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Genome Screen to Identify Susceptibility Genes for Parkinson Disease in a Sample without *parkin* Mutations

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Parkinson disease (PD) is a common neurodegenerative disorder characterized by bradykinesia, resting tremor, muscular rigidity, and postural instability, as well as by a clinically significant response to treatment with levodopa. Mutations in the *α-synuclein* gene have been found to result in autosomal dominant PD, and mutations in the *parkin* gene produce autosomal recessive juvenile-onset PD. We have studied 203 sibling pairs with PD who were evaluated by a rigorous neurological assessment based on (*a*) inclusion criteria consisting of clinical features highly associated with autopsy-confirmed PD and (*b*) exclusion criteria highly associated with other, non-PD pathological diagnoses. Families with positive LOD scores for a marker in an intron of the *parkin* gene were prioritized for *parkin*-gene testing, and mutations in the *parkin* gene were identified in 22 families. To reduce genetic heterogeneity, these families were not included in subsequent genome-screen analysis. Thus, a total of 160 multiplex families without evidence of a *parkin* mutation were used in multipoint nonparametric linkage analysis to identify PD-susceptibility genes. Two models of PD affection status were considered: model I included only those individuals with a more stringent diagnosis of verified PD (96 sibling pairs from 90 families), whereas model II included all examined individuals as affected, regardless of their final diagnostic classification (170 sibling pairs from 160 families). Under model I, the highest LOD scores were observed on chromosome X (LOD score 2.1) and on chromosome 2 (LOD score 1.9). Analyses performed with all available sibling pairs (model II) found even greater evidence of linkage to chromosome X (LOD score 2.7) and to chromosome 2 (LOD score 2.5). Evidence of linkage was also found to chromosomes 4, 5, and 13 (LOD scores >1.5). Our findings are consistent with those of other linkage studies that have reported linkage to chromosomes 5 and X.

Introduction

Parkinson disease (PD [MIM 168600]) is a common neurodegenerative disorder affecting >1% of 55-year-old individuals and >3% of those >75 years of age (de Rijk et al. 1997). It is characterized by bradykinesia, resting tremor, muscular rigidity, and postural instability, as well as by a clinically significant response to treatment with levodopa (Gasser 2001). The pathology of PD involves the degeneration of brain dopaminergic pathways, mostly in the substantia nigra but also in other regions of the brain, and the presence of Lewy bodies in the substantia nigra (Gibb and Lees 1989; Fearnley and Lees 1991).

To better understand the role of genetics in PD, many investigators have initiated studies to estimate familial aggregation of PD in first- and/or second-degree relatives of patients with PD. Studies around the world have provided evidence that genetic risk factors are involved in the pathogenesis of the idiopathic form of PD. Estimates of the relative risk to first-degree relatives of an affected individual range from 2.7 (Kuopio et al. 2001) to 3.5 (Payami et al. 1994) in the United States and are 2.9 in Finland (Autere et al. 2000), 6.7 in Iceland (Sveinbjornsdottir et al. 2000), 7.7 in France (Preux et al. 2000), 3.2 in three centers within Europe (Elbaz et al. 1999), 5.0 in Canada (Uitti et al. 1997), 13.4 in Italy (De Michele et al. 1995), and 7.1 in Germany (Vieregge et al. 1994).

Several families with Mendelian segregation of PD have been reported. At present, four genes implicated in autosomal dominant forms of parkinsonism have been identified or localized. The alpha-synuclein gene was identified by studying a large Italian kindred in which PD was pathologically confirmed (Polymeropoulos et al. 1997). The same alpha-synuclein mutation, G209A, observed in the Italian kindred was later found in three Greek families, most of which can trace their ancestry to a very small geographical area on the Pelo-

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ponnisos in southern Greece (Polymeropoulos et al. 1997). Alpha-synuclein mutations in eight additional individuals from six different families from central and southwestern Greece have been reported more recently (Bostantjopoulou et al. 2001). In view of the close historical ties to southern Italy, these mutation results suggest the presence of a founder effect (Gasser 2001). Subsequently, another mutation, G88C, in the gene was identified in a German family with autosomal PD (Kruger et al. 1998). But, since no other mutations in alpha-synuclein have been identified in the large number of patients with sporadic or familial PD that have been screened (Chan et al. 1998; Farrer et al. 1998; Vaughan et al. 1998; Pastor et al. 2001), alpha-synuclein is most likely not a major risk factor in familial PD. A mutation, Ile93Met, in the ubiquitin carboxy-terminal hydrolase-L1 gene was identified in two individuals with PD who were members of a German pedigree (Leroy et al. 1998). The PARK3 locus on chromosome 2p13 has been linked in a subset of families with German ancestry (Gasser et al. 1998), although the responsible gene has not yet been identified. Last, the PARK4 locus on chromosome 4p14-16.3 has been linked in a four-generation pedigree (Farrer et al. 1999). Despite these promising findings, pedigrees with autosomal dominant PD are rare and seem to represent only a small number of families with PD (The French Parkinson's Disease Genetics Study Group 1998).

