

Genome-wide linkage analysis and evidence of gene-by-gene interactions in a sample of 362 multiplex Parkinson disease families

Nathan Pankratz¹, William C. Nichols², Sean K. Uniacke², Cheryl Halter¹, Jill Murrell¹, Alice Rudolph³, Clifford W. Shults⁴, P. Michael Conneally¹, Tatiana Foroud^{1,*} and the Parkinson Study Group

¹Department of Medical and Molecular Genetics, Indiana University Medical Center, Indianapolis, IN, USA,

²Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA,

³Department of Neurology, University of Rochester, Rochester, NY, USA and ⁴Department of Neurosciences, University of California, La Jolla, CA and the VA San Diego Healthcare System, San Diego, CA, USA

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Parkinson disease (PD) is the second most common neurodegenerative disorder. We studied 754 affected individuals, comprising 425 sibling pairs, to identify PD susceptibility genes. Screening of the *parkin* gene was performed in a subset of the sample having earlier age of PD onset or a positive LOD score with a marker in the *parkin* gene. All subjects were evaluated using a rigorous neurological assessment. Two diagnostic models were considered for genome-wide, non-parametric linkage analyses. Model I included only those individuals with a more stringent diagnosis of verified PD (216 sibling pairs) and resulted in a maximum LOD score of 3.4 on chromosome 2. Model II included all affected individuals (425 sibling pairs) and yielded a LOD score of 3.1 on the X chromosome. Our large sample was then employed to test for gene-by-gene (epistatic) interactions. A genome screen using the 23 families with PD patients having a mutation in only one allele of the *parkin* gene detected evidence of linkage to chromosome 10 (LOD = 2.3). The 85 families with a very strong family history of PD were employed in a genome screen and, in addition to strong evidence of linkage to chromosome 2 (LOD = 4.9), also produced a LOD of 2.4 on chromosome 14. A genome screen performed in the 277 families without a strong family history of PD detected linkage to chromosomes 10 (LOD = 2.4) and X (LOD = 3.2). These findings demonstrate consistent evidence of linkage to chromosomes 2 and X and also support the hypothesis that gene-by-gene interactions are important in PD susceptibility.

INTRODUCTION

Parkinson disease (PD; MIM 168600) is a common neurodegenerative disorder affecting more than 1% of 55-year-old individuals and more than 3% of those over 75 years of age (1). It is characterized by bradykinesia, resting tremor, muscular rigidity and postural instability, as well as a clinically significant response to treatment with levodopa (2). The cardinal pathological feature of PD is the loss of dopaminergic neurons in the substantia nigra. A second characteristic pathological feature is the presence of intracytoplasmic inclusions, called Lewy bodies, in nigral and extranigral neurons (3,4).

Recent studies have consistently found that genetic factors are involved in the pathogenesis of idiopathic PD. Results suggest the risk of PD to be anywhere from two to 14 times higher for first-degree relatives of an affected individual as compared with the risk in members of unaffected families (5–13). Mutations in four genes, all having a role in the ubiquitin–proteasome pathway, have been found in families segregating autosomal dominant or autosomal recessive PD. Mutations in alpha-synuclein (PARK1) were identified in families with early-onset, autosomal dominant PD (14). Extensive molecular studies found alpha-synuclein mutations in only a small number of PD families, suggesting that this gene is not a major risk factor for PD (15–18). Subsequently, mutations in the *parkin* gene

*To whom correspondence should be addressed at: Indiana University School of Medicine, Department of Medical and Molecular Genetics (IB 130), 975 West Walnut Street, Indianapolis, IN 46202-5251, USA. Tel: +1 3172781291; Fax: +1 3172742387; Email: tforoud@iupui.edu

Table 1. Study sample

Model	Number of			Percentage of subjects VPD	Percentage of male	Age at onset Mean \pm SD
	Subjects	Families	Pairs			
Full sample (model II)	754	362	425	70%	60%	61.0 \pm 12.1
Families with verified PD (model I)	399	194	216	100%	59%	59.8 \pm 12.1
Families with ≥ 1 <i>parkin</i> mutation(s)	91	39	68	71%	59%	48.7 \pm 15.1
<i>Parkin</i> heterozygotes	50	23	31	69%	68%	56.5 \pm 13.8
<i>Parkin</i> -negative families	663	323	357	70%	60%	62.7 \pm 10.6
Strong family history ^a	183	85	113	100%	59%	58.3 \pm 12.0
Weak family history	571	277	312	62%	60%	61.9 \pm 12.1

^aStrong family history is defined as families with verified PD and having at least four affected family members or an affected sibling pair with an affected parent.

