

PARK6-Linked Parkinsonism Occurs in Several European Families

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The *Parkin* gene on 6q25.2–27 is responsible for about 50% of autosomal recessive juvenile parkinsonism and less than 20% of sporadic early-onset cases. We recently mapped a novel locus for early-onset parkinsonism (PARK6) on chromosome 1p35–p36 in a large family from Sicily. We now confirm linkage to PARK6 in eight additional families with *Parkin*-negative autosomal recessive juvenile parkinsonism from four different European countries. The maximum cumulative pairwise LOD score was 5.39 for marker D1S478. Multipoint linkage analysis gave the highest cumulative LOD score of 6.29 for marker D1S478. Haplotype construction and determination of the smallest region of homozygosity in one consanguineous family has reduced the candidate interval to a 9cM region between markers D1S483 and D1S2674. No common haplotype could be detected, excluding a common founder effect. These families share some clinical features with the phenotype reported for European *Parkin*-positive cases, with a wide range of ages at onset (up to 68 yrs) and slow progression. However, features typical of autosomal recessive juvenile parkinsonism, including dystonia at onset and sleep benefit, were not observed in PARK6-linked families, thus making the clinical presentation of late-onset cases indistinguishable from idiopathic Parkinson's disease. PARK6 appears to be an important locus for early-onset parkinsonism in European *Parkin*-negative patients.

Ann Neurol 2002;51:14–18

Parkinson's disease (PD) is a common neurodegenerative disorder presenting with rigidity, bradykinesia, tremor, and postural instability. Neuropathological studies have demonstrated massive degeneration of dopaminergic neurons in the pars compacta of the substantia nigra (SNpc) and in other areas of the brain, and the occurrence of Lewy bodies (cytoplasmic eosinophilic inclusions).¹ Most PD cases are sporadic and the primary cause of the disease is still unknown. However, it is now clear that genetic factors play an important role in the etiology of the disease. So far, four different loci have been identified in families with autosomal dominant PD.² Autosomal recessive juvenile parkinsonism (ARJP) shares many clinical features with PD but is characterized by early onset of parkinsonian

symptoms (usually below 40 years), slow disease progression, good response to L-DOPA and early L-DOPA-induced dyskinesias. Hyperreflexia, dystonia at onset, and sleep benefit may be present.³ Pathological studies have shown a more selective degeneration of dopaminergic neurons in the SNpc and the absence of Lewy bodies.⁴ A gene for ARJP (*Parkin*) has been identified on human chromosome 6q25.2–27.⁵ The *Parkin* gene codes for a protein with ubiquitin-ligase activity, absent in the brain of patients with 6q-linked ARJP.^{6,7} A wide range of independently occurring mutations has been detected in sporadic and autosomal recessive cases with early-onset parkinsonism.⁸ The phenotype-genotype correlation showed that some patients with *Parkin* mutations had a later age of onset

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Received Jun 6, 2001, and in revised form Sep 5. Accepted for publication Sep 6, 2001.

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(up to 65 years) and were clinically indistinguishable from patients with idiopathic PD.^{9,10} In a recent study on the prevalence of Parkin gene in Europe, mutations were detected in about 50% of ARJP families and only 18% of sporadic cases with early-onset parkinsonism.⁹ We have recently performed a genome-wide search in a large family from Sicily and mapped a novel locus for early-onset parkinsonism (PARK6) to a 12.5cm interval on human chromosome 1p35–p36, between flanking markers D1S483 and D1S247.¹¹ The clinical presentation was very similar to idiopathic PD, the only distinctive features being an earlier age of onset (range 32–48 years) and a slower progression of the disease. In order to assess the role of PARK6 in determining Parkin-negative ARJP in Europe, we performed linkage analysis in 28 European ARJP families in which Parkin mutations had been excluded. Further aims of this study were to evaluate the possibility of founder effects and to draw genotype-phenotype correlates.