Autosomal recessive juvenile parkinsonism (ARJP [MIM 600116]) is a distinct clinical and genetic entity within familial PD. It is characterized by typical PD features and an early (<40 years) age at onset, slow progression of the disease, sustained response to levodopa, early levodopa-induced complications (fluctuations and dyskinesias) that are often severe, hyperreflexia, and mild dystonia, mainly in the feet (Yamamura et al. 1973; Ishikawa and Tsuji 1996). Among the genes implicated in familial PD, the largest number of mutations have been found in the *parkin* gene (Kitada et al. 1998), and mutations in this gene might account for PD in as many as 50% of familial patients with ARJP (Lücking et al. 2000). Two other loci have been implicated in autosomal recessive early-onset parkinsonism: PARK6 has been localized on chromosome 1p35-36 in a single Italian family (Valente et al. 2001), and PARK7 has been mapped to chromosome 1p37 in a consanguineous pedigree from a genetically isolated population in the southwestern region of the Netherlands (van Duijn et al. 2001).

There is clear evidence of a single Mendelian gene in a minority of families with PD. However, evidence of a genetic contribution to more typical, late-onset PD has not been universal. A report of a low concordance rate of PD in a sample of World War II veteran twins has been interpreted to imply that the role of genes in PD susceptibility is minimal. Further analyses of these data suggest greater twin concordance among twins with early-onset PD (Tanner et al. 1999). A major limitation of most twin studies is that they are usually cross-sectional in nature. In the case of PD, in which the age at onset is quite variable, a cross-sectional study may fail to identify concordant twin pairs with widely differing ages at onset. In one instance, the age at onset of PD in a pair of MZ twins differed by 20 years (Dickson et al. 2001). Functional imaging of the brain has suggested that some apparently normal cotwins actually have decreased function of the nigrostriatal dopaminergic system and may be presymptomatic, implying that the concordance rates for both MZ and DZ twins may be higher than previously estimated (Burn et al. 1992; Piccini et al. 1997).

The purpose of the present study was to identify genes contributing to PD susceptibility, particularly among families with more typical, later-onset PD. Since, to date, mutations in *parkin* are the most common inherited defect identified in PD, our initial efforts were focused on identifying those families likely to have *parkin* mutations, so that they could be further screened and eliminated from the genome-screen analyses. Our intention was to reduce the genetic heterogeneity in our sample and, thereby, to increase our power to detect non-*parkin* PD-susceptibility genes. Our study provides evidence of PD-susceptibility loci in several chromosomal regions.

Subjects and Methods

Subjects

Families consisting of at least one pair of living siblings diagnosed with PD were recruited through 60 Parkinson Study Group (PSG) sites located throughout North America. The sample was primarily white (94%), although Hispanics (6%) also participated. All study participants completed a uniform clinical evaluation that consisted of parts II and III of the Unified Parkinson Disease Rating Scale (Lang and Fahn 1989). A diagnostic checklist also was completed by a PSG movement-disorder specialist, with inclusion criteria consisting of clinical features highly associated with autopsy-confirmed PD and with exclusion criteria highly associated with other, non-PD pathological diagnoses (see the Appendix) (Hughes et al. 1992*a*, 1992*b*). Responses on the diagnostic checklist were then used to classify study subjects as having verified PD (285 subjects) or nonverified PD (99 subjects). Peripheral blood was obtained from all individuals after appropriate written informed consent approved by each individual institution's institutional review board was completed. DNA was prepared by standard methods (Madisen et al. 1987).

Table 1

Demographics of Sample

Model	No. of Families	No. of Pairs	% Male	Mean ± SD Age at Onset (years)
I (183 subjects)	90	96	60	62.3 ± 9.7 (range 32–83)
II (325 subjects)	160	170	61	62.8 ± 10.7 (range 18–83)

One of the advantages of our study is the use of the diagnostic checklist for the classification of disease status. The inclusion criteria, consisting of clinical features highly associated with autopsy-confirmed PD, in conjunction with the exclusion criteria, consisting of clinical features highly associated with other, non-PD pathological diagnoses, provide the stringent diagnostic criteria essential for the successful identification of PD-susceptibility genes. The high interrater and intersite reliability of the diagnostic instrument provided further reassurance that error in diagnosis was kept at a minimum (Siemers et al. 1998).

parkin *Screening*

A marker (D6S305) in intron 7 of the *parkin* gene was genotyped in all study subjects. Families with positive LOD scores at this marker, under an autosomal recessive model of disease inheritance ($n = 68$), and families with an affected family member with an age at onset of <45 years ($n = 21$) were screened for *parkin* mutations, by both direct-sequencing and fluorescent-dosage analysis (Nichols et al., in press). Twenty different *parkin* mutations were identified in 22 of the 74 families analyzed. Genetic analyses were performed with and without the families with a *parkin* mutation.

