GAAAGTCAGGTCACAGTGACCTG-3′ and 5′-TATGATCAGGTCACTGTGACCTGACT-3′; and CRE oligonucleotide, 5′-TCGAGCAAAATTGACGTCATGGTAATTAC-3′ and 5′-TCGAGTAATTACCATGACGTCAATTTTGC-3′. The AP-1 binding site oligonucleotide was obtained from Oncogene Science. Monoclonal antibodies (500 ng) to the ER DNA binding domain (clone ER33; Affinity BioReagents) or to the ER ligand binding domain (C11, 2D8.B4, and B5; Eli Lilly and Co.), or a nonspecific immunoglobulin G2a antibody (Eli Lilly and Co.), were included in antibody mobility-shift experiments.

15. We thank D. L. Phillips and L. Short for providing the ER antibodies and estrogen metabolite binding data; B. Katzenellenbogen for the ER constructs; G. L. Greene for the human progesterone receptor cDNA clone pHPR-65; and J. D. Termine, J. Hock, and A. H. Tashjian Jr. for discussions and comments on the manuscript.

19 March 1996; accepted 9 July 1996

Tourette Syndrome: Prediction of Phenotypic Variation in Monozygotic Twins by Caudate Nucleus D2 Receptor Binding

Steven S. Wolf, Douglas W. Jones, Michael B. Knable, Julia G. Gorey, Kan Sam Lee, Thomas M. Hyde, Richard Coppola, Daniel R. Weinberger*

Tourette syndrome, a chronic tic disorder with autosomal dominant inheritance, exhibits considerable phenotypic variability even within monozygotic twin pairs. The origins of this variability remain unclear. Recent findings have implicated the caudate nucleus as a locus of pathology, and pharmacological evidence supports dopaminergic involvement. Within monozygotic twins discordant for Tourette syndrome severity, differences in D2 dopamine receptor binding in the head of the caudate nucleus predicted differences in phenotypic severity ($r = 0.99$); this relation was not observed in putamen. These data may link Tourette syndrome with a spectrum of neuropsychiatric disorders that involve associative striatal circuitry.

Georges Gilles de la Tourette originally described Tourette syndrome (TS) in 1885 as a neuropsychiatric disorder characterized by chronic motor and vocal tics that begin in childhood (*1*). The tics are not strictly involuntary but commonly involve a compelling urge to perform rapid, sudden movements or vocalizations. TS is found in all cultures and racial groups; although an accurate prevalence rate for TS has not been established, it is increasingly recognized as being a relatively common disorder (*2*). Family studies suggest that TS is inherited as a single autosomal dominant gene with incomplete penetrance (*3*). Twin studies reveal higher concordance rates for TS within monozygotic twin pairs than within dizygotic pairs, supporting a primary genetic contribution. However, discordance for symptom severity between identical twins with TS indicates that nongenetic factors, including possible prenatal influences, modify the clinical expression of this disorder (*4*).

Several lines of evidence suggest that dopaminergic function is a factor in the phenotypic expression of TS, including the observed efficacy of dopamine D2 receptor antagonists such as haloperidol and pimozide and the exacerbation of symptoms by dopamimetic agents (for example, L-dopa and methylphenidate) (*2*). Although research has focused on the basal ganglia as a likely locus of pathology (*2*), direct evidence for an abnormality of the striatal dopaminergic system is limited (*5*). Thus far, investigations have not revealed an alteration of dopamine D2 receptor density in TS patients compared with persons without the disease (*6*), and linkage analyses have not identified a major gene effect of the dopamine receptor loci or of genes involved in dopamine metabolism and transport (*7*). Although the cause of TS does not appear to be an abnormality of dopamine receptors, the efficacy of antidopaminergic drugs suggests that differences in receptor function, perhaps in specific brain regions, could be a factor in phenotypic variance between individuals. One brain area rich in D2 receptors that has recently been shown to be functionally and morphometrically abnormal in TS is the striatum (*8*).

We studied identical twins to explore the role of dopamine receptor function as a modifying factor that might account for differences in clinical expression of TS. Identical twins discordant for severity of TS provide a powerful statistical design to discern extragenetic factors that correlate with phenotypic variation because genetic factors are controlled for. If, for example, differences in striatal dopamine function modulate the clinical expression of TS, they should be more apparent in the more symptomatic twin.

Five sets of monozygotic twins concordant for the diagnosis of TS but discordant for symptom severity (*9*) (Table 1) were studied with single–photon emission computed tomography (SPECT). Striatal binding of [I^{123}]iodobenzamide ([^{123}I]IBZM), a potent D2 receptor antagonist (*10*), was measured through use of anatomical regions of interest (ROIs) drawn on coregistered magnetic resonance imaging (MRI) scans (*11*) (Fig. 1). To analyze the time course ROI data, we used an integral method that yielded a measure proportional to D2 receptor availability (*12*) (Fig. 2).