(PARK2) were found to cause autosomal recessive, juvenile-onset PD (19). Multiple reports of point mutations and exon rearrangements in the *parkin* gene, including both deletions and duplications, have been identified in patients with PD (19–28). The ubiquitin carboxy-terminal hydrolase L1 gene (UCHL1; PARK5) has been implicated in autosomal dominant PD. Mutations in two siblings were reported in a single German family; however, further analyses in several other samples have failed to identify mutations in any other individuals (29–31). DJ-1 (PARK7) was found recently to play a causative role in autosomal recessive PD (32), and so the frequency of DJ-1 mutations in other samples is not yet known. Genetic analyses have detected linkage to several other chromosomal regions, although the genes have not yet been identified [PARK3 (33) PARK4 (34) PARK6 (35) PARK8 (36)].

Studies of non-Mendelian families have implicated additional genes and chromosomal regions. Xu *et al.* (37) reported that homozygosity for a 7048G7049 polymorphism in intron 6 of the *Nurr1* gene (also known as NR4A2) was significantly more common among familial PD and sporadic PD as compared with healthy controls. Further studies in an independent sample suggested that heterozygosity for the polymorphism conferred an increased risk for PD (38). Subsequently, mutations in exon 1 of the *Nurr1* gene have been reported in a subset of familial PD subjects (39). Recently, mtDNA haplogroups J and K have been found to significantly decrease the risk of PD (40). A mutation was recently identified in two German patients with PD in the synphilin-1 gene (41). Studies using late-onset families from Iceland identified a novel locus, termed PARK10, located at 1p32 (42).

To identify additional PD susceptibility genes, we recruited a sample of multiplex PD families. Previously, a genome screen performed in 182 families consisting of 203 PD sibling pairs identified linkage to regions on chromosomes 2 and X (43). Additional analyses were then performed using only those pedigrees with a strong family history of PD. With an autosomal dominant model of disease inheritance, this subset of 65 families generated a LOD score of 5.1 at chromosome 2q36–37 (44). We have now doubled our sample through the recruitment and genetic analysis of an additional 180 families, consisting of 222 PD sibling pairs.

Since *parkin* mutations are the most common inherited defect in PD, we identified families in our sample that were likely to have *parkin* mutations. Through the prioritization of families with a positive LOD score at the *parkin* gene and/or an early

age of onset (<50 years), mutations in the *parkin* gene were identified in 17% of our familial sample (20). Affected individuals in more than half of our *parkin* mutation positive families were heterozygous for a single *parkin* mutation. Other studies have also reported affected individuals heterozygous for a single *parkin* mutation (21,25,27,45–48). This raises the possibility that additional susceptibility alleles at other loci might be necessary for disease. The importance of gene-by-gene (epistatic) interactions has been postulated as a crucial mechanism for genetically complex disorders. We have tested this hypothesis in our expanded dataset, now more than doubled in size, that provides consistent evidence for PD susceptibility loci on chromosomes 2 and X.

RESULTS

A sample of 754 affected individuals, consisting of 425 sibling pairs from 362 families, were employed in the analysis. Model I included only those individuals with a more stringent diagnosis of verified PD (216 sibling pairs from 194 families), hence only families with at least two members fulfilling criteria for verified PD are included in the genetic analyses. Model II included all examined individuals as affected, regardless of their final diagnostic classification (425 sibling pairs from 362 families). The majority of families consisted of a single pair of affected siblings. Under model I, there were 183 families with a single pair of affected siblings and 11 families with three affected siblings. There were also two families in which additional affected family members were sampled. Under the broader disease definition employed in model II, there were 335 families with two affected siblings, 24 families with three affected siblings and three families with four affected siblings. There were nine families in which additional affected family members were sampled. The average age at onset of PD was earlier for the sample employed in the model I analyses (mean = 59.8) than for those individuals exclusive to the broader model II definition (mean = 62.6; $P = 0.002$). The characteristics of the study population are described in Table 1.

As described previously (22), families producing a positive LOD score (LOD > 0) with a microsatellite marker in intron 7 of the *parkin* gene (D6S305) using either an autosomal dominant or autosomal recessive model of disease inheritance were screened for *parkin* mutations. In addition, the

Table 2. Regions with LOD scores $\geq 2.2^a$ ($n = 362$ families; 425 affected sibling pairs)

Chromosome	Position ^b	Before removing families with <i>parkin</i> mutations		After removing families with <i>parkin</i> mutations	
		Model I	Model II	Model I	Model II
2	238 cM (223–244) ^c	3.4	2.1	3.2	1.4
X	109 cM (101–121)	1.1	2.6	1.2	3.1

^aCriteria for suggestive evidence of linkage (LOD ≥ 2.2) (49).

