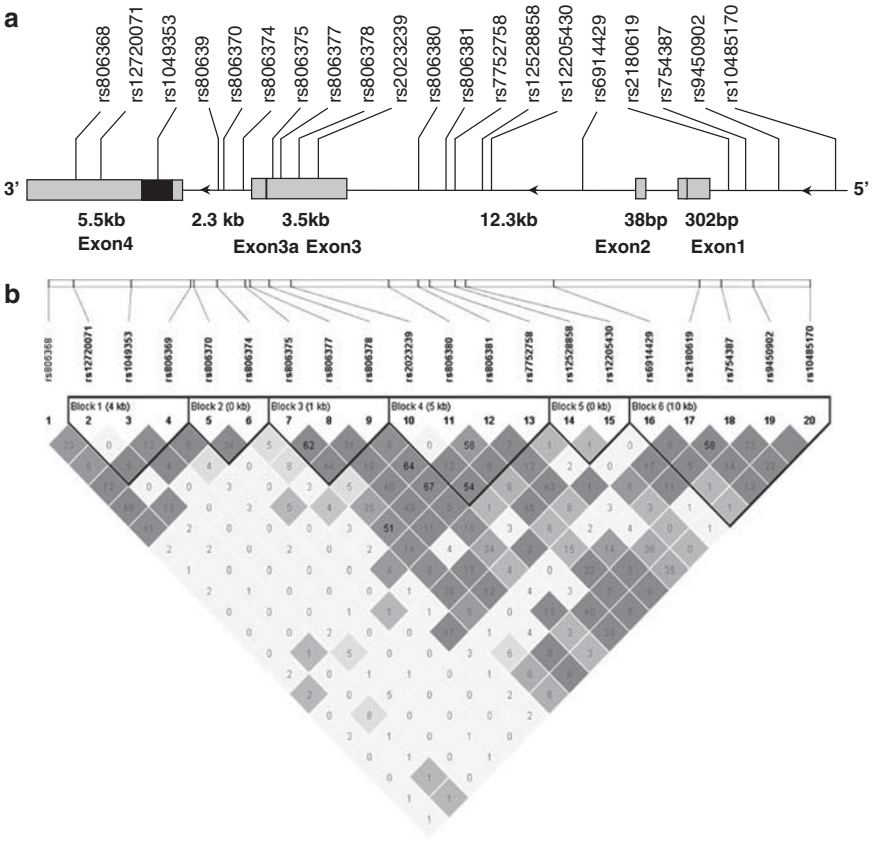

Figure 1 *CNR1* gene structure and linkage disequilibrium. (**a**) Gene structure of CNR1. Alternative splicing is known to occur at CNR1 and five transcripts have been identified.[41] The dark filled boxes represent the coding region. (**b**) Linkage disequilibrium among the 20 tag SNPs. Standard color scheme is used (Haploview 4.1). Bright red represents $D' = 1$ and LOD $\geqslant 2$; shades of pink/red, $D' < 1$ and LOD $\geqslant 2$; blue, $D' = 1$ and LOD < 2; white, $D' < 1$ and LOD < 2. The values in the boxes represent r^2. The haplotype blocks were constructed using the solid spine of LD method. The color reproduction of this figure is available on the html full text version of the manuscript.

assays (Applied Biosystems Inc, Foster City, CA, USA). Genotype calling was confirmed by two independent researchers and 5% of the total sample was re-genotyped to assure concordance. The concordance rate for this study was 99.86% and discordant genotypes were treated as missing in the statistical analysis. A total of 20 tagSNPs ($r^2 \geqslant 0.8$, minor allele frequency $\geqslant 0.05$) covering 100% of the common variation in the *CNR1* gene region (~ 32.5 kbp) were included in the study (Figure 1).

Data analysis

The statistical analyses were performed using SPSS 15.0 (SPSS, Chicago, IL, USA). Categorical variables were tested using a χ^2-test and continuous variables (for example, AIMS score) were tested using analysis of variance or analysis of covariance. *Post hoc* analyses were carried out only for SNPs with a trend or significant associations with TD. Allelic and haplotypic comparisons were made using UNPHASED 3.0.[35] Only haplotypes with frequencies $>5\%$ were included in the analyses. Linkage disequilibrium (LD) and tagSNPs ($r^2 \geqslant 0.8$, minor allele frequency $\geqslant 0.05$) were determined using Haploview 4.1.[36] LD blocks were constructed using the method solid spine of LD. Power calculations were carried out using Quanto 1.2.4.[37] Assuming a minor allele frequency of 0.25 in a sample size of $n = 191$, we had $>80\%$ power to detect an odds ratio (OR) of 2.0 in a log-additive model.

