DBH −1021C→T Does Not Modify Risk or Age at Onset in Parkinson's Disease

Lani S. Chun, BS,[1] Ali Samii, MD,[1,2]
Carolyn M. Hutter, MS,[3] Alida Griffith, MD,[4]
John W. Roberts, MD,[5] Berta C. Leis, PhD, RN,[4]
Anthony D. Mosley, MD, MS,[4] P. Luke Wander, BFA,[1]
Karen L. Edwards, PhD,[3] Haydeh Payami, PhD,[6] and
Cyrus P. Zabetian, MD, MS[1,7]

DBH is a candidate gene in Parkinson's disease (PD) and contains a putative functional polymorphism (-1021C→T) that has been reported to modify PD susceptibility. We examined −1021C→T in a sample of 1,244 PD patients and 1,186 unrelated control subjects. There was no significant difference in allele ($p = 0.14$) or genotype ($p = 0.26$) frequencies between the two groups. A similar result was obtained after pooling our data with those previously published. Furthermore, we found no evidence for an effect of genotype on age at onset among patients. Our findings argue against DBH −1021C→T as a risk factor or age at onset modifier in PD.

Parkinson's disease (PD) is characterized by loss of dopaminergic neurons in the substantia nigra and noradrenergic (NA) neurons in the locus ceruleus.[1] The clinical significance of central NA neuronal loss in PD is still debated, but one possible consequence is to render dopaminergic neurons more vulnerable to toxic insult.[2] Support for this hypothesis comes from studies on animal models of PD in which prior lesioning of the locus ceruleus markedly enhances 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine– and 6-hydroxydopamine–mediated injury of nigral dopaminergic neurons.[3,4]

From the [1]Department of Neurology, University of Washington School of Medicine; [2]Parkinson's Disease Research Education and Clinical Center, VA Puget Sound Health Care System; [3]Department of Epidemiology, University of Washington School of Public Health and Community Medicine, Seattle; [4]Booth Gardner Parkinson's Care Center, Evergreen Hospital Medical Center, Kirkland; [5]Virginia Mason Medical Center, Seattle, WA; [6]Genomics Institute, Wadsworth Center, New York State Department of Health, Albany, NY; and [7]Geriatric Research Education and Clinical Center, VA Puget Sound Health Care System, Seattle, WA.

Address correspondence to Dr Zabetian, Geriatric Research Education and Clinical Center S-182, VA Puget Sound Health Care System, 1660 South Columbian Way, Seattle, WA 98108. E-mail: zabetian@u.washington.edu

Thus, factors that modulate NA transmission might influence susceptibility or progression of PD.

The DBH gene encodes the enzyme dopamine β-hydroxylase that converts dopamine to noradrenaline. Plasma activity levels of the enzyme vary 100-fold among European Americans, and a putative functional polymorphism (-1021C→T) in the DBH promoter accounts for approximately 50% of the variance.[5] Because the circulating enzyme is derived from neuronal sources,[6] −1021C→T might also serve as a marker for locus ceruleus dopamine β-hydroxylase content, which could affect NA outflow. Furthermore, DBH resides within a region on chromosome 9q with suggestive evidence of linkage for PD affection status and age at onset (AAO).[7,8] Thus, DBH is of interest as both a biological and positional candidate gene in PD.

In the only study published to date that has assessed the contribution of DBH −1021C→T to PD risk, Healy and colleagues[9] reported that the T/T genotype, which is associated with low levels of plasma dopamine β-hydroxylase activity, was protective against PD. We sought to replicate this finding in a large sample of well-characterized PD patients and control subjects.

