SHORT REPORT

Genetic polymorphism of dopamine D2 receptors in Parkinson's disease and interactions with cigarette smoking and MAO-B intron 13 polymorphism

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Abstract

Genetic polymorphisms of dopamine D2 receptors (DRD2) may be susceptibility factors for Parkinson's disease due to their influence on dopamine response and association with cigarette smoking, which is inversely related to risk of Parkinson's disease. Relations of TaqIA and TaqIB DRD2 genotypes with Parkinson's disease were investigated and tested for interactive effects with smoking and the monoamine oxidase B (MAO-B) intron 13 polymorphism previously found to be related to smoking. Study subjects were 152 cases of idiopathic Parkinson's disease and 231 controls. The smoking history of all genotyped subjects was known. Subjects of genotype B12 were more frequent among cases than controls (27% and 23.8%, respectively), and were more frequent among "ever smokers" than "never smokers", among controls (27.8% and 17.2%, respectively), although these associations were not statistically significant. Neither TaqIA or TaqIB genotypes modified the inverse relation of smoking and Parkinson's disease. When genotypes for DRD2 were considered in combination with genotypes for intron 13 of MAO-B, genotype combinations with high risk of Parkinson's disease were found; although the MAO-B/DRD2 interaction did not reach statistical significance after Bonferroni correction for multiple comparisons, these results are suggestive of a possible synergism between MAOB and DRD2 genes with respect to Parkinson's disease. (J Neurol Neurosurg Psychiatry 2000;69:535-537)

Keywords: Parkinson's disease, genetic polymorphisms, dopamine D2 receptors, Monoamine oxidase B

Differences among individual subjects in the structure and expression of dopamine receptor genes affect dopamine responses and may be involved in determining genetic predisposition to Parkinson's disease.

The A1 allele of the TaqI A polymorphism of the D2 dopamine receptor (DRD2) gene has been shown to be associated with low DRD2 density in human brain both from in vitro^{1 2} and in vivo studies,^{3 4} compared with the A2 allele. Additionally, the A1 allele of the TaqI A polymorphism has been reported to be associated with various addictive behaviours, including tobacco smoking.^{5 6} The TaqIB polymorphism of DRD2 has not been studied as widely as TaqIA, although recently Spitz *et al*⁷ found that the TaqIB polymorphism was a better marker for smoking behaviour than TaqIA. The well known inverse relation between cigarette smoking and Parkinson's disease⁸ provides rationale for studying DRD2 polymorphisms in relation to the disease. Previous studies of polymorphisms of the D2 receptor and Parkinson's disease have yielded contradictory results.⁹⁻¹²

We have recently found an association of the allele G of the G/A polymorphism in intron 13 of MAO-B with Parkinson's disease,¹³ and an interaction of this polymorphism with smoking with respect to Parkinson's disease.¹⁴

In this study we tested for associations of TaqIA and TaqIB DRD2 polymorphisms with Parkinson's disease, associations of these polymorphisms with smoking, interactions with cigarette smoking, and interaction with MAO-B intron 13 polymorphism and risk of Parkinson's disease.

Materials and methods

One hundred and fifty two newly diagnosed patients with idiopathic Parkinson's disease (92 men and 60 women, aged 37 to 88 years), were identified by neurology and general medical practice clinics of Group Health Cooperative (GHC) from the Puget Sound area in western Washington State. Inclusion criteria for the cases were the presence of at least two of the four cardinal signs of Parkinson's disease: bradykinesia, resting tremor, cogwheel rigidity, and postural reflex impairment. Exclusion criteria were the use of certain medications during the 12 months preceding symptom onset, history of multiple cerebrovascular events, or

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Table 1 DRD2 genotype distribution in Parkinson's disease cases and controls and distribution of MAO-B intron 13 and DRD2 genotype combinations in Parkinson's disease cases and controls

Genotype	Cases	%	Controls	%	Total	%	OR	(95% CI)
Taq I A:								
A22	84	67.2	135	66.8	219	67.0	1.00	
A12	37	29.6	59	29.2	96	29.3	1.01	(0.62–1.65)
A11	4	3.2	8	4.0	12	3.7	0.80	(0.23–2.75)
Taq I B:								
B22	109	71.7	172	74.5	281	73.4	1.00	
B12	42	27.6	55	23.8	97	25.3	1.21	(0.76–1.92)
B11	1	0.7	4	1.7	5	1.3	0.39	(0.04–3.58)
Genotype combination								
MAO-B/DRD2 TaqIA:								
A+AA/A11+A12	16	13	44	22	60	18	1.00	
G+GG+GA/A11+A12	25	20	23	11	48	15	2.99	(1.33–6.68)
A+AA/A22	36	29	63	31	99	30	1.57	(0.78–3.17)
G+GG+GA/A22	48	38	72	36	120	37	1.83	(0.93–3.61)
MAO-B/DRD2 TaqIB:								
A+AA/B11+B12	16	11	38	16	54	14	1.00	
G+GG+GA/B11+B12	27	18	21	9	48	13	3.05	(1.35–6.91)
A+AA/B22	52	34	87	38	139	36	1.42	(0.72–2.79)
G+GG+GA/B22	57	37	85	37	142	37	1.59	(0.81–3.12)