Genotyping

A genome screen was completed by use of 400 dinucleotide markers, from the ABI Prism Linkage Mapping Set (Applied Biosystems), that had an average heterozygosity of 79% and an average intermarker spacing of 8.6 cM. The average information content for the 23 chromosomes was 0.65 when estimated every 1 cM and was 0.71 when estimated at a marker (Mapmaker/Sibs; Kruglyak and Lander 1995). In brief, 30 ng of genomic DNA was PCR amplified by use of each marker, in a 10-μl reaction. After PCR, the PCR products were pooled by use of equal amounts of each PCR. One microliter of this multiplexed mix was added to 10 μl of formamide containing the GENESCAN-400HD ROX size standard (Applied Biosystems). Genotypes were determined by an ABI 3700 DNA Analyzer (Applied Biosystems) and GENESCAN 3.5, GENOTYPER 3.6, and GENEMAPPER 1.1 software.

All genotypic data were evaluated for Mendelian inheritance of marker alleles, by the program Pedcheck (O'Connell and Weeks 1998). The marker genotypic data were used to verify the full-sibling relationships among the subjects, by the computer program RELATIVE (Goring and Ott 1997). Three half-sibling pairs were eliminated from further analyses, because the sharing of marker alleles identical by descent was significantly lower than that which would be expected for full siblings.

Statistical Analysis

A total of 160 families ($n = 325$ individuals) who had no evidence of a *parkin* mutation and who had two or more affected siblings were available for genetic analyses. Of the two models of affection considered for this sample, model I included only those individuals with a more stringent diagnosis of verified PD ($n = 96$ sibling pairs, from 90 families), whereas model II included all examined individuals as affected, regardless of their final diagnostic classification ($n = 170$ sibling pairs, from 160 families). The majority of families consisted of a single pair of affected siblings. Under model I, there were 87 families with a single affected sibling pair and 3 families with three affected siblings. Under the broader disease definition employed in model II, there were 155 families with two affected siblings and 5 families with three affected siblings. The average age at onset of PD did not differ significantly ($P = .47$) between the sample employed in the model I analyses and the sample of individuals included in the more inclusive model II definition. The characteristics of the study population are listed in table 1.

Multipoint nonparametric linkage analysis was performed for both models of affection status, by the maximum-likelihood method implemented in the computer program Mapmaker/SIBS (Kruglyak and Lander 1995). Analysis was performed both with dominance variance fixed at zero and with dominance variance free to vary. Analyses were performed by employing all possible sibling pairs from families of size greater than two.

For completeness, genomewide linkage analyses also were performed with the entire collected sample, including those families with a known *parkin* mutation ($n = 182$ families). In addition, to identify any potential loci acting epistatically with the *parkin* gene, a genomewide linkage analysis also was performed in a sample limited to the 22 families with a known *parkin* mutation.

Results

Results of the genome screen of the families with *parkin* mutations were reviewed ($n = 160$ families), and chromosomal regions with LOD scores >1.5 were identified for further evaluation (figs. 1 and 2 and table 2). Information content, both at linked markers and in the linked intermarker regions, was comparable to that observed in the full genome. When only PD sibling pairs meeting the stricter PD diagnostic criteria were included in the analyses (model I), three chromosomal regions had LOD scores >1.5 (fig. 1). The highest LOD scores were observed on chromosome X (LOD score 2.1), near marker DXS1001 (fig. 3), and on chromosome 2 (LOD score 1.9), near marker D2S206 (fig. 4). Three markers in this 21-cM region on chromosome X (i.e., markers DXS1106, DXS8055, and DXS1001) yielded LOD scores >1.5. Subsequent analyses suggested that the majority of the evidence of linkage to this region is derived from 35 brother-brother pairs (LOD score 1.8). Analyses of the 17 sister-sister pairs produced a maximum LOD score of 0.9 in this region of Xq. Linkage analyses employing the mixed-sex sibships ($n = 44$ sib pairs) yielded a maximum LOD score of 0.3. In addition, a LOD score of 1.6 was found for chromosome 5, near marker D5S471.