Iodobenzamide binding to D2 receptors in the caudate nucleus was greater in all five of the more affected TS patients compared with their less affected siblings (sign test, $P = 0.03$), and mean caudate nucleus binding values differed by 17% between twins (mean ± SEM: 1.49 ± 0.16 compared with 1.25 ±

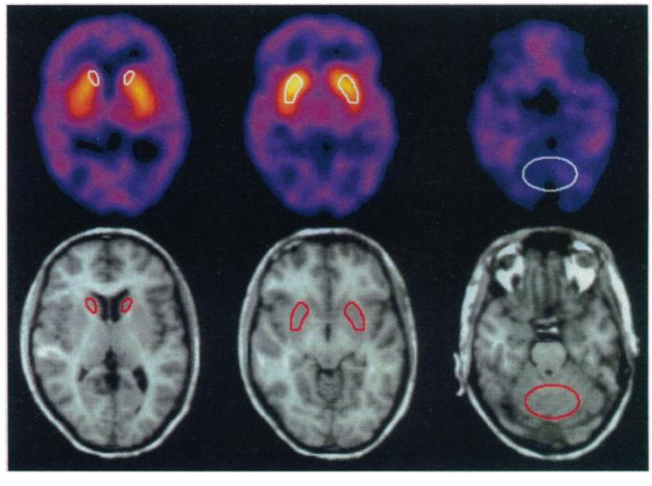

Fig. 1. Placement of ROIs for measurement of [^{123}I]IBZM binding. The 4-hour time-averaged SPECT image (top row) was used for coregistration of the MRI scan (bottom row). ROIs (in red), created on the MRI images for the head of the caudate nucleus (left), putamen (middle), and cerebellum (right), are shown transposed to the summed SPECT image (in white). Actual binding measurements were taken from individual time points (*11*).

Clinical Brain Disorders Branch, Intramural Research Program, National Institute of Mental Health (NIMH), National Institutes of Health, NIMH Neuroscience Center at St. Elizabeths, 2700 Martin Luther King Jr. Avenue, SE, Washington, DC 20032, USA.

*To whom correspondence should be addressed. E-mail: weinberd@dirpc.nimh.nih.gov

Table 1. Clinical ratings of symptom severity for more affected compared with less affected TS twins.

Twin pair		Shapiro symptom checklist		Yale global tic severity		Yale tic count		Aggregate score	
Sex	Age (years)	More affected	Less affected	More affected	Less affected	More affected	Less affected	More affected	Less affected
Male	31	22	18	72	54	8	8	102	80
Male	31	32	17	63	39	34	16	129	72
Male	18	15	5	29	14	12	1	56	20
Female	42	18	11	32	22	5	1	55	34
Male	25	17	0	59	13	18	1	94	14
Mean ± SE	29 ± 4	21 ± 3*	10 ± 3*	51 ± 9*	28 ± 8*	15 ± 5†	5 ± 3†	87 ± 14*	44 ± 14*

*$P = 0.04$. †$P = 0.07$.

0.10; Wilcoxon test, $P = 0.04$). In contrast, no significant differences were observed in the putamen (2.03 ± 0.11 compared with 2.05 ± 0.24, $P = 0.89$) (*13*). Intrapair differences in binding and the corresponding differences in clinical ratings correlated highly, again exclusively for binding in the caudate nucleus (Table 2). The correlation ($r = 0.99$) with the aggregate clinical score suggests that differences in D2 binding in the caudate nucleus account for almost all variance in intrapair phenotype. The credibility of this finding is strengthened by the absence of any significant results in the adjacent putamen, eliminating a role for confounding cohort effects and systematic errors.

The present findings also cannot be attributed to regional cerebral blood flow effects. We studied all subjects with [^{99m}Tc]-labeled HMPAO (hexamethylpropyleneamine oxime) SPECT and found no significant differences within pairs for either caudate nucleus or putamen (*14*). It is also unlikely that our findings are due to medication-induced dopamine receptor up-regulation because subjects were neuroleptic-free for an extended period before the study (9). Partial volume effects probably account for the consistently lower binding data for caudate nucleus compared with putamen in all subjects but would not affect within-pair comparisons for either structure. In contrast, small but significant reductions in caudate nucleus volume have been observed in the more severely affected of monozygotic TS twin pairs (8). However, volume reductions would likely reduce the apparent IBZM binding, in contradiction to our findings; the difference in binding in caudate nucleus may therefore have been even greater than we observed. A strong within-pair concordance was also observed in the time to attain peak IBZM binding (*15*). Considering the many factors (including cerebral blood flow, peripheral metabolism, and blood-brain permeability) that determine radioligand kinetics, this finding further confirms the biological similarity within monozygotic twin pairs.