another explanation for parkinsonism symptoms (for example, brain injury, brain tumour, encephalitis). Two hundred and thirty one control subjects (149 men and 82 women, aged 45 to 84 years), were identified from GHC enrollees without histories of Parkinson's disease or other neurodegenerative disorders. Controls were matched as a group to the cases by birth decade, sex, and year of enrollment in GHC. All subjects were of non-Hispanic Caucasian ethnicity. Subjects were considered as "ever smokers" if they had smoked at least 100 cigarettes in their lifetime.

Study subjects were volunteers who were informed of the purpose of the study. Study forms and procedures were approved by the institutional review board committees on human subjects research at the University of Washington and GHC Center for Health Studies.

TaqIA and TaqIB genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as previously described by Grandy *et al*¹⁵ and Spitz *et al*⁷ respectively.

A total of 383 subjects (152 cases and 231 controls) were genotyped for Taq I B polymorphism, whereas 327 subjects (125 cases and 202 controls) were genotyped for Taq I A. Study subjects genotyped for DRD2 were the same subjects of our previous study on MAO-B,¹⁴ with the addition of 54 new cases and 85 controls. Data on association of MAO-B with Parkinson's disease are not reported here, as they were already reported in our previous articles.^{13,14}

For both TaqIA and TaqIB polymorphisms we presented the results obtained by pooling subjects homozygous 11 with subjects heterozygous 12 if the results we obtained for 11 were similar to the results for 12, as the frequency of subjects homozygous 11 is very low (3.7% for A11 and 1.3% for B11). Odds ratios (ORs), and 95% confidence intervals (95% CIs) were calculated to test for association of genotypes and Parkinson's disease and for association of genotypes and smoking. To test for statistical significance of the associations and interactions, logistic regression and likelihood ratio tests were performed in models that corrected

for age and sex. As eight different statistical tests were performed as a total on the two DRD2 polymorphisms, we used the Bonferroni correction and divided the $\alpha=0.05$ level of significance by 8; every p value > 0.00625 was considered as non-significant.

Results

The distribution of genotypes followed the expected Hardy-Weinberg equilibrium. The frequency for the A1 allele of the Taq I A polymorphism was 0.180 in cases and 0.186 in controls; for Taq I B polymorphism, the frequency of allele B1 was 0.140 in cases and 0.136 in controls. As shown in the table, no significant differences in Parkinson's disease risk were present for the genotypes A11, A12, or A22 of the Taq I A polymorphism, nor for the B11, B12, or B22 genotypes of the TaqIB polymorphism. The associations with Parkinson's disease were also tested in a logistic regression model and were non-significant. For the association of TaqIA with smoking, the odds ratios for ever compared with never smoked were: 1.00, 0.94 (95%CI 0.43–2.03), and 1.1 (95%CI 0.15–8.17) for genotypes A22, A12, and A11, respectively in cases. In controls the odds ratios for genotypes A22, A12, and A11 were: 1.00, 1.1 (0.58–2.08), and 0.61 (0.15–2.53). For TaqIB polymorphism the odds ratios for ever versus never smoked were, in cases: 1.00 and 0.93 (95%CI 0.45–1.89) for B22 and B12 genotypes, respectively (OR could not be calculated for B11 as only one subject was present in cases with this genotype). For controls the OR was: 1.00, 1.79 (0.92–3.48), and 0.23 (0.02–2.19), for B22, B12, and B11, respectively. The association with smoking was not significant from a likelihood ratio test in a logistic regression model.

We confirmed the inverse association between smoking and Parkinson's disease; 53% of the cases never smoked compared with 38% of the controls, with a relative risk of 0.55 for Parkinson's disease for subjects who ever smoked compared with subjects who never smoked. The presence of a possible interaction between smoking and Taq I A or Taq I B genotype on risk for Parkinson's disease was tested

in a logistic regression model by the likelihood ratio test, and was non-significant ($p=0.55$).

As shown in the table, particular combinations of genotypes for MAO-B and DRD2 resulted in increased risk for Parkinson's disease, in particular where the allele G for MAO-B cooccurred with the allele A1 or B1 of DRD2. Interactions between MAO-B and TaqIA or TaqIB genotypes of DRD2 were tested in logistic regression models, resulting in $\chi^2=3.63$, $p=0.058$ for TaqIA and $\chi^2=4.38$, $p=0.038$ for TaqIB. These values were not significant after Bonferroni corrections.