Subjects and Methods

Patients

Twenty-eight parkinsonian families were included in the study. Twenty-five had been collected by the European Consortium on Genetic Susceptibility in Parkinson's Disease; 3 other families were selected from the Movement Disorder Database of the Catholic University in Rome. All families were negative for mutations in the Parkin gene. All patients fulfilled the UK Brain Bank criteria,¹² and in addition, the following criteria for early-onset familial PD were met: (1) inheritance compatible with autosomal recessive transmission; (2) age of onset of 45 years or younger in at least one affected member in the family. Out of 28 families, 8 were British, 6 were Dutch, 6 were Italian, 5 were German, and 3 were French. There were at least 2 affected siblings in each nuclear family. After obtaining informed consent, blood was obtained from 62 affected individuals and 48 clinically unaffected first-degree relatives.

DNA and Linkage Analysis

DNA was extracted from peripheral leukocytes using standard protocols. All affected individuals from the 28 families were genotyped for 11 microsatellite markers spanning the PARK6 region on chromosome 1p35–p36. The 11 loci are ordered as follows: *tel*-D1S483-*0cM*-D1S199-*3.2cM*-D1S2732-*0cM*-D1S2828-*0cM*-D1S478-*0.6cM*-D1S2702-*3.6cM*-D1S2734-*0cM*-D1S2698-*1.6cM*-D1S2674-*0.8cM*-D1S2885-*2.7cM*-D1S247-*cen*. Microsatellite markers were amplified from genomic DNA using the PCR technique as specified by the manufacturers, and electrophoresed on a capillary using a 3100 DNA Sequencer (Applied Biosystems, Foster City, CA). DNA fragment size analysis was performed semiautomatically using the Genescan and Genotyper software (Applied Biosystems, Foster City, CA) to determine genotypes. Haplotypes were manually constructed. In families where the affected individuals shared identical haplotypes within the PARK6 region, all available unaffected family members were genotyped, and haplotypes

were phased based on the minimum number of recombinants. Pairwise LOD scores were obtained using the FASTLINK version of the MLINK program^{13,14} under the assumption of equal male-female recombination rate, autosomal recessive inheritance, reduced penetrance (0.90), a gene frequency of 0.001 and equal allele frequencies for each marker. Cumulative multipoint LOD scores were generated by use of the SIMWALK2 program.¹⁵ Pairwise and multipoint LOD scores were calculated in all 28 families using affected individuals only. In the 8 families showing evidence of linkage with PARK6, LOD scores were recalculated adding the available unaffected family members. Marker order and genetic distances were based on the chromosome 1 genetic map of the Center for Medical Genetics, Marshfield Medical Research Foundation.

Results

Linkage Analysis

Linkage to PARK6 was first tested in the 62 affected members of the 28 families. Eight families generated positive LOD scores at all recombination fractions (θ), and haplotype construction showed identical haplotypes shared by the affected members of each family. The other families generated negative LOD scores at all recombination fractions and affected individuals had different haplotypes across the entire 12.5cM region; these were therefore excluded from further analysis.

In the 8 families showing evidence of linkage with PARK6, the 11 markers were genotyped in all available unaffected family members. Figure 1 shows simplified pedigrees of these families and haplotypes for the 11 markers spanning the candidate interval on chromosome 1p35–p36. Linkage analysis including unaffected family members gave a cumulative maximum pairwise LOD score of 5.39 at $\theta = 0.00$ for marker D1S478 (Table). Adding the Sicilian family to the linkage calculation, the cumulative maximum pairwise LOD score reached 9.40 at $\theta = 0.00$ for the same marker (data not shown). The maximum cumulative multipoint LOD score obtained on the 8 families using 6 markers (D1S483, D1S478, D1S2734, D1S2674, D1S2885, and D1S247) was 6.29 at locus D1S478 (Fig 2).

Haplotype Analysis

No recombination events were detected at the telomeric end of the region, which is still defined by the flanking marker D1S483 by a recombination between markers D1S483 and D1S199 identified in the Sicilian family.¹¹ Families DE-028 and IT-GR showed homozygosity across the entire region, except for markers D1S2885 and D1S247 (family DE-028) and for markers D1S2674, D1S2885, and D1S247 (family IT-GR). This is of great interest especially in the latter family (IT-GR), where the parents of the 2 affected siblings were first-degree cousins, indicating homozygosity by descent in the affected individuals. The lack of homozygosity at D1S2674 and at more centromeric