Subjects and Methods

Subjects
The study population comprised 1,244 PD patients (mean AAO, 57.9 ± 12.2 years; mean age at enrollment, 67.9 ± 10.6 years; male, 69.9%) and 1,186 unrelated control subjects (mean age at enrollment, 66.9 ± 14.1 years; male, 46.3%) of self-defined European American or "white" ancestry. All patients met standardized clinical diagnostic criteria for PD[10] as determined by a movement disorder specialist, and 16.2% reported having one or more first-degree relatives with PD. The PD cohort was consecutively recruited at six movement disorder clinics in the Portland, OR, and Seattle, WA, areas. Control subjects had no history of parkinsonism and were either spouses of PD patients or individuals recruited from the community. The institutional review boards at each participating site approved the study, and all subjects gave informed consent.

Genotyping and Data Analysis
DBH −1021C→T (rs1611115) was genotyped by TaqMan assay on an ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Quality control of genotyping was assessed by sequencing 47 randomly selected DNA samples.

We used Pearson χ^2 tests to test for deviation from Hardy–Weinberg equilibrium and for association between −1021C→T alleles and PD. Logistic regression was used to test for an association between genotype and PD risk. We adjusted for sex and age at enrollment divided into five categories (<50, 50–60, 60–70, 70–80, and >80 years). We also performed a pooled association analysis combining our data with those of Healy and colleagues.[9]

Linear regression was used to compare mean AAO for cases by genotype. To further examine the relation between

genotype and AAO, we stratified the sample into three age categories based on quantiles of AAO (<53 years: n = 402 cases and 195 control subjects; 53–64 years: n = 436 cases and 279 control subjects; >64 years: n = 406 cases and 712 control subjects). Categories were defined using AAO for cases and age at enrollment for control subjects. Logistic regression was used to test for association between genotype and case–control status in each age category and for an interaction between genotype and age category.

Power calculations were performed using Genetic Power Calculator[11] software assuming a T-allele frequency of 0.2 and a disease prevalence of 0.015. All other statistical analyses were performed using STATA version 8.

Results

TaqMan and sequencing-derived DBH −1021C→T genotype calls were concordant for all 47 samples assessed by both methods. There was no significant deviation from Hardy–Weinberg equilibrium in either cases (p = 0.85) or control subjects (p = 0.43).

There were no significant overall differences in genotype or allele frequency between cases and control subjects (Table). With or without adjustment for age and sex, we did not observe an underrepresentation of the T/T genotype among patients with PD (T/T vs C/C: adjusted odds ratio [OR], 1.29; 95% confidence interval [CI], 0.85–1.96; see the Table). Similarly, we found no evidence of an effect for the T allele on disease risk under an adjusted log-additive model (OR, 1.12; 95% CI, 0.97–1.31; p = 0.10). In the pooled data set, there was no significant association between genotype (χ^2 = 1.49; p = 0.48) or allele (χ^2 = 0.16; p = 0.69) and PD (see the Table).

The mean AAO did not significantly differ between genotype groups (mean ± standard deviation [SD] for all genotypes, 57.9 ± 12.2 years; C/C: 58.1 ± 11.9 years; C/T: 57.1 ± 12.8 years; T/T: 59.8 ± 12.2

years; p = 0.16). There was no association between genotype and disease status within any of the three age categories (<53 years: p = 0.17; 53–64 years: p = 0.82; >64 years: p = 0.59) and no evidence of an interaction between genotype and age category (p = 0.68).

Because the effect of DBH −1021C→T on plasma dopamine β-hydroxylase activity has previously been shown to be additive,[5] we assumed an additive model for PD risk in performing power calculations. Our sample provided 99% power (α = 0.05) to detect an effect on PD risk equal to or greater than that Healy and colleagues[9] reported, and 80% power for a T/T genotype relative risk of 0.64.

Discussion

In contrast with a previous study,[9] our data suggest that DBH −1021C→T does not modify susceptibility to PD. Although there was a substantial difference in sex ratios between cases and control subjects in our sample, adjusting for sex in a logistic regression model had little effect on the results of the analysis (see the Table). In addition, we observed no evidence that −1021C→T impacts AAO in PD because there was no significant difference in mean AAO between genotype groups within patients and no difference in genotype frequencies between cases and control subjects in any of the three age quantiles.