Results

All the SNPs tested in this study (except for rs806374) were in Hardy–Weinberg equilibrium ($p > 0.05$, Table 2). The SNP rs806374 deviated from Hardy–Weinberg equilibrium because of the association of the SNP with presence of TD (TD-Y). This SNP was in Hardy–Weinberg equilibrium in patients without TD (TD-N, $P = 0.798$). Furthermore, in another schizophrenia patient sample this SNP is in Hardy–Weinberg equilibrium.[38] Variable amount of LD between the SNPs was observed. An interesting observation was the presence of a region of high recombination between rs806374 (marker 6) and rs806375 (marker 7), separating the coding region in exon 4 from the non-coding exons in the 5' untranslated region (Figure 1). This high recombination region is also evident in the SNP information available from the HapMap database (http://hapmap.ncbi.nlm.nih.gov). A significant difference in the distribution of mean

Table 2 SNPs in the *CNR1* gene associated with tardive dyskinesia at genotypic and/or allelic level ($P<0.1$)

Serial no	*SNP*	*HWE*	*Allele*	*TD-Y*	*TD-N*	P-*value*	*Genotype*	*TD-Y*	*TD-N*	P-*value*
1	rs806368	0.224	T	111 (0.75)	178 (0.78)	0.492	TT	46 (62.2)	69 (60.5)	0.085
			C	37 (0.25)	50 (0.22)		TC	19 (25.7)	40 (35.1)	
							CC	9 (12.2)	5 (4.4)	
2	rs806370	0.503	T	23 (0.16)	21 (0.10)	0.082	TT	4 (5.5)	0	0.280[a]
			C	123 (0.84)	197 (0.90)		TC	15 (20.5)	21 (19.3)	
							CC	54 (74.0)	88 (80.7)	
3	rs806374	0.0318	T	82 (0.57)	147 (0.70)	0.012	TT	29 (40.3)	52 (49.5)	0.012
			C	62 (0.43)	63 (0.30)		TC	24 (33.3)	43 (41.0)	
							CC	19 (26.4)	10 (9.5)	
4	rs9450902	0.454	G	9 (0.06)	28 (0.12)	0.056	GG	1 (1.4)	2 (1.7)	0.049[a]
			C	137 (0.94)	206 (0.88)		GC	7 (9.6)	24 (20.5)	
							CC	65 (89.0)	91 (77.8)	
5	rs10485170	0.361	T	137 (0.94)	202 (0.89)	0.08	TT	65 (89.0)	90 (78.9)	0.074[a]
			C	9 (0.06)	26 (0.11)		TC	7 (9.6)	22 (19.3)	
							CC	1 (1.4)	2 (1.8)	

Abbreviations: HWE, Hardy–Weinberg equilibrium; SNP, single nucleotide polymorphism; TD-N, tardive dyskinesia absent; TD-Y, tardive dyskinesia present.
[a]Calculated using 2×2 table collapsing the category with low cell value with the heterozygous group.

age and gender was observed among patients with and without TD (Table 1). There was a significant correlation between AIMS score and age (Pearson's correlation coefficient = 0.290, $P<0.01$), and females had a numerically higher AIMS score compared with males (7.47 ± 7.6 vs 5.47 ± 7.5, $P=0.117$). Based on these observations, AIMS score comparisons across genotypic groups reported below were corrected for the effect of age and gender using analysis of covariance.