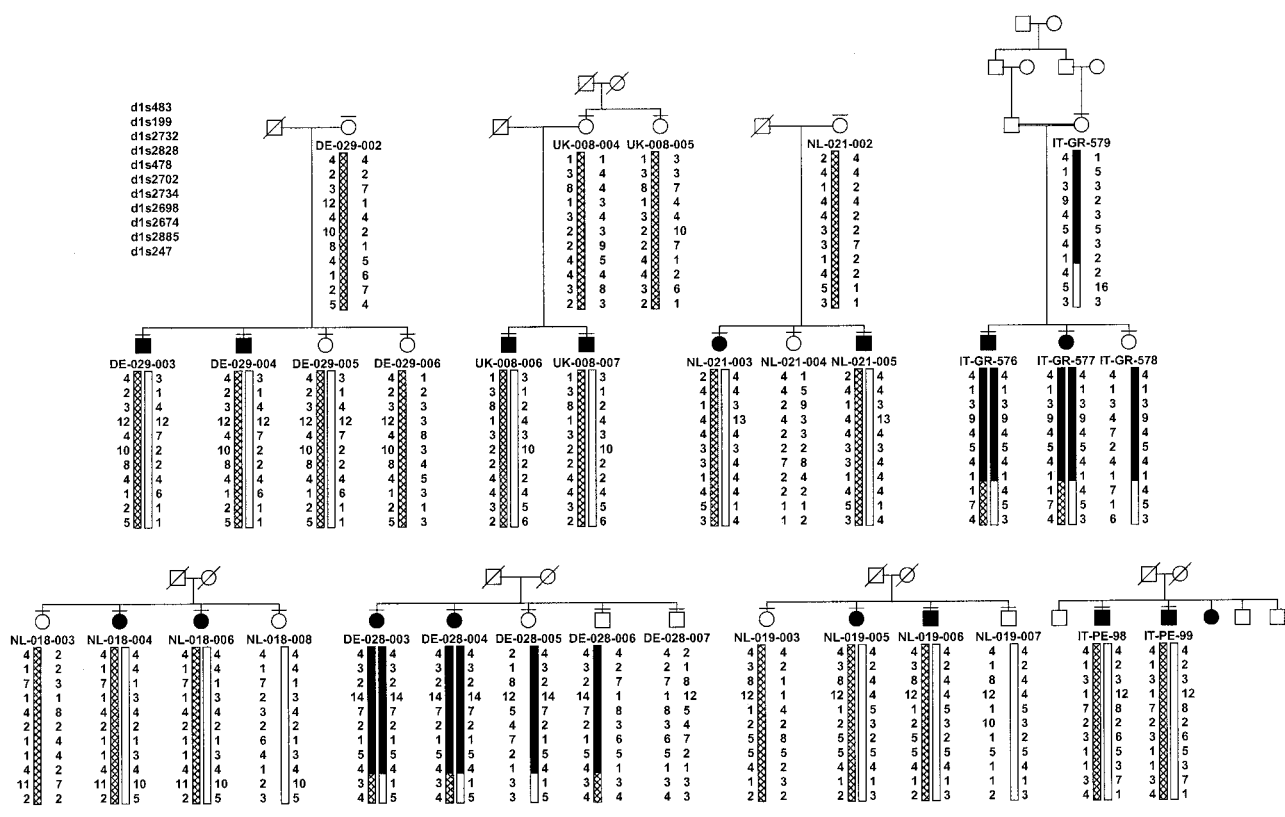


Fig 1. Dutch (NL-018, NL-019, and NL-021), German (DE-028 and DE-029), British (UK-008), and Italian (IT-GR and IT-PE) families with haplotypes of the PARK6 region on 1p35–p36. Genotyped individuals are marked with a thin horizontal line above symbols. A black symbol denoted affected family members, while a diagonal line across the symbol represents deceased members. Haplotypes shared by the affected members of each family are indicated by bars; homozygous haplotypes are marked by a black bar.

markers in this family suggests a recombination event between this marker and D1S2698. This is confirmed by the negative LOD scores obtained for family IT-GR only at loci D1S2674, D1S2885, and D1S247 (at $\theta = 0.00$: -1.10 , -1.11 , and -1.40 , respectively) and allows the candidate interval to be reduced to a 9cm region between flanking markers D1S483 and D1S2674.

Each family shows a different haplotype, excluding a common founder effect and suggesting independent mutational events (see Fig 1).