Analyses were also performed with all available sibling pairs (model II). With this larger sample of sibling pairs, LOD scores >1.5 were observed for chromosomes 2, 4, 13, and X (table 2 and fig. 2). The maximum LOD score obtained for the entire genome screen was for chromosome X (LOD score 2.7), near marker DXS1106 (fig. 3). Three markers in this 23-cM region (i.e., markers DXS1106, DXS8055, and DXS1001) yielded LOD scores >1.5. Subsequent analyses suggest that the majority of the evidence of linkage to this region is derived from 66 brother-brother pairs (LOD score 1.9). Analyses of the 29 sister-sister pairs produced a maximum LOD score of 0.65 for this region of Xq. Linkage analyses employing the mixed-sex sibships ($n = 75$ sib pairs) yielded a maximum LOD score of 0.24.

The maximum LOD score for chromosome 2, when the broader disease definition (model II) was used, was 2.5. Similar to what was seen in the model I analyses, the maximum LOD score for chromosome 2 was near marker D2S206 (fig. 4). Three markers in this region of chromosome 2, spanning 24 cM, produced LOD scores ≥ 1.5 (i.e., D2S396, D2S206, and D2S338). Thus, the two chromosomes with the highest LOD scores under the more restrictive model I diagnosis also had the highest LOD scores under the broader disease definition.

Two additional chromosomal regions had LOD scores above our initial threshold of 1.5. A maximum LOD score of 1.6 was found for chromosome 4, near marker D4S1597, and a maximum LOD score of 1.5 was found for chromosome 13, near marker D13S171.

Linkage analyses also were performed in the entire collected sample ($n = 182$ families), including the 22 families with an identified *parkin* mutation (table 2). For chromosomes 4, 5, and X, both the LOD score under both model I and that under model II were lower than those for the analyses performed without families with *parkin* mutations. For chromosome 13, the complete sample had a higher LOD score under model II only (LOD score 2.1 vs. LOD score 1.5). Analyses performed with the entire sample did identify one additional chromosomal region, on chromosome 10 (LOD score 1.6), that met our initial linkage threshold. Interestingly, for chromosome 2, the entire sample produced a substantially higher LOD score under model I (LOD score 3.1 vs. LOD score 1.9). To further examine the potential role of a PD-susceptibility locus on chromosome 2, as well as potential epistatic interactions between *parkin* and other loci, a genome screen was performed with only the 22 families with *parkin* mutations (fig. 5). In this small subset of the data, there was no evidence of a PD-susceptibility gene on chromosome 2; however, there was

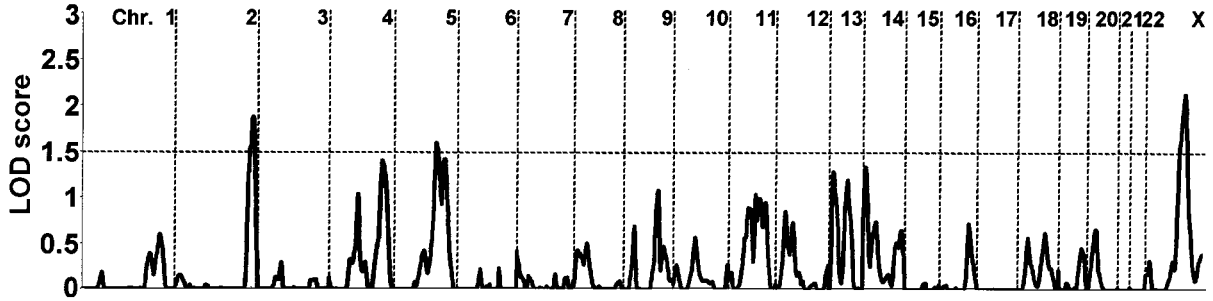

Figure 1 Multipoint LOD-score graph summarizing results of genome screen of chromosomes 1–22 and X, under the narrower, model I definition of PD diagnosis.

some evidence of linkage to chromosomes 8 (LOD score 1.6), 12 (LOD score 2.0), and 19 (LOD score 1.7).

Discussion

Three other studies are currently ongoing that seek to identify PD-susceptibility genes. The GenePD study, analyzing data from 113 sibling pairs, has reported LOD scores of 0.9–1.3 for chromosomes 1, 9, 10, and 15 (DeStefano et al. 2001). A second study, analyzing 174 extended pedigrees, including 378 affected individuals, has reported LOD scores of 1.5–2.5 for chromosomes 5, 8, 9, 14, 17, and X in families with late-onset PD (Scott et al. 2001). The deCODE study in Iceland has reported a LOD score of 4.9 on chromosome 1, as well as evidence of linkage to chromosomes 5, 7, 13, 14, and X (Hicks et al. 2001).

We have completed a genome screen in a large sample of 170 sibling pairs with PD that were ascertained from 160 families. Unlike previous PD-linkage studies, ours has attempted to reduce genetic heterogeneity by screening all families by use of a marker in the *parkin* gene, to identify families likely to have *parkin* mutations. This has allowed us to identify 22 families with *parkin* mutations who were then removed from subsequent genome-screen analyses. Linkage analyses performed with and without the families with *parkin* mutations suggest that most of the LOD scores that we have reported increased with the removal of the families with known *parkin* mutations (table 2).