We observed a significant association of the SNP rs806374 with TD ($P=0.012$, Table 2, Figure 2). Carriers of the CC genotype (CC vs TT + TC, OR = 3.4 (1.5–7.8), $P=0.003$) and the C-allele (OR = 1.76 (1.13–2.75), $P=0.012$) had a higher risk of developing TD. Furthermore, schizophrenia patients with the CC genotype had more severe TD as compared with patients with either TC or TT genotypes (CC vs TT + TC, 9.52 ± 9.2 vs 5.62 ± 6.9; $P=0.046$). The genotypic association statistics were corrected for the effect of age and gender using logistic regression. The SNP rs806374 was still significantly associated with TD ($P=0.036$), and carriers of the CC genotype (OR = 3.1 (1.3–7.3), $P=0.010$) had a higher risk of developing TD. As the patient sample collected in Toronto, Canada were on a combination of atypical and typical antipsychotics at the time for the assessment for TD we checked if the trends are similar to those observed in the overall sample as well as the sample of United States patients only. In the Toronto sample we observed a trend similar to that observed in the overall as well as the USA sample only. The CC genotype was overrepresented in patients with TD (22.9%) compared with patients without TD (12%). However, this observation was not statistically significant (Supplementary Table 1).

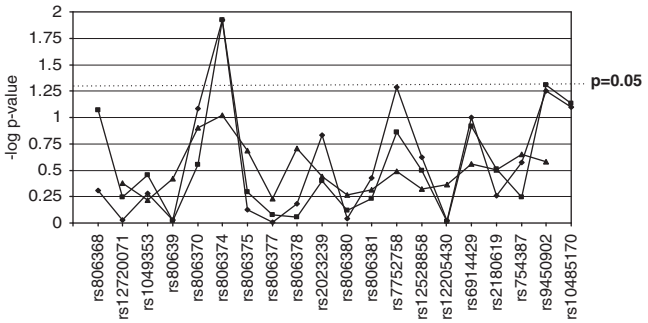

Figure 2 Summary of results for allelic —◆—, genotypic —■— and haplotypic —▲— (three marker sliding window) association between the SNPs and TD. The values on the *y*-axis are negative logarithm of the *P*-value (−log p) observed for each association statistics. The SNP rs806374 exhibits both allelic and genotypic association whereas rs9450902 and rs10485170 exhibit only allelic association.

The SNP rs9450902, present in the promoter region of *CNR1*, exhibited a marginal genotypic association ($P=0.049$) and a trend of allelic association ($P=0.056$) with TD (Table 2). Similar non-significant trends were also observed with rs10485170, which is in LD with rs9450902 ($D'=1$, $r^2=1$, Figure 1). None of the remaining 17 SNPs were associated with TD (Supplementary Table 2, Figure 2).

Haplotypes were compared using a three SNP sliding window across the *CNR1* gene. None of the haplotypes achieved significance ($p>0.05$); however, the sliding window including the SNP rs806374 showed a trend of association with TD (rs806370-rs806374-rs806375; $P=0.096$, Figure 2).

We also compared AIMS scores across genotypic groups for the 20 tagSNPs genotyped in this study. No significant difference in the distribution of mean AIMS score across genotypic groups (correcting for age and gender) was observed ($P>0.05$, Supplementary Table 3).

To account for multiple testing we calculated the effective number of independent tests using single nucleotide polymorphism spectral decomposition (SNPSpD;[39] http://gump.qimr.edu.au/general/daleN/SNPSpD/). The effective number of independent marker loci (MeffLi)[40] was 14.035, whereas the experiment-wide significance threshold required to keep type I error rate at 5% was 0.0036. After this correction for multiple tests, the overall genotypic association of rs806374 with TD was not significant (genotypic corrected $P_{(14.035\times0.012)}=0.168$). However, the *post hoc* test of the recessive model for the CC genotype remained significant ($P_{(14.035\times0.003)}=0.042$).

Discussion

This is the first study investigating the association of *CNR1* gene with occurrence of TD. Using 20 tagSNPs, we captured 100% of the known common variations in the *CNR1* gene region. Of the 20 tagSNPs, spanning ~32.5 kb, we observed a significant association of an intronic SNP rs806374 with TD (CC vs TT + TC, OR = 3.4 (1.5–7.8), $P=0.003$). Furthermore, increased severity of TD was observed in individuals who carried the risk genotype (CC vs TT + TC, 9.52 ± 9.2 vs 5.62 ± 6.9, $P=0.046$). A trend of association was also observed, with rs9450902 present ~4.5 kb upstream of the *CNR1* transcription start site.[41]