Genotype-Phenotype Correlation

Eight of 28 families tested showed evidence of linkage with PARK6. Of these families, 3 were Dutch, 2 were

Table. Cumulative Pairwise LOD Scores Between the Disease and Markers on Chromosome 1p35–p36 in the 8 Linked Families

	LOD Scores at $\theta =$						
Markers	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D1S483	2.78	2.72	2.43	2.07	1.33	0.67	0.20
D1S199	4.43	4.31	3.83	3.29	2.13	1.09	0.35
D1S2732	3.85	3.78	3.43	2.94	1.91	0.96	0.28
D1S2828	4.96	4.85	4.36	3.72	2.41	1.24	0.39
D1S478	5.39	5.25	4.68	3.96	2.54	1.30	0.40
D1S2702	4.45	4.35	3.90	3.32	2.14	1.09	0.33
D1S2734	5.17	5.05	4.54	3.86	2.46	1.23	0.36
D1S2698	3.55	3.46	3.10	2.62	1.67	0.84	0.25
D1S2674	2.89	3.40	3.48	3.08	2.00	0.97	0.25
D1S2885	1.69	2.22	2.41	2.17	1.41	0.68	0.18
D1S247	2.33	2.86	3.01	2.70	1.78	0.87	0.23

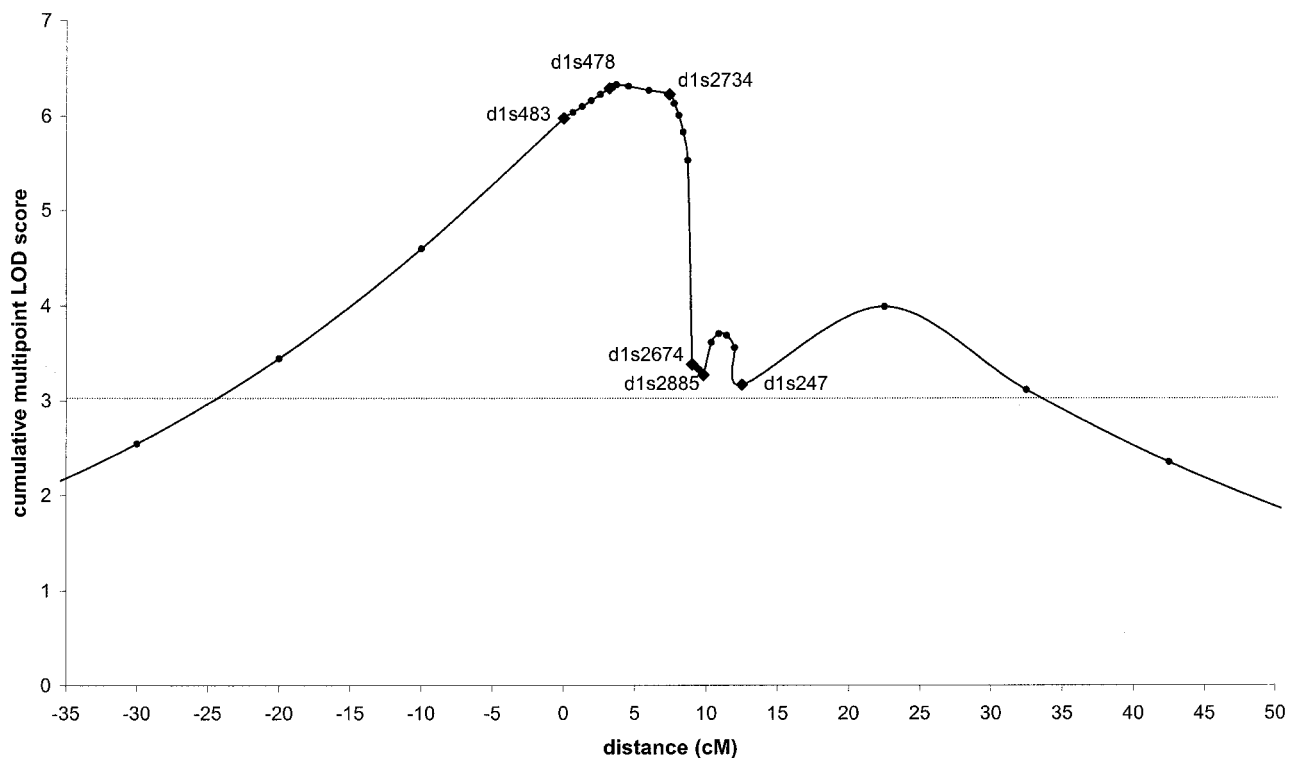


Fig 2. Multipoint analysis of six markers spanning the PARK6 region in the 8 linked families. The highest cumulative multipoint LOD score was obtained at marker locus D1S478 (6.29 at $\theta = 0.00$).

