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Report

Significant Linkage of Parkinson Disease to Chromosome 2q36-37

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Parkinson disease (PD) is the second most common neurodegenerative disorder, surpassed in frequency only by Alzheimer disease. Elsewhere we have reported linkage to chromosome 2q in a sample of sibling pairs with PD. We have now expanded our sample to include 150 families meeting our strictest diagnostic definition of verified PD. To further delineate the chromosome 2q linkage, we have performed analyses using only those pedigrees with the strongest family history of PD. Linkage analyses in this subset of 65 pedigrees generated a LOD score of 5.1, which was obtained using an autosomal dominant model of disease transmission. This result strongly suggests that variation in a gene on chromosome 2q36-37 contributes to PD susceptibility.

Parkinson disease (PD [MIM 168600]) is a common neurodegenerative disorder, affecting 3% of those >75 years of age (de Rijk et al. 1997). It is associated with resting tremor, postural rigidity, and progressive accumulation of protein inclusions containing ubiquitin and α-synuclein in the substantia nigra. The first causative PD mutation was identified in the α-synuclein gene on chromosome 4; however, only two mutations in a very small number of families have thus far been reported (Gwinn-Hardy 2002). Subsequently, mutations in the *parkin* gene on chromosome 6 were reported to result in autosomal recessive, juvenile parkinsonism (ARJP [MIM 600116]). Mutations in this gene may account for PD in as many as 50% of familial cases of ARJP (Lücking et al. 2000) and may also result in individuals with idiopathic late-onset PD, particularly those with a positive family history (Foroud et al. 2003). Recently, mutations in the DJ-1 gene on chromosome 1q were found to result in autosomal recessive, early-onset PD (Bonifati et al. 2002). In addition, linkage to four other chromosomal regions has been reported in families segregating either an autosomal dominant or an autosomal recessive form of PD; however, the causative genes have not yet been identified (Gwinn-Hardy 2002). We have recently identified linkage to chromosome 2q36–37 in a sample of 170 affected sibling pairs (Pankratz et al. 2002).

To identify susceptibility genes for PD, affected sibling pairs and more extended multiplex families with PD were recruited through the movement-disorder specialists of the Parkinson Study Group (PSG), a network of 60 centers located throughout North America. During an in-person study visit, the Diagnostic Checklist (Nichols et al. 2002; Pankratz et al. 2002) was completed by the neurologist; inclusion criteria consisted of clinical features highly associated with autopsy-confirmed PD, and exclusion criteria consisted of features highly associated with other non-PD pathological diagnoses (Hughes et al. 1992*a*, 1992*b*). Responses on the Diagnostic Checklist were used to classify study participants as having verified PD (417 subjects) or nonverified PD (163 subjects). Peripheral blood was obtained from all individuals after they completed the appropriate written informed consent form approved by each center's institutional review board.

A marker in intron 7 of the *parkin* gene (D6S305) was genotyped to identify families more likely to have a mutation in this known PD-susceptibility gene. Families having a positive LOD score at this marker (as determined using an autosomal recessive model of disease

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inheritance [$n = 94$]), along with families that included at least one affected individual with an age at onset $\leqslant 50$ years ($n = 66$; overlap $n = 30$), were screened for *parkin* mutations, using both direct sequencing and fluorescent-dosage PCR analysis (Nichols et al. 2002). Linkage analyses on chromosome 2 were performed with and without the 47 families determined to have *parkin* mutations.

Thirty dinucleotide repeat markers from the ABI Prism Linkage Mapping Set (Applied Biosystems), with an average heterozygosity of 80% and an average intermarker spacing of 8.7 cM, were genotyped on chromosome 2. Marker order and the genetic distances between markers were estimated from the sex-averaged genetic maps from Marshfield Genetic Laboratory, ABI (ABI Linkage Mapping Set 2), and deCode (Kong et al. 2002). The maximum LOD score was within 0.01 for all analyses using the different maps. Therefore, only results obtained using the maps generated from Marshfield are reported. PCR amplification was performed for each of the markers, and the products were separated by electrophoresis, using an ABI 3700 DNA Analyzer (Applied Biosystems). Genotypes were determined by Genescan 3.5, Genotyper 3.6, and Genemapper 2.0 software. Mendelian errors in the genotypic data were detected using the program PedCheck (O'Connell and Weeks 1998), and the marker genotypic data were used to verify all reported family relationships among the subjects, using the computer program Relative (Goring and Ott 1997). Allele frequencies were estimated from the full PD cohort of 1,352 chromosomes, using the Userm13 module from the Mendel software package (Lange et al. 1988). Nonparametric multipoint LOD scores were calculated using Mapmaker/Sibs (Kruglyak and Lander 1995).