Healy and colleagues[9] examined DBH −1021C→T genotype and allele frequencies in a PD cohort (n = 809) and two independent control cohorts (Control A: n = 637; Control B: n = 450) of European ancestry. In the primary analysis of the PD and Control A groups, there was a significant overall difference in genotype frequency (χ^2 = 9.1; p = 0.01) and an under-

Table. Association Testing of *DBH* -1021C→T in Parkinson's Disease

Allele or Genotype	This Study								Healy and colleagues[9]			Pooled Data				
	Cases, n (%)	Control Subjects, n (%)	OR[a]	95% CI	p[b]	OR[c]	95% CI	p[d]	Cases, n (%)	Control A, n (%)	Control B, n (%)	Cases, n (%)	Control Subjects, n (%)	OR[a]	95% CI	p[e]
C	1,932 (77.7)	1,883 (79.4)		Reference					1,313 (81.1)	987 (77.5)	707 (78.6)	3,245 (79.0)	3,577 (78.7)		Reference	
T	556 (22.3)	489 (20.6)	1.11	0.96–1.27	0.14				305 (18.9)	287 (22.5)	193 (21.4)	861 (21.0)	969 (21.3)	0.98	0.88–1.09	0.69
C/C	749 (60.2)	743 (62.6)		Reference			Reference		529 (65.4)	390 (61.2)	283 (62.9)	1,278 (62.3)	1,416 (62.3)		Reference	
C/T	434 (34.9)	397 (33.5)	1.08	0.92–1.29	0.35	1.13	0.93–1.35	0.20	255 (31.5)	207 (32.5)	141 (31.3)	689 (33.6)	745 (32.8)	1.02	0.90–1.17	0.71
T/T	61 (4.9)	46 (3.9)	1.32	0.89–1.95	0.17	1.29	0.85–1.96	0.23	25 (3.1)	40 (6.3)	26 (5.8)	86 (4.2)	112 (4.9)	0.85	0.64–1.14	0.28

[a]Unadjusted.
[b]For the overall unadjusted comparison of genotype groups, p = 0.30.
[c]Adjusted for sex and age at enrollment in 10 year categories (<50, 50–60, 60–70, 70–80, and >80 years).
[d]For the overall adjusted comparison of genotype groups, p = 0.26.
[e]For the overall comparison of genotype groups, p = 0.48.
CI = confidence interval; OR = odds ratio.

representation of both the T allele (OR, 0.80; 95% CI, 0.67–0.97) and T/T genotype (T/T vs C/C: OR, 0.46; 95% CI, 0.27–0.80) among PD patients. The authors then reported that a similar deficit in T/T homozygotes seen among PD patients in comparison with Control B subjects (T/T vs C/C: OR, 0.51; CI, 0.28–0.94) validated their findings. However, the assertion that the latter results validate $-1021C \rightarrow T$ as a risk factor for PD should be tempered by the following facts: (1) an independent PD cohort was not included; and (2) if one performs χ^2 analyses of the PD and Control B data, there are no significant overall differences in genotype ($p = 0.07$) or allele ($p = 0.12$) frequencies. Finally, a pooled analysis of their PD and control groups (A and B) and our case–control sample did not support an association of $-1021C \rightarrow T$ with PD risk (see the Table).