German, 2 were Italian, and 1 was from the UK. Consanguinity between parents of affected siblings was detected only in 1 family from central Italy (IT-GR). Affected individuals were observed in only one generation in all families. Mean age of onset was 42.1 ± 9.4 years in the 16 affected individuals, and at least 1 of the 2 affected siblings had an onset before age of 45 years. However, 4 affected individuals (from family UK-008 and from the 3 Dutch families) had an age of onset above 45 years, with the latest onset at 68 years in individual NL-018-004. Clinical features were indistinguishable from Parkin-positive ARJP, especially of non-Japanese origin, being characterized in all patients by a mild to moderate parkinsonian syndrome with good response to L-DOPA and slow progression (mean disease duration = 12 ± 6.9 years). L-DOPA-induced dyskinesias occurred in 8 out of 16 patients (50%). Dystonia at onset and sleep benefit, mainly reported in Japanese ARJP subjects, were not detected in any of the 16 patients. Hyperreflexia was observed only in 2 of the 16 patients.

Twenty of 28 families did not show evidence of linkage to the PARK6 locus. The mean age of onset in the 46 affected individuals was 46.4 ± 9.1 years. Eighteen of these individuals from 16 families had an age of onset above 45 years. This figure is slightly higher compared to that in PARK6-linked families. Clinical

presentation, progression of disease, response to L-DOPA treatment and occurrence of distinct features, including L-DOPA-induced dyskinesias and hyperreflexia, were similar to those in PARK6-linked families, preventing any attempt to subclassify the two groups of patients on clinical ground alone.

Discussion

The Parkin gene on chromosome 6q is responsible only for about 50% of cases of autosomal recessive parkinsonism with onset below 45 years, indicating genetic heterogeneity. We have recently mapped a novel locus for early-onset parkinsonism (PARK6) on the short arm of chromosome 1 in a large consanguineous family from Sicily. We now confirm linkage to PARK6 in 8 additional families from 4 different European countries. No common haplotype could be detected in the linked families, suggesting independent mutational events. These results indicate that PARK6 is not restricted to Italy, thus confirming the role of this locus in determining the parkinsonian phenotype in European Parkin-negative cases. It has to be highlighted that the PARK6-associated phenotype does not resemble the typical ARJP presentation, as it is often seen in Japanese patients. The clinical presentation is more similar to the phenotype described in Parkin-positive non-Japanese ARJP patients, with a broader clinical

spectrum and range of ages at onset. Four of 16 affected individuals had an age of onset above 45 years, with the highest onset at 68 years. In these late-onset cases, clinical features are indistinguishable from idiopathic PD. Thus, PARK6 is also responsible for a subset of cases diagnosed as idiopathic PD. However, no pathological data on PARK6-positive patients are available at present. It is still unclear whether PARK6 shares the same neuropathological features as Parkin (a more selective neuron loss than idiopathic PD and absence of Lewy bodies) or idiopathic PD (with presence of Lewy bodies), or whether it represents a distinct disorder within familial parkinsonisms.

Parental consanguinity was detected only in 1 (IT-GR) of 8 families. The 2 affected siblings in this family were homozygous across the entire linked region except for the three centromeric markers (see Fig 1). This finding, which strongly suggests a recombination between markers D1S2698 and D1S2674, allows the refinement of the candidate interval to 9.0cm between markers D1S483 and D1S2674. Homozygosity was also detected in another family from Germany (DE-028) without any obvious consanguinity. However, parents of the 2 affected siblings were originating from a restricted geographic area and the existence of a common ancestor whose memory has been lost in the family cannot be ruled out.