Our results support the hypothesis that dopamine D2 receptor "supersensitivity" explains the phenotypic variation in TS. Relatively large clinical variations in the expression of TS may result from subtle changes in receptor availability, at least within monozygotic twins in whom most potential confounders are held constant. By analogy, a similar phenomenon may also explain the fluctuations in clinical severity typically observed over time in individuals with TS. The mechanism of this increased D2 receptor binding is uncertain and may involve increased receptor density, receptor-ligand affinity, endogenous dopamine concentrations, or any combination of these factors. Previous dopamine receptor imaging studies in which unrelated groups were compared found no significant differences between TS patients and persons without the disease (6). Most likely, this lack of an effect is due to relatively large interindividual variations in radioligand binding (*16*). Because we studied monozygotic twins, the effect of interindividual variability was substantially reduced; the matched-pair design controlled for genetic factors and many environmental influences.

These results complement earlier findings of striatal pathology in TS (*5*, 8) and point to a concomitant sensitivity of dopamine receptor function within the head of the caudate nucleus. Although neural networks involved in TS have not been identified, functional brain imaging studies of persons with obsessive-compulsive disorder, a frequently comorbid condition, implicate the head of the caudate nucleus together with its projections from orbitofrontal and cingulate cortices (*17*). Our finding that

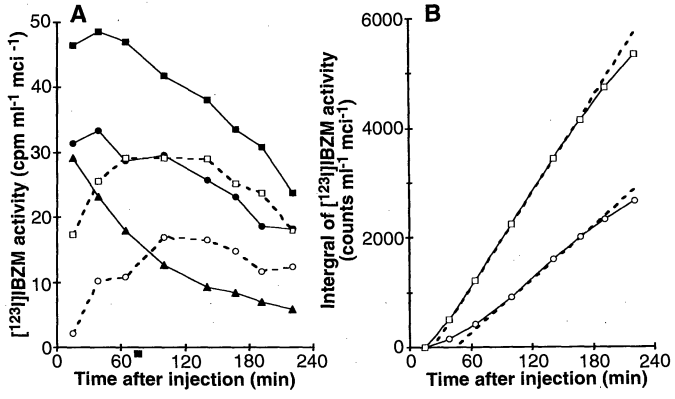

Fig. 2. Representative analysis of [^{123}I]IBZM uptake over time. (**A**) Typical [^{123}I]IBZM time-activity curves from one patient (solid lines) illustrating total uptake in putamen (■), caudate nucleus (●), and cerebellum (▲). Specific binding curves (dashed lines), calculated by subtracting cerebellar measurements from total uptake in putamen (□) and caudate nucleus (○), are also shown. (**B**) Time integrals of the [^{123}I]IBZM-specific binding curves shown in (A), for putamen (□) and caudate nucleus (○). The least squares linear-regression lines, used to calculate peak specific binding from the linear portion of the integral, are indicated by dashed lines (*12*).

Table 2. Correlations within monozygotic pairs of differences in clinical scores and [^{123}I]IBZM binding. The first row is aggregate score data; the subsequent three rows are post hoc analyses of component scales.

Scale	Caudate nucleus		Putamen	
	Pearson's r	P	Pearson's r	P
Aggregate score	0.99	<0.001	0.22	0.72
Shapiro symptom checklist	0.95	<0.01	0.38	0.52
Yale global tic severity	0.93	<0.02	0.02	0.97
Yale tic count	0.88	<0.05	0.40	0.50

tics also relate to caudate nucleus function adds to the evidence that TS and obsessive-compulsive disorder are overlapping neurobehavioral conditions.

The caudate nucleus is a key node in certain behavior-linked neural circuits, which are distinct from putamenal motor circuits (*18*). Our study may aid in understanding the curious motor behaviors of TS because it links these behaviors to associative, nonmotor striatal circuits and distinguishes them, as long suspected, from traditional hyperkinetic movement disorders that are linked to motor striatal circuits. Although there is insufficient basic neuroscience data to explain precisely how variation in D2 receptor availability in the head of the caudate nucleus accounts for the variable expression of TS, we speculate that involvement of the caudate nucleus may be related to the "compulsive" component of tics, a unique feature that helps to distinguish tics from other hyperkinetic movements. Dopaminergic dysfunction in the caudate nucleus may well be the common link between the ideational and motor components of TS.