No functional effect of rs806374 is known although it is present in a region of high recombination and close to the alternative promoter described by Zhang *et al*.[41] Furthermore, *in silico* prediction using a 31-bp sequence including the rs806374 T>C predicts allele specific binding of transcription factors (MatInspector, Genomatix Software GmbH, Munich, Germany). The C-allele is ancestral and presence of the C-allele creates a binding site for a homeodomain containing transcription factor NK2 homeobox 3 (NKX2-3). The *NKX2-3* gene is associated with the development of gut and spleen[42] and has been associated with ulcerative colitis and Crohn's disease across several populations.[43–45] The presence of the T-allele of rs806374 creates a binding site for two transcription factors, namely POU class 6 homeobox 1 (BRN5 or POU6F1) and homeobox 9 (*HOXB9*). BRN5 (brain-5) is widely expressed, exhibiting highest expression levels in the developing rat brain and spinal cord during embryogenesis. In the adult rat brain, diffuse expression of Brn-5 is observed, along with expression in kidney, lung, heart, adrenal, skin, testis and anterior pituitary.[46] *HOXB9* is expressed throughout the body and is involved in cell proliferation and differentiation. Increased expression of this gene has been observed in leukemia, prostate cancer, breast cancer and lung cancer (OMIM,[47]). Although this allele specific transcription factor binding is predicted, there is no evidence directly implicating these transcription factors in the control or differentiation of motor circuits. Thus, it is possible that the observed association is due to a yet unidentified functional SNP that is in linkage disequilibrium with rs806374. Considering the high expression of the *CNR1* gene in the basal ganglia circuits, recent observations of the important role played by *CNR1* in mediating D1 and D2 mediated behaviors in rats suggest that the *CNR1* gene *per se* may have a role in TD and possibly other movement disorders.[18,20,23]

Limitations of this study include unavailability of lifetime antipsychotic treatment details (class of drug, dose and duration) and substance abuse. Treatment of patients with atypical antipsychotic such as clozapine can ameliorate TD. In this regard our Toronto sample was treated with a combination of atypical and typical antipsychotics, however, the majority of the patients (~90%) did not receive clozapine. Additional limitations were the relatively small sample size and collection of samples from three different clinical sites.[25] Furthermore, the overall associations were not significant after correction for multiple testing, although the recessive model for rs806374 remained significant. In this study we show an association with the TD diagnosis status as well as the severity measured by the total AIMS score. It would be interesting to discern whether the present association is due to a particular subtype of TD (orofaciolingual or limb-truncal), however, the information on the subscales of the AIMS score was not available for our patients. The observations made in this study need to be replicated in a larger, well characterized and independent sample set.

In conclusion, we provide preliminary evidence suggesting that a polymorphism, rs806374, in the *CNR1* gene may be associated with TD. Extensive investigation of this gene in TD and other movement disorders such as Parkinson disease and Huntington's disease may help us in understanding their genesis.

Conflict of interest

AKT/CCZ/DJM: report no competing interests. HYM has received grants or is a consultant to Abbott Labs, ACADIA, Bristol Myers Squibb, Eli Lilly, Janssen, Pfizer, Astra Zeneca, Glaxo Smith Kline, Memory, Cephalon, Minster, Aryx and BiolineRx. HYM is a shareholder of ACADIA. JAL reports that he serves on the Advisory Board of Bioline, GlaxoSmithKline, Intracellular Therapies, Eli Lilly, Pierre Fabre, Psychogenics and Wyeth. He does not receive financial compensation or salary support for his participation as an advisor. He receives grant support from Allon, Forest Labs, Merck and Pfizer; he holds a patent from Repligen. JLK has been a consultant to GSK, Sanofi-Aventis and Dainippon-Sumitomo.