In this study, the strongest evidence of linkage was reported for chromosome X. Evidence of linkage was observed both under the more restrictive (model I) and under the broader (model II) disease definitions. The maximum LOD score in each analysis was at a different marker; however, these markers are only 13 cM apart and likely represent the same underlying susceptibility gene. It is interesting that, in our sample, most of the evidence of linkage to this region on chromosome X came from the brother-brother pairs. It is intriguing to speculate that this linkage to chromosome X might explain the slightly higher incidence of PD among males (Tanner et al. 1992).

Table 2

Regions with LOD Scores ≥1.5

Chromosome	Position[a] (cM)	LOD Score: With Families with *parkin* Mutations, Model I	With Families with *parkin* Mutations, Model II	Without Families with *parkin* Mutations, Model I	Without Families with *parkin* Mutations, Model II
2	236	3.1	2.5	1.9	2.5
4	160	1.1	1.4	1.4	1.6
5	128	1.3	.7	1.6	.5
10	68	.6	1.6	.6	1.3
13	13-22	1.2	2.1	1.3	1.5
X	105-122	1.4	2.5	2.1	2.7

[a] Based on the sex-averaged genetic maps from the Center for Medical Genetics, Marshfield Medical Research Foundation.

Two other PD studies appear to report linkage to this same region of Xq21-25 (Hicks et al. 2001; Scott et al. 2001). Unfortunately, few details were provided, in either study, regarding the markers near the maximum LOD score. Also, neither study reported whether the evidence of linkage was limited to a sample subset consisting of brother pairs, as is the case in our study. Thus, it is difficult to verify whether the results from these other studies might represent converging lines of evidence supporting the presence of a PD-susceptibility gene on chromosome X.

Interestingly, X-linked dystonia parkinsonism (XDP), which has been reported at high incidence in Panay, The Philippines, has been linked to Xq13.1 (Lee et al. 2001). It is an adult-onset, highly penetrant, X-linked disorder that primarily afflicts men (male:female ratio 123:1). It is a severe, progressive disorder with onset typically occurring, with dystonic movements, during the 3rd or 4th decade. Approximately a decade after disease onset, the dystonia typically coexists or is replaced by parkinsonism. Neuropathology reveals pronounced atrophy of the

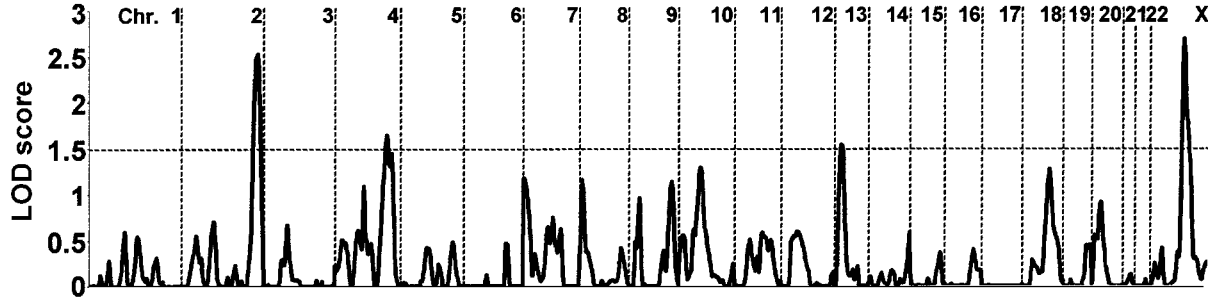

Figure 2 Multipoint LOD-score graph summarizing results of genome screen of chromosomes 1–22 and X, under the broader, model II definition of PD diagnosis.

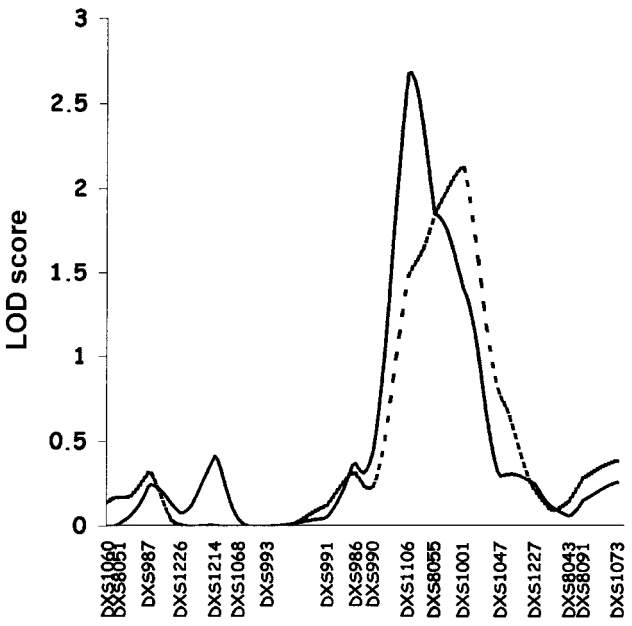

Figure 3 Multipoint LOD-score graph of chromosome X, under models I (*dashed line*) and II (*solid line*).