REFERENCES AND NOTES

1. G. Gilles de la Tourette, *Arch. Neurol.* **9**, 19 (1885).
2. M. M. Robertson, *Br. J. Psychiatry* **154**, 147 (1989); R. Kurlan, *Arch. Neurol.* **51**, 1145 (1994); T. M. Hyde and D. R. Weinberger, *J. Am. Med. Assoc.* **273**, 498 (1995).
3. D. L. Pauls and J. F. Leckman, *N. Engl. J. Med.* **315**, 993 (1986).
4. R. A. Price, K. K. Kidd, D. J. Cohen, D. L. Pauls, J. F. Leckman, *Arch. Gen. Psychiatry* **42**, 815 (1985); T. M. Hyde, B. A. Aaronson, C. Randolph, K. C. Rickler, D. R. Weinberger, *Neurology* **42**, 652 (1992); J. F. Leckman and B. S. Peterson, *Biol. Psychiatry* **34**, 425 (1993).
5. H. S. Singer, I.-H. Hahn, T. H. Moran, *Ann. Neurol.* **30**, 558 (1991); R. T. Malison *et al.*, *Am. J. Psychiatry* **152**, 1359 (1995).
6. H. S. Singer *et al.*, in *Advances in Neurology*, T. Chase, A. Friedhoff, D. Cohen, Eds., (Raven, New York, 1992), vol. 58, pp. 233–238; N. Turjanski *et al.*, *J. Neurol. Neurosurg. Psychiatry* **57**, 688 (1994); M. S. George *et al.*, *Psychiatry Res.* **55**, 193 (1994).
7. E. J. Devor *et al.*, *Hum. Hered.* **40**, 105 (1990); J. Galernter *et al.*, *Arch. Gen. Psychiatry* **47**, 1073 (1990); P. M. Brett, M. M. Robertson, H. M. D. Gurling, *Lancet* **341**, 1225 (1993); J. Galernter *et al.*, *Am. J. Psychiatry* **150**, 449 (1993); P. M. Brett, D. Curtis, M. M. Robertson, H. M. D. Gurling, *Biol. Psychiatry* **37**, 533 (1995); J. Galernter *et al.*, *Genomics* **30**, 495 (1995).
8. B. Peterson *et al.*, *Neurology* **43**, 941 (1993); H. S. Singer *et al.*, *ibid.*, p. 950; T. M. Hyde *et al.*, *ibid.* **45**, 1176 (1995); J. Moriarty *et al.*, *Br. J. Psychiatry* **167**, 249 (1995).
9. Twin pairs were solicited through the national newsletter of the Tourette Syndrome Association. Of nearly 100 responding twin pairs, 5 pairs fulfilled all selection criteria (adult, monozygotic, neuroleptic-free, and discordant for symptom severity). Complete matching of 19 red cell antigens (National Reference Laboratory for Blood Serology, American Red Cross, Rockville, MD) confirmed monozygosity with a probability of >97% [F. Vogel and A. G. Motulsky, *Human Genetics* (Springer-Verlag, New York, 1986) pp. 578–585]. Five participants were neuroleptic-naïve, three were neuroleptic-free for 3 years or more, and two discontinued low dosages of pimozide (1 mg/day) and haloperidol (1 mg/day), respectively, 6 weeks before the study. Diagnosis of TS followed published guidelines [S. Fahn *et al.*, *Arch. Neurol.* **50**, 1013 (1993)]. Patients were rated for symptom severity with an aggregate clinical score calculated as the sum of the following complementary scales: Shapiro Symptom Check List, Yale Global Tic Severity Rating Scale, and Yale Tic Count (performed immediately before SPECT scanning). Written informed consent was obtained from all participants under a protocol approved by the Institutional Review Board of the National Institute of Mental Health.
10. H. F. Kung *et al.*, *J. Nucl. Med.* **30**, 88 (1989). Recent evidence indicates that IBZM also binds to D3 dopamine receptors (M. P. Kung, unpublished data). Although D3 receptor density is relatively low compared with that of D2 receptors in the regions we studied [A. M. Murray, H. L. Ryoo, E. Gurevich, J. N. Joyce, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 11271 (1994)], our measurements of IBZM binding may include some small fraction due to D3 receptor binding.
11. [^{123}I]IBZM was prepared [M.-P. Kung and H. F. Kung, *J. Labelled Comps. Radiopharm.* **27**, 691 (1989)] and administered intravenously (mean dose: 5.27 ± 0.77 mCi). Multiple SPECT scans [CERASPECT; Digital Scintigraphics, Waltham, MA; full width at half-maximum (FWHM), 11.5 mm; 64 1.67-mm slices; 15 min per scan] began 15 min after injection and continued over 4 hours. Individual scans were coregistered in three orthogonal planes to templates created on the 4-hour time-averaged image. To define anatomical ROIs, we also coregistered volume magnetic resonance imaging (MRI) scans (GE 1.5T Signa; spoiled gradient recalled acquisition in steady state; repetition time, 24 ms; time to echo, 5 ms) with the time-averaged image. ROIs sampling the head of the caudate nucleus, the body of putamen, and the cerebellum were drawn on five contiguous transverse MRI slices and transferred to corresponding SPECT images. Activity concentration (cpm/ml) was measured for the volume encompassed by each set of ROIs, corrected for decay, and normalized to injected dose.
12. M. B. Knable *et al.*, *J. Nucl. Med.* **36**, 1216 (1995).
13. Individual caudate nucleus binding values by twin pair (more severe, less severe) were as follows: 1.51, 1.50; 1.67, 1.31; 1.12, 0.93; 1.19, 1.15; and 1.97, 1.34. Putamen values were as follows: 2.11, 2.77; 2.12, 2.32; 2.03, 1.60; 1.60, 1.43; and 2.27, 2.14.
14. Participants underwent a single 30-min SPECT scan (7.5 mm FWHM) beginning 15 min after intravenous administration of [^{99m}Tc]HMPAO (mean dose: 14.9 ± 0.2 mCi). Attenuation-corrected scans were coregistered and analyzed as described (*11*) except that these data were normalized to the whole slice. Striatal blood flow measurements revealed no significant differences between more and less affected twins for either caudate nucleus (mean ± SEM: 90 ± 3 compared with 88 ± 4) or putamen (127 ± 2 compared with 128 ± 2) (Wilcoxon test, $P = 0.69$ for both).
15. The time of peak specific [^{123}I]IBZM binding was estimated from the midpoint of the linear portion in the integral method (*12*) and ranged from 75 to 125 min after injection. Within-pair concordance on this measure was high [unbiased intraclass correlation coefficient, ICC(U), = 0.94 for caudate nucleus, $P < 0.0005$; ICC(U) = 0.82 for putamen, $P < 0.005$] [J. J. Bartko and W. T. Carpenter Jr., *J. Nerv. Ment. Dis.* **163**, 307 (1976)].
16. A statistical power analysis revealed that, given our means and standard deviations for caudate nucleus IBZM binding data, a sample size of 33 persons in each group would be required for 90% power to observe a significant difference in a nontwin design. A similar analysis for the putamen data revealed that more than 9000 persons in each group would be required for this region.
17. T. R. Insel, *Arch. Gen. Psychiatry* **49**, 739 (1992).
18. G. E. Alexander, M. R. DeLong, P. L. Strick, *Annu. Rev. Neurosci.* **9**, 357 (1986).