Lack of reproducibility of findings among genetic association studies has been attributed to a number of factors including inadequate power in attempts at replication, variation in study design, and population stratification.[12–14] We considered the role of each of these in our failure to replicate Healy and colleagues[9] findings. Our study had adequate power (99%) to detect a risk effect of the magnitude they observed, so sample size was unlikely a major issue. For design, PD was defined using the same clinical diagnostic criteria[10] in both studies, though approximately one third of the cases in their study, and none in ours, was confirmed by autopsy.[9] However, a recent clinicopathological series utilizing these same diagnostic criteria, applied by movement disorder specialists, reported a positive predictive value of 98.6% for PD.[15] Thus, the rate of misdiagnosis between studies was probably equally low. Selection of control subjects differed substantially in that the majority (60%) of their Control A group were actually patients with "various neurological disorders," and their Control B group was entirely female and nearly a decade younger (mean age, 45 years) than the mean AAO (54.3 years) of their PD group. These confounding factors were not accounted for in their analysis. Lastly, neither study tested for population stratification, which can lead to false-positive or negative associations if cases and control subjects are drawn differentially from subpopulations that differ in disease prevalence and marker allele frequencies.[16] However, because $-1021C \rightarrow T$ allele frequencies are quite similar across intercontinental populations,[5] this was likely of little consequence in either study.

With the limited success of genetic association studies in complex disease, stringent criteria that favor strong protection from bias and extensive replication have been proposed for evaluating data on candidate susceptibility genes.[17] We propose that the "candidacy" of DBH as a risk factor in PD be placed on hold until further supportive evidence becomes available.

References

1. Forno LS. Neuropathology of Parkinson's disease. J Neuropathol Exp Neurol 1996;55:259–272.
2. Gesi M, Soldani P, Giorgi FS, et al. The role of the locus coeruleus in the development of Parkinson's disease. Neurosci Biobehav Rev 2000;24:655–668.
3. Bing G, Zhang Y, Watanabe Y, et al. Locus coeruleus lesions potentiate neurotoxic effects of MPTP in dopaminergic neurons of the substantia nigra. Brain Res 1994;668:261–265.
4. Mavridis M, Degryse AD, Lategan AJ, et al. Effects of locus coeruleus lesions on parkinsonian signs, striatal dopamine and substantia nigra cell loss after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in monkeys: a possible role for the locus coeruleus in the progression of Parkinson's disease. Neuroscience 1991;41:507–523.
5. Zabetian CP, Anderson GM, Buxbaum SG, et al. A quantitative-trait analysis of human plasma-dopamine beta-hydroxylase activity: evidence for a major functional polymorphism at the DBH locus. Am J Hum Genet 2001;68:515–522.
6. Weinshilboum R, Axelrod J. Serum dopamine—hydroxylase: decrease after chemical sympathectomy. Science 1971;173:931–934.
7. DeStefano AL, Golbe LI, Mark MH, et al. Genome-wide scan for Parkinson's disease: the GenePD Study. Neurology 2001;57:1124–1126.
8. DeStefano AL, Lew MF, Golbe LI, et al. PARK3 influences age at onset in Parkinson disease: a genome scan in the GenePD study. Am J Hum Genet 2002;70:1089–1095.
9. Healy DG, Abou-Sleiman PM, Ozawa T, et al. A functional polymorphism regulating dopamine beta-hydroxylase influences against Parkinson's disease. Ann Neurol 2004;55:443–446.
10. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51:745–752.
11. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003;19:149–150.
12. Marchini J, Cardon LR, Phillips MS, Donnelly P. The effects of human population structure on large genetic association studies. Nat Genet 2004;36:512–517.
13. Tabor HK, Risch NJ, Myers RM. Opinion: candidate-gene approaches for studying complex genetic traits: practical considerations. Nat Rev Genet 2002;3:391–397.
14. Trikalinos TA, Ntzani EE, Contopoulos-Ioannidis DG, Ioannidis JP. Establishment of genetic associations for complex diseases is independent of early study findings. Eur J Hum Genet 2004;12:762–769.
15. Hughes AJ, Daniel SE, Ben-Shlomo Y, Lees AJ. The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. Brain 2002;125:861–870.
16. Cardon LR, Palmer LJ. Population stratification and spurious allelic association. Lancet 2003;361:598–604.
17. Ioannidis JP. Commentary: grading the credibility of molecular evidence for complex diseases. Int J Epidemiol 2006;35:572–578, 593–596.