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References

1 Casey DE. Neuroleptic drug-induced extrapyramidal syndromes and tardive dyskinesia. *Schizophr Res* 1991; **4**: 109–120.
2 Leucht S, Wahlbeck K, Hamann J, Kissling W. New generation antipsychotics versus low-potency conventional antipsychotics: a systematic review and meta-analysis. *Lancet* 2003; **361**: 1581–1589.
3 Miller DD, Caroff SN, Davis SM, Rosenheck RA, McEvoy JP, Saltz BL *et al.* Extrapyramidal side-effects of antipsychotics in a randomised trial. *Br J Psychiatry* 2008; **193**: 279–288.
4 Correll CU, Schenk EM. Tardive dyskinesia and new antipsychotics. *Curr Opin Psychiatry* 2008; **21**: 151–156.
5 Tarsy D, Baldessarini RJ. Epidemiology of tardive dyskinesia: is risk declining with modern antipsychotics? *Mov Disord* 2006; **21**: 589–598.
6 Gerlach J. Improving outcome in schizophrenia: the potential importance of EPS and neuroleptic dysphoria. *Ann Clin Psychiatry* 2002; **14**: 47–57.
7 Marsalek M. Tardive drug-induced extrapyramidal syndromes. *Pharmacopsychiatry* 2000; **33** (Suppl 1): 14–33.
8 Muller DJ, Schulze TG, Knapp M, Held T, Krauss H, Weber T *et al.* Familial occurrence of tardive dyskinesia. *Acta Psychiatr Scand* 2001; **104**: 375–379.
9 Muller DJ, Shinkai T, De Luca V, Kennedy JL. Clinical implications of pharmacogenomics for tardive dyskinesia. *Pharmacogenomics J* 2004; **4**: 77–87.
10 Thelma B, Srivastava V, Tiwari AK. Genetic underpinnings of tardive dyskinesia: passing the baton to pharmacogenetics. *Pharmacogenomics* 2008; **9**: 1285–1306.
11 Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat Med* 2008; **14**: 923–930.
12 Hudson BD, Hebert TE, Kelly ME. Ligand- and heterodimer-directed signaling of the CB(1) cannabinoid receptor. *Mol Pharmacol* 2010; **77**: 1–9.
13 van der Stelt M, Di Marzo V. The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders. *Eur J Pharmacol* 2003; **480**: 133–150.
14 Matyas F, Yanovsky Y, Mackie K, Kelsch W, Misgeld U, Freund TF. Subcellular localization of type 1 cannabinoid receptors in the rat basal ganglia. *Neuroscience* 2006; **137**: 337–361.
15 Fernandez-Ruiz J. The endocannabinoid system as a target for the treatment of motor dysfunction. *Br J Pharmacol* 2009; **156**: 1029–1040.
16 Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. *Trends Neurosci* 1989; **12**: 366–375.
17 Surmeier DJ, Ding J, Day M, Wang Z, Shen W. D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci* 2007; **30**: 228–235.
18 Kreitzer AC, Malenka RC. Striatal plasticity and basal ganglia circuit function. *Neuron* 2008; **60**: 543–554.
19 Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc Natl Acad Sci USA* 1999; **96**: 5780–5785.
20 Martin AB, Fernandez-Espejo E, Ferrer B, Gorriti MA, Bilbao A, Navarro M *et al.* Expression and function of CB1 receptor in the rat striatum: localization and effects on D1 and D2 dopamine receptor-mediated motor behaviors. *Neuropsychopharmacology* 2008; **33**: 1667–1679.
21 Rodriguez de Fonseca F, Del Arco I, Martin-Calderon JL, Gorriti MA, Navarro M. Role of the endogenous cannabinoid system in the regulation of motor activity. *Neurobiol Dis* 1998; **5** (6 Part B): 483–501.
22 Giuffrida A, Parsons LH, Kerr TM, Rodriguez de Fonseca F, Navarro M, Piomelli D. Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nat Neurosci* 1999; **2**: 358–363.
23 Kreitzer AC, Malenka RC. Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature* 2007; **445**: 643–647.
24 Shen W, Flajolet M, Greengard P, Surmeier DJ. Dichotomous dopaminergic control of striatal synaptic plasticity. *Science* 2008; **321**: 848–851.
25 Zai CC, Hwang RW, De Luca V, Muller DJ, King N, Zai GC *et al.* Association study of tardive dyskinesia and twelve DRD2 polymorphisms in schizophrenia patients. *Int J Neuropsychopharmacol* 2007; **10**: 639–651.