caudate and putamen, mostly in the cases with long-standing illness (Lee et al. 2001). The gene has been mapped to an <350-kb region in the DXS7117–DXS559 region (Nemeth et al. 1999), <20 cM from the region identified in our study. Our linkage finding may seem to be too far from the XDP linkage to represent the same gene. However, when, in our entire sample (n = 182 families), we review the data for chromosome 6, we find that the maximum LOD score (LOD score 1.1) occurs ~25 cM proximal to the actual location of the *parkin* gene. This is the case even when a marker is genotyped in exon 7 of the *parkin* gene. Thus, we believe that the *XDP* gene remains a possible positional candidate gene.

We have used complementary methods of analysis to ensure maximum power to detect loci contributing to PD susceptibility. Holmans (1993) has shown that analysis of affected-sib-pair data under the assumption of dominance variance, when Holmans's "possible triangle" is applied, appears to allow for a more sensitive test for putative genes acting in a recessive fashion than does analysis when dominance variance is fixed at zero. Several studies have reported higher estimates of relative risk for PD among siblings of affected individuals, compared with the risk of PD among the parents or offspring of affected individuals (Autere et al. 2000; Sveinbjornsdottir et al. 2000), suggesting evidence of at least some recessively acting PD-susceptibility genes. This would appear to be supported both by the recessively acting genes already implicated, such as *parkin,* and by the recent linkage findings on chromosome 1 (Vallente et al. 2001; van Duijn et al. 2001). We also have observed empirical data suggesting that LOD scores at a recessively acting locus increase when dominance variance is free to vary. Before the families with *parkin* mutations were removed, the LOD score for the *parkin* locus in the entire sample increased from 0.6 to 1.1 when dominance variance was free to vary; likewise, when the sample was limited to only the 22 families with *parkin* mutations, the LOD score increased from 5.0 to 7.6 (fig. 5).

In our study, the linkage findings for chromosome 2q are consistent with a recessively acting susceptibility locus. Allowing the dominance variance to vary increased the LOD score from 1.2 to 1.9 under model I and from 2.1 to 2.5 under model II. The linkage finding in this region was supported by three markers, all with LOD scores >1.5. None of the other three genomewide linkage studies (DeStefano et al. 2001; Hicks et al. 2001; Scott et al. 2001) have reported any evidence of linkage to chromosome 2.

The linkage to chromosome 4q32 was supported by four markers with a LOD score >1.0. In addition, another region, ~70 cM away, at 4q21, had a maximum LOD score of 1.1. None of the other three studies have reported linkage to either region (DeStefano et al. 2001; Hicks et al. 2001; Scott et al. 2001). The strongest candidate gene in this region is alpha-synuclein, which is near marker D4S1534 in the 4q21 region. Although only a few families with mutations in the alpha-synuclein gene have been identified (Chan et al. 1998; Farrer et al. 1998; Vaughan et al. 1998; Pastor et al. 2001), several recent studies have reported an association between PD and haplotypes in the promoter region of the alpha-synuclein gene (Kruger et al. 1999; Farrer et al. 2001*b*).

The evidence of linkage to chromosome 5q23 is supported by four markers with a LOD score >1.0. Similar to the linkage finding on chromosome 2, the results of analyses performed on chromosome 5, under model I, suggest that this locus also may be a recessively acting susceptibility locus; the maximum LOD score increased from 0.9 to 1.6 when linkage analyses were performed with dominance variance allowed to vary. Unlike the other linkage findings reported in this study, for this region there is a large disparity between the maximum LOD score under model I and that under model II. The higher LOD scores under model I may provide insight with regard to the putative gene's mode of action. "Parkinsonism" is a term referring to all clinical states characterized by tremor, muscle rigidity, and slowed movement (termed "bradykinesia") (Koller and Hubble 1992) and includes secondary forms of parkinsonism, such as those resulting from drug exposure and stroke. PD is the most common form of parkinsonism. We anticipate that model II includes some subjects with parkinsonism but