Discussion

The allelic frequencies we found in this study for Taq I A and Taq I B polymorphisms of DRD2 for both cases and controls were consistent with those previously given in the literature, as the frequency of allele A1 and B1 alleles had been previously reported as 0.20 and 0.16, respectively, among Caucasians.¹⁶ The TaqIA and TaqIB polymorphisms did not seem to be associated with Parkinson's disease in this study. Contrary to some previous reports,⁵⁻⁷ but in agreement with others,¹⁷ we did not find associations of TaqIA or TaqIB polymorphisms of DRD2 with smoking in either cases of Parkinson's disease or controls. Also, we did not find evidence that either the TaqIA or the TaqIB polymorphism modified the inverse relation between smoking and Parkinson's disease. The results from MAO-B and DRD2 genotype combinations, although not significant after correcting for multiple testing, are suggestive of a possible synergism on Parkinson's disease risk between MAOB and DRD2 genes, which are both involved in the dopamine pathway. The effect of the MAO-B polymorphism in functional terms is not known. Studies of larger populations and functional studies on the effects of these genetic variants will help clarify the interpretation of these results.

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- 1 Noble EP, Blum K, Ritchie T, *et al.* Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. *Arch Gen Psychiatry* 1991;48:648-54.
- 2 Thompson J, Thomas N, Singleton A, *et al.* D2 dopamine receptor gene (DRD2) TaqI A polymorphism: reduced dopaminergic D2 receptor binding in the human striatum associated with the A1 allele. *Pharmacogenetics* 1997;7:479-84.
- 3 Pohjalainen T, Rinne J, Nagren K, *et al.* Genetic determinants of human D2 dopamine receptor binding characteristics in vivo. *Am J Hum Genet* 1996;59:A387.
- 4 Pohjalainen T, Rinne JO, Nagren K, *et al.* The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. *Mol Psychiatry* 1998;3:256-60.
- 5 Comings DE, Ferry L, Bradshaw-Robinson S, *et al.* The dopamine D2 receptor (DRD2) gene: a genetic risk factor in smoking. *Pharmacogenetics* 1996;6:73-9.
- 6 Noble EP, St-Jeor ST, Ritchie T, *et al.* D2 dopamine receptor gene and cigarette smoking: a reward gene? *Med Hypotheses* 1994;42:257-60.
- 7 Spitz MR, Shi H, Yang F, *et al.* Case-control study of the D2 dopamine receptor gene and smoking status in lung cancer patients. *J Natl Cancer Inst* 1998;90:358-63.
- 8 Morens DM, Grandinetti A, Reed D, *et al.* Cigarette smoking and protection from Parkinson's disease: false association or etiologic clue? *Neurology* 1995;45:1041-51.
- 9 Plante-Bordeneuve V, Taussig D, Thomas F, *et al.* Evaluation of four candidate genes encoding proteins of the dopamine pathway in familial and sporadic Parkinson's disease. *Neurology* 1997;48:1589-93.
- 10 Nanko S, Ueki A, Hattori M, *et al.* No allelic association between Parkinson's disease and d2, d3, d4 receptor gene polymorphisms. *Am J Med Genet* 1994;54:361-4.
- 11 Comings DE, Comings BG, Muhleman D, *et al.* The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. *JAMA* 1991;266:1793-800.
- 12 Oliveri RL, Annesi G, Zappia M, *et al.* Dopamine D2 receptor gene polymorphism and the risk of levodopa-induced dyskinesias in PD. *Neurology* 1999;53:1425-30.
- 13 Costa P, Checkoway H, Levy D, *et al.* Association of a polymorphism in intron 13 of the monoamine oxidase B gene with Parkinson's disease. *Am J Med Genet* 1997;74:154-6.
- 14 Checkoway H, Franklin GM, Costa-Mallen P, *et al.* Modification of the association between cigarette smoking and Parkinson's disease by a genetic polymorphism of monoamine oxidase type B. *Neurology* 1998;50:1458-61.
- 15 Grandy DK, Zhang Y, Civelli O. PCR detection of the TaqA RFLP at the DRD2 locus. *Hum Mol Genet* 1993;2:2197.
- 16 Grandy DK, Litt M, Allen L, *et al.* The human dopamine D2 receptor gene is located on chromosome 11 at q22-q23 and identifies a TaqI RFLP. *Am J Hum Genet* 1989;45:778-85.
- 17 Lerman C, Caporaso NE, Audrain J, *et al.* Evidence suggesting the role of specific genetic factors in cigarette smoking. *Health Psychol* 1999;18:14-20.