The identification of an unaffected individual (DE-029-005) sharing the same haplotypes with the 2 affected siblings can be explained assuming a reduced penetrance of the underlying gene. However, this individual is 54 years old and might not yet have developed the disease.

A large number of genes map within the 9cm candidate interval identified in our family, but none of them represents a striking candidate for parkinsonism. An interesting gene mapping to the region is a gene coding for the serotonin receptor subtype 1D (HTR1D). In fact, it has been shown that serotonin receptors play an important role in serotonin-mediated striatal dopamine release and that serotonergic-mediated mechanisms are damaged in the parkinsonian brain.^{16,17} However, the linked region is still too large to allow searching for candidate genes. Additional work is needed to test a larger group of ARJP Parkinson-negative families for linkage to PARK6 to further refine the linked interval and ease the identification of candidate genes.

N.W. Wood is supported by The Parkinson's Disease Society (UK) and the Brain Research Trust; B. Dallapiccola and A. Filla are supported by the Italian Ministry of Health. This project was supported by Telethon grants to A.R. Bentivoglio (C38) and to A. Albanese

(E1165), by Italian MURST grant (Cofin. 2000) to G. De Michele, by the Deutsche Forschungsgemeinschaft (grant Ga 402/9-1 to T. Gasser) and by MRC program grant G9706148 to N.W. Wood.

We are grateful to Drs. Antonio Pizzuti, Jenny Vaughan, and Eric Sobel for their useful contributions to this work.

References

1. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain* 1991;114:2283–2301.
2. Kitada T, Asakawa S, Matsumine H, et al. Progress in the clinical and molecular genetics of familial parkinsonism. *Neurogenetics* 2000;2:207–218.
3. Ishikawa A, Tsuji S. Clinical analysis of 17 patients in 12 Japanese families with autosomal recessive type juvenile parkinsonism. *Neurology* 1996;47:160–166.
4. Takahashi H, Ohama E, Suzuki S, et al. Familial juvenile parkinsonism: clinical and pathological study in a family. *Neurology* 1994;44:437–441.
5. Kitada T, Asakawa S, Hattori N, et al. Mutations in the *parkin* gene cause autosomal recessive juvenile parkinsonism. *Nature* 1998;392:605–608.
6. Shimura H, Hattori N, Kubo S, et al. Immunohistochemical and subcellular localization of Parkin protein: absence of protein in autosomal recessive juvenile parkinsonism patients. *Ann Neurol* 1999;45:668–672.
7. Shimura H, Hattori N, Kubo S, et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat Genet* 2000;25:302–305.
8. Abbas N, Lücking CB, Ricard S, et al. A wide variety of mutations in the *parkin* gene are responsible for autosomal recessive parkinsonism in Europe. *Hum Mol Genet* 1999;8:567–574.
9. Lücking CB, Dürr A, Bonifati V, et al. Association between early-onset Parkinson's disease and mutations in the *parkin* gene. *N Engl J Med* 2000;342:1560–1567.
10. Klein C, Pramstaller PP, Kis B, et al. *Parkin* deletions in a family with adult-onset, tremor-dominant parkinsonism: expanding the phenotype. *Ann Neurol* 2000;48:65–71.
11. Valente EM, Bentivoglio AR, Ialongo T, et al. Localization of a novel locus for autosomal recessive early onset parkinsonism (PARK6) on human chromosome 1p35–p36. *Am J Hum Genet* 2001;68:895–900.
12. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* 1992;55:181–184.
13. Cottingham Jr RW, Idury RM, Schaffer AA. Faster sequential genetic linkage computations. *Am J Hum Genet* 1993;53:252–263.
14. Dwarkadas S, Schaffer AA, Cottingham Jr RW, et al. Parallelization of general linkage analysis problems. *Hum Hered* 1994;44:127–141.
15. Sobel E, Lange K. Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. *Am J Hum Genet* 1996;58:1323–1337.
16. Volpi R, Caffarra P, Scaglioni A, et al. Defective 5-HT1 receptor-mediated neurotransmission in the control of growth hormone secretion in Parkinson's disease. *Neuropsychobiology* 1997;35:79–83.
17. Sershen H, Hashim A, Lajtha A. Serotonin-mediated striatal dopamine release involves the dopamine uptake site and the serotonin receptor. *Brain Res Bull* 2000;53:353–357.