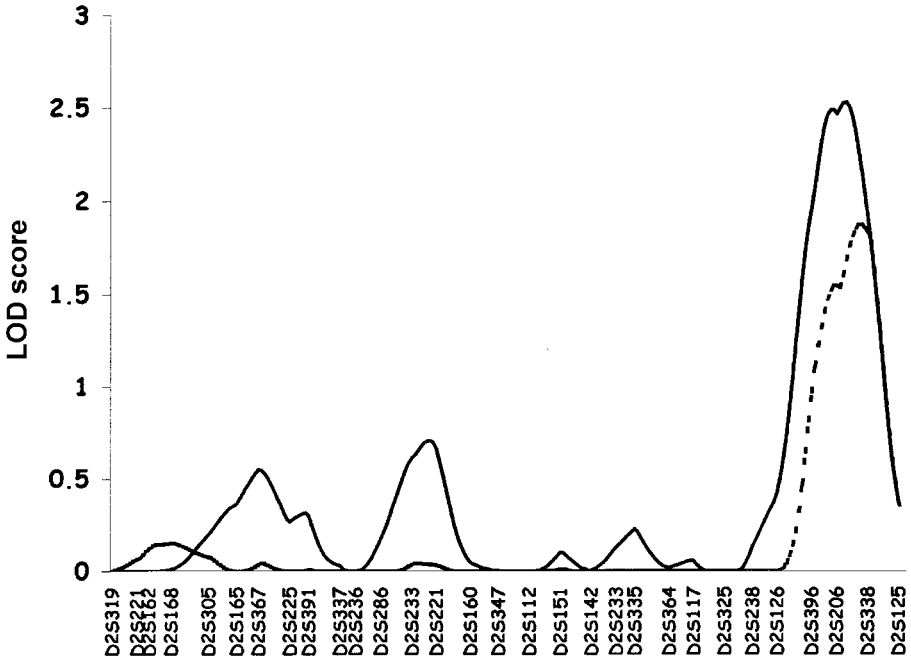

Figure 4 Multipoint-LOD-score graph of chromosome 2, under model I (*dashed line*) and II (*solid line*)

without PD. Perhaps the 5q23 locus is a PD-specific susceptibility locus, whereas most of the other loci mapped in this study may confer more-general parkinsonism susceptibility. Alternatively, the same genes may be contributing to both model I and model II; however, analyses performed with the broader disease model (i.e., model II) may result in higher LOD scores simply because the sample size is greater, as is the power to detect genetic effects.

Two other studies have reported linkage to 5q23 (Hicks et al. 2001; Scott et al. 2001). Scott et al. reported a multipoint LOD score of 1.5 for marker D5S816, which is <10 cM from marker D5S471, where the maximum LOD score was found. Within this cytogenetic band is a gene that is an excellent potential candidate, the synphilin-1 gene (*SNCAIP*). Normal parkin ubiquitinates synphilin-1 as well as the glycosylated form of alpha-synuclein. It is proposed that, without proper ubiquitination, these molecules do not properly degrade and that the accumulation of these proteins thus becomes toxic. Notably, all three of these proteins are present in the intracytoplasmic inclusions called "Lewy bodies" (Chung et al. 2001). No mutations in *SNCAIP* have been discovered in a small sample of patients with PD who have been screened in other studies (Bandopadhyay et al. 2001; Farrer et al. 2001*a*).

Linkage near the centromere of chromosome 13 is supported by two markers with LOD scores >1.0. Hicks et al. (2001) have reported linkage near this region, although the exact position and magnitude of their linkage finding has not been reported. One attractive candidate gene in this region is the copper-transporting P-type ATPase gene (*ATP7B*), which, if mutated, results in the autosomal recessive disorder Wilson disease. Cox et al. (1972) referred to the two "typical" forms of Wilson disease as being the "Slavic" type and the "juvenile" type, with the latter being a differential diagnosis considered for patients presenting with early-onset parkinsonism. The Slavic type has a late age at onset and is predominantly a neurologic disease that, like PD, affects the basal ganglia and can manifest tremor, dysarthria, poor motor coordination, and dementia. Approximately 1% of the general population are thought to be carriers of Wilson disease (Riordan and Williams 2001). These individuals typically do not have any clinical sequelae; however, they do have problems with copper metabolism. This is significant, because copper concentration in the cerebrospinal fluid has been reported at significantly increased levels in patients with idiopathic PD, compared with that in control subjects (Pall et al. 1987). Furthermore, alpha-synuclein has been shown to undergo self-oligomerization in the presence of copper(II) (Paik et al. 2000; Kim et al. 2001; Uversky et al. 2001), which may lead to the protein aggregation and neurodegeneration found in both PD and Alzheimer disease.