We previously reported evidence of linkage to chromosome 2q (LOD = 3.1) in a sample of 104 families in which the affected individuals met the strictest diagnosis of PD (i.e., verified PD) (table 1; Pankratz et al. 2002). This sample included 90 families without a *parkin* mutation and 14 families with mutations in one or both *parkin* alleles. We have subsequently ascertained an additional 96 individuals from 46 families that meet our strictest diagnostic criteria. These families provide additional evidence of linkage to chromosome 2q36-37. Combined analyses of the 150 families yielded suggestive evidence of linkage in the region of three adjacent markers (D2S396, D2S206, and D2S338) and a resulting maximum LOD score of 3.5 (table 1). A total of 25 families with *parkin* mutations were identified in the initial and subsequently ascertained samples, including 14 families with a mutation in only one of their two *parkin* alleles, three families with homozygous *parkin* mutations, and eight families whose members were compound heterozygotes. When the 25 families with mutations in the *parkin* gene were removed from the sample, the LOD score was reduced to 2.5. The increased LOD score that resulted from the inclusion of the families that were positive for the *parkin* mutation may suggest a potential epistatic or additive interaction between the *parkin* gene and a chromosome 2q susceptibility locus. Most reported *parkin* mutations have been found only in patients with PD, but at least one of the mutations observed in our cohort (Ser167Asn) has been observed in a few control individuals who did not have PD (Oliveri et al. 2001). Therefore, it is possible that a mutation in only one of the two *parkin* alleles is not sufficient to cause PD. Rather, a mutation in a second PD susceptibility locus may be required for manifestation of disease.

Subsequent analyses were designed to further elucidate the role of the chromosome 2q susceptibility locus. A subset of families was identified that met the strictest diagnostic criteria of verified PD and also had a stronger family history of PD. We defined this subset as those families having at least four first-, second-, or third-degree relatives reported to have PD but not necessarily examined ($n = 40$ families) and those families that included an affected sibling pair who also had a parent reportedly diagnosed with PD ($n = 49$). The overlap between these two groups was substantial ($n = 24$). A total of 65 families with verified PD were thus identified who were considered to have a more extensive family history. On the basis of the results of the previous analyses suggesting a potential interaction between *parkin* and the locus on 2q, kindreds meeting these family his-

Table 1

Demographic Information and LOD Scores for Families with Verified PD

Sample	No. of Families	No. of Sibling Pairs	% Male	Mean ± SD Age at Onset (years)	LOD Score: Including Families with *parkin* Mutations	LOD Score: Excluding Families with *parkin* Mutations
Original	104	116	61	60.4 ± 11.8	3.05[a]	1.87[a]
Expanded	150	170	60	60.0 ± 12.1	3.51[a]	2.47[a]
Strong family history	65	77	57	58.0 ± 12.2	5.14[b]	4.12[b]

[a] Nonparametric LOD scores computed with Mapmaker/Sibs.

[b] Parametric LOD scores computed using an autosomal dominant model with 80% penetrance, 0.005 disease allele frequency, and a 3% phenocopy rate.

tory criteria were included in the analysis regardless of the presence or absence of a *parkin* mutation(s).