26 Zai CC, Romano-Silva MA, Hwang R, Zai GC, Deluca V, Muller DJ *et al.* Genetic study of eight AKT1 gene polymorphisms and their interaction with DRD2 gene polymorphisms in tardive dyskinesia. *Schizophr Res* 2008; **106**: 248–252.
27 Zai CC, Tiwari AK, De Luca V, Muller DJ, Bulgin N, Hwang R *et al.* Genetic study of BDNF, DRD3, and their interaction in tardive dyskinesia. *Eur Neuropsychopharmacol* 2009; **19**: 317–328.
28 Zai CC, Tiwari AK, Basile V, de Luca V, Muller DJ, Voineskos AN *et al.* Oxidative stress in tardive dyskinesia: genetic association study and meta-analysis of NADPH quinine oxidoreductase 1 (NQO1) and superoxide dismutase 2 (SOD2, MnSOD) genes. *Prog Neuropsychopharmacol Biol Psychiatry* 2010; **34**: 50–56.
29 Guy W. *ECDEU Assessment Manual for Psychopharmacology* (revised edn.) Department of Health, Education and Welfare: Washington, DC, 1976.
30 Schooler NR, Kane JM. Research diagnoses for tardive dyskinesia. *Arch Gen Psychiatry* 1982; **39**: 486–487.
31 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.
32 Lieberman J, Kane JM, Woerner M, Weinhold P, Basavaraju N, Kurucz J *et al.* Prevalence of tardive dyskinesia in elderly samples. *Psychopharmacol Bull* 1984; **20**: 382–386.
33 Lieberman J, Johns C, Cooper T, Pollack S, Kane J. Clozapine pharmacology and tardive dyskinesia. *Psychopharmacology (Berl)* 1989; **99**(Suppl): S54–S59.
34 Lahiri DK, Nurnberger Jr JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991; **19**: 5444.
35 Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol* 2003; **25**: 115–121.
36 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**: 263–265.
37 Gauderman WJ, Morrison JM. QUANTO 1.1: a computer program for power and sample size calculations for genetic-epidemiology studies, http://hydra.usc.edu/gxe, 2006.
38 Tiwari AK, Zai CC, Likhodi O, Lisker A, Singh D, Souza RP, *et al.* A common polymorphism in the cannabinoid receptor 1 (CNR1) gene is associated with antipsychotic-induced weight gain in Schizophrenia. *Neuropsychopharmacology* 2010; **35**: 1315–1324.
39 Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* 2004; **74**: 765–769.
40 Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity* 2005; **95**: 221–227.
41 Zhang PW, Ishiguro H, Ohtsuki T, Hess J, Carillo F, Walther D *et al.* Human cannabinoid receptor 1: 5′ exons, candidate regulatory regions, polymorphisms, haplotypes and association with polysubstance abuse. *Mol Psychiatry* 2004; **9**: 916–931.
42 Pabst O, Forster R, Lipp M, Engel H, Arnold HH. NKX2.3 is required for MAdCAM-1 expression and homing of lymphocytes in spleen and mucosa-associated lymphoid tissue. *EMBO J* 2000; **19**: 2015–2023.
43 Fisher SA, Tremelling M, Anderson CA, Gwilliam R, Bumpstead S, Prescott NJ *et al.* Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. *Nat Genet* 2008; **40**: 710–712.
44 Franke A, Balschun T, Karlsen TH, Hedderich J, May S, Lu T *et al.* Replication of signals from recent studies of Crohn's disease identifies

previously unknown disease loci for ulcerative colitis. *Nat Genet* 2008; **40**: 713–715.
45 Van Limbergen J, Wilson DC, Satsangi J. The genetics of Crohn's disease. *Annu Rev Genomics Hum Genet* 2009; **10**: 89–116.
46 Andersen B, Schonemann MD, Pearse II RV, Jenne K, Sugarman J, Rosenfeld MG. Brn-5 is a divergent POU domain factor highly expressed in layer IV of the neocortex. *J Biol Chem* 1993; **268**: 23390–23398.
47 Hayashida T, Takahashi F, Chiba N, Brachtel E, Takahashi M, Godin-Heymann N *et al.* HOXB9, a gene overexpressed in breast cancer, promotes tumorigenicity and lung metastasis. *Proc Natl Acad Sci USA* 2010; **107**: 1100–1105.

Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (http://www.nature.com/tpj)