Similar to studies of other complex genetic disorders, previous genetic studies of PD have reported several linkage findings that were not replicated in our study. Most notable is the linkage to chromosome 1, reported by Hicks et al. (2001), in a sample of 117 affected individ-

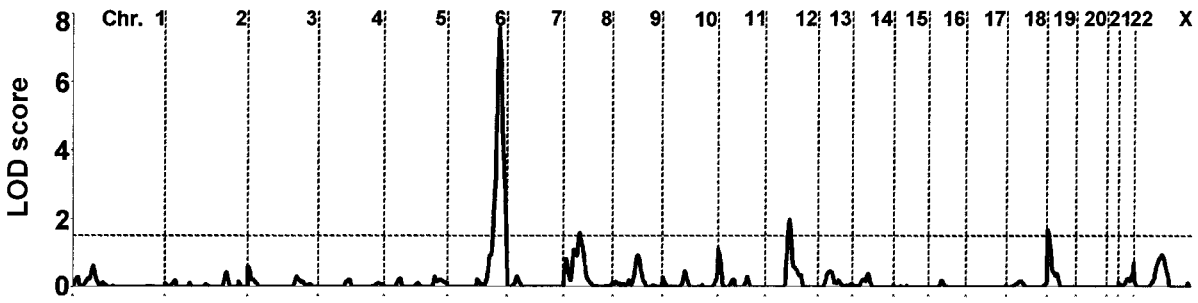

Figure 5 Multipoint LOD-score graph summarizing results of genome screen of chromosomes 1–22 and X, for the 22 families with *parkin* mutations.

uals from the genetically isolated population of Iceland. In that study, the result for chromosome 1 was the most significant linkage result, with a LOD score of 4.9. However, we found no evidence of linkage to this region (LOD score <0.6, both under model I and under model II). Importantly, no evidence of linkage near the centromere of chromosome 1 was reported in the two other genomewide studies of PD. It is therefore possible that a PD-susceptibility gene on chromosome 1 is present at high frequency only in the Icelandic population.

The study by Scott et al. (2001) found the most significant linkage on chromosome 17, near the *tau* gene. The strength of this linkage finding was increased when analyses were limited to a subset of families in which at least one individual in the kindred was not responsive to levodopa treatment. Since a positive response to this dopamine precursor is very common among individuals with PD, Scott et al. considered this potential phenotypic heterogeneity to be indicative of genotypic heterogeneity. The most notable improvements to LOD scores were for chromosomes 3, 9, and 17. Exploratory analyses were performed in our sample of only nine families, containing 11 sibling pairs, in which at least one member of the family was not responsive to levodopa. In this very small data set as well, as in analyses performed with either model I or model II, we found no evidence of linkage to *tau* (LOD scores 0.2, 0.0, and 0.0, respectively). Unlike our study of a sample primarily comprising sibling pairs, the study by Scott et al. (2001) included many extended pedigrees. Thus, it is possible that some of the families in that study are segregating a mutation in *tau*, which might produce an autosomal dominant pattern of PD inheritance.

In our sample of 170 affected sibling pairs, we have identified two chromosomal regions with particularly strong evidence of linkage. The findings for chromosomes 2 and X suggest the presence of loci that contribute to PD susceptibility, both in our analysis employing a narrow disease definition and in our analysis employing a more broader disease classification. These findings may suggest loci contributing to parkinsonism susceptibility—rather than simply to PD susceptibility per se. Importantly, our study has provided additional evidence of linkage, in an independent sample, to chromosomes 5 and X. We are continuing to recruit families with multiple living members diagnosed with PD, to replicate the results found for chromosomal regions identified in these analyses. An important advantage of our study is the identification and removal, prior to the genome-screen analyses, of families with *parkin* mutations. This has reduced genetic heterogeneity in our sample and, in most instances, has increased the LOD score for linked chromosomal regions.

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Appendix

Inclusion and Exclusion Criteria

Inclusion Criteria

Age at onset >20 years
Bradykinesia
Clinician's overall impression that the subject has PD is >50%
At least one of the following:
  Muscular rigidity
  Rest tremor
  Postural instability
At least two of the following:

Persistent asymmetry of signs
Progressive disorder
Rest tremor
>50% Levodopa response
Levodopa-induced chorea
Levadopa response for ⩾5 years
Clinical course of ⩾10 years

Exclusion Criteria

Unexplained upper motor-neuron signs
History of repeated strokes with stepwise progression of parkinsonian features
History of encephalitis
History of oculogyric crisis
Clinical features/course suggesting Alzheimer disease or other dementia distinct from PD
Cortical sensory deficits or apraxia
Parkinsonism likely due to antidopaminergic drug use
Sustained remission of parkinsonian symptoms
Strictly unilateral features after 3 years
Supranuclear gaze palsy with down gaze <50% of normal
Cerebellar signs
Symptomatic orthostatic hypotension early in the course of the disease
Failure to respond to large dosage of levodopa
Brain imaging (by computed tomography or magnetic-resonance imaging) reveals presence of structural lesion(s) likely causing or contributing to parkinsonism

Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

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 PD [MIM 168600] and ARJP [MIM 600116])

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