19. We gratefully acknowledge the assistance of E. Watsky, H. Leonard, and K. Rickler. This work was partially funded through an award from the Tourette Syndrome Association (T.M.H.).

7 March 1996; accepted 28 June 1996

KUZ, a Conserved Metalloprotease-Disintegrin Protein with Two Roles in *Drosophila* Neurogenesis

Jenny Rooke, Duojia Pan, Tian Xu,* Gerald M. Rubin

During neurogenesis in *Drosophila* both neurons and nonneuronal cells are produced from a population of initially equivalent cells. The *kuzbanian* (*kuz*) gene described here is essential for the partitioning of neural and nonneuronal cells during development of both the central and peripheral nervous systems in *Drosophila*. Mosaic analyses indicated that *kuz* is required for cells to receive signals inhibiting the neural fate. These analyses further revealed that the development of a neuron requires a *kuz*-mediated positive signal from neighboring cells. The *kuz* gene encodes a metalloprotease-disintegrin protein with a highly conserved bovine homolog, raising the possibility that *kuz* homologs may act in similar processes during mammalian neurogenesis.

Neurogenesis in the fruit fly *Drosophila melanogaster* requires that cells from initially equivalent populations be selected to adopt different fates (*1*). In both the central and peripheral nervous systems, the selection of neural cells occurs in a stepwise process controlled by two groups of genes (*2*). First, genes of the proneural class confer equivalent neural potential on groups of cells. Subsequently, members of the neurogenic gene class ensure that only a single cell in each group is allowed to achieve its neural potential, whereas the others become epi-

J. Rooke and T. Xu, Department of Genetics, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, CT 06536, USA.
D. Pan and G. M. Rubin, Howard Hughes Medical Institute and Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA.

*To whom correspondence should be addressed.