It has been shown that employing a single-gene model for a disease with potentially complex inheritance can result in a potential gain in power, even if the disease model is incorrectly specified (Abreu et al. 1999; Hodge et al. 2002). Therefore, after review of our families with a strong family history of PD, an autosomal dominant model of PD susceptibility was postulated with 80% penetrance, a disease allele frequency of 0.005, and a 3% phenocopy rate. Two-point LOD scores were calculated using the MLINK program of the LINKAGE software package (Lathrop and Lalouel 1984). This model yielded a maximum two-point LOD score of 3.0 for $\theta = 0$ at marker D2S206. As with the nonparametric analyses, the flanking markers D2S396 (LOD = 2.9) and D2S338 (LOD = 2.4) also provided evidence of linkage under this model. The program Allegro (Gudbjartsson et al. 2000) was then used to perform multipoint linkage analysis, using this same parametric disease model. A maximum multipoint LOD score of 5.1 was computed at the marker D2S206 (fig. 1). Variation in marker allele frequencies resulted in only minor changes in the maximum LOD score. The 11 families positive for *parkin* mutation contributed a summed LOD score of 1.0. Given this relatively small sample of *parkin*-positive families with verified PD, it will be important to further study *parkin*-positive families to test whether there is evidence for an epistatic or additive interaction between the *parkin* locus and a PD susceptibility gene on chromosome 2q.

Elsewhere, linkage to an autosomal dominant PD locus on chromosome 2p (PARK3 [MIM 602404]) was identified in a sample of families of European origin (Gasser et al. 1998). More recently, analyses of affected sibling pairs provided evidence that the PARK3 locus might affect age at onset in late-onset PD (DeStefano et al. 2002). However, our linkage to 2q36-37 is ~140 cM away and therefore represents linkage to a gene that is distinct from the PARK3 locus.

In summary, our data provide strong evidence that a susceptibility gene for PD is located within a 20-cM region near the q terminus of chromosome 2. Future studies will focus on identifying genes in this chromosomal region, which might be tested as candidates for their role in PD susceptibility.

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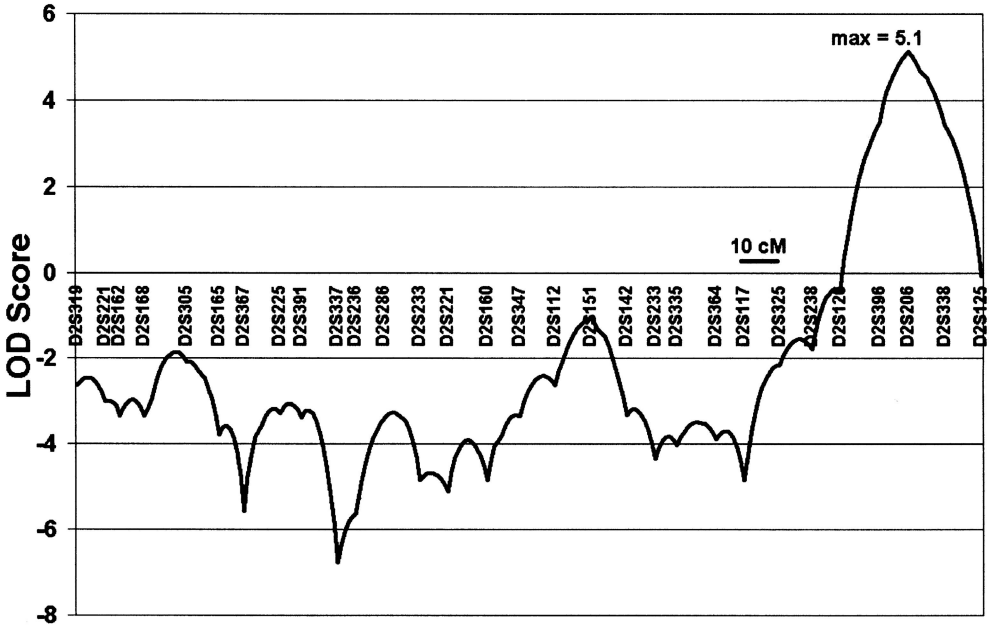

Figure 1 Autosomal dominant multipoint LOD score plot for the 65 families with a strong family history of PD

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Electronic-Database Information

URLs for data presented herein are as follows:

Applied Biosystems, http://home.appliedbiosystems.com
Center for Medical Genetics, Marshfield Medical Research Foundation http://research.marshfieldclinic.org/genetics/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for ARJP, PD, and PARK3)

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