^bMap distances are based on the sex-averaged genetic maps from Marshfield Genetic Laboratory.

^c1-LOD support interval.

parkin gene was also examined in any family having at least one member with an age of onset of 50 years or less. This resulted in the identification of *parkin* mutations in 39 of the 173 families screened. Importantly, 16 families had mutations in both *parkin* alleles, while in the other 23 families, a mutation was identified in only one of the two *parkin* alleles. After families with *parkin* mutations were removed from the full sample, the maximum LOD score in the region of the *parkin* locus was 0.4. These results suggest that most families with a *parkin* mutation have been identified in the sample.

Genome screen

As shown in Table 2, non-parametric linkage analysis was performed with model I, using only those sibling pairs meeting rigorous study criteria for verified PD (Fig. 1A), as well as with model II, in which the full sample of PD sibling pairs was employed (Fig. 1B). Results are also shown with and without the 39 families with an identified *parkin* mutation.

Similar to our previous report (43), chromosomes 2 and X provided the greatest evidence of linkage. Linkage to chromosome 2 was greater using the more restrictive PD diagnostic classification (model I; LOD = 3.4; genome-wide P -value = 0.03). In addition, inclusion of the families with *parkin* mutations resulted in higher LOD scores. For all models, three markers on chromosome 2, spanning 17 cM, retained at least 70% of the maximum LOD score (D2S396, D2S206, D2S338) and the 1-LOD support interval encompassed 21 cM. Interestingly, the 18 Hispanic families in the sample provide a substantial portion of the linkage evidence. Analysis of the sample without these families and using allele frequencies based on only the non-Hispanic sample resulted in lower LOD scores for model I (LOD = 2.5 with the inclusion of the *parkin* positive families; LOD = 2.3 when the *parkin* positive families are removed). There was no common haplotype identified in the linked families.

The evidence of linkage to the X chromosome was greater when the broader disease definition was employed (model II; LOD = 3.1; genome-wide P -value = 0.04). Three markers in a 19 cM region on the X chromosome (DXS1106, DXS8055, DXS1001) retained at least 70% of the maximum LOD score and the 1-LOD encompassed 20 cM. Results were not substantially altered when the analysis was performed with the Hispanic families removed from the dataset.

Analyses of *parkin*-positive families

While mutations in both *parkin* alleles clearly result in PD, we hypothesize that a *parkin* mutation in only one allele may not be sufficient for disease. Therefore, a genome screen was performed, using only those families with a single *parkin* mutation, to detect epistatic interactions that may jointly contribute to PD susceptibility. A maximum LOD score of 7.2 was obtained in the subset of 23 families with a *parkin* mutation on only one allele (Fig. 2A). The only other chromosomal region that exceeded Lander and Kruglyak's threshold for suggestive linkage (LOD ≥ 2.2) (49) was on chromosome 10 near the marker D10S196 (LOD = 2.3) and the 1-LOD support interval encompassed 29 cM.

Analyses after stratification by the presence or absence of a strong family history of PD

We have previously shown that the evidence for a chromosome 2q36–37 susceptibility gene was primarily due to families with verified PD that had a strong family history of PD, defined as at least four affected family members or an affected sibling pair with an affected parent. In our expanded sample of 362 families, there were 85 families meeting these criteria. A genome screen performed in these 85 families continued to provide strong evidence of linkage to chromosome 2q (LOD = 4.9; Fig. 2B). In addition, analysis of this sample resulted in a LOD score of 1.9 at the *parkin* locus and a LOD score of 2.4 on chromosome 14 and the 1-LOD support interval encompassed 39 cM.

Analyses were also performed after removing from the sample those 85 families with a strong family history of PD. The genome screen in the remaining sample of 277 families resulted in a LOD score of 3.2 on the X chromosome and the 1-LOD support interval encompassed 22 cM. In addition, linkage was also observed to the region on chromosome 10 noted above to have potential epistatic effects in the families with a single *parkin* mutation (LOD = 2.4; Fig. 2C) and the 1-LOD support interval encompassed 28 cM.

DISCUSSION

In our sample of 362 multiplex PD families, we continued to have strong evidence for PD susceptibility genes on chromosomes 2 and X. Our sample included more than twice as many individuals affected with Parkinson disease as any other genome screen published to date (42,43,50,51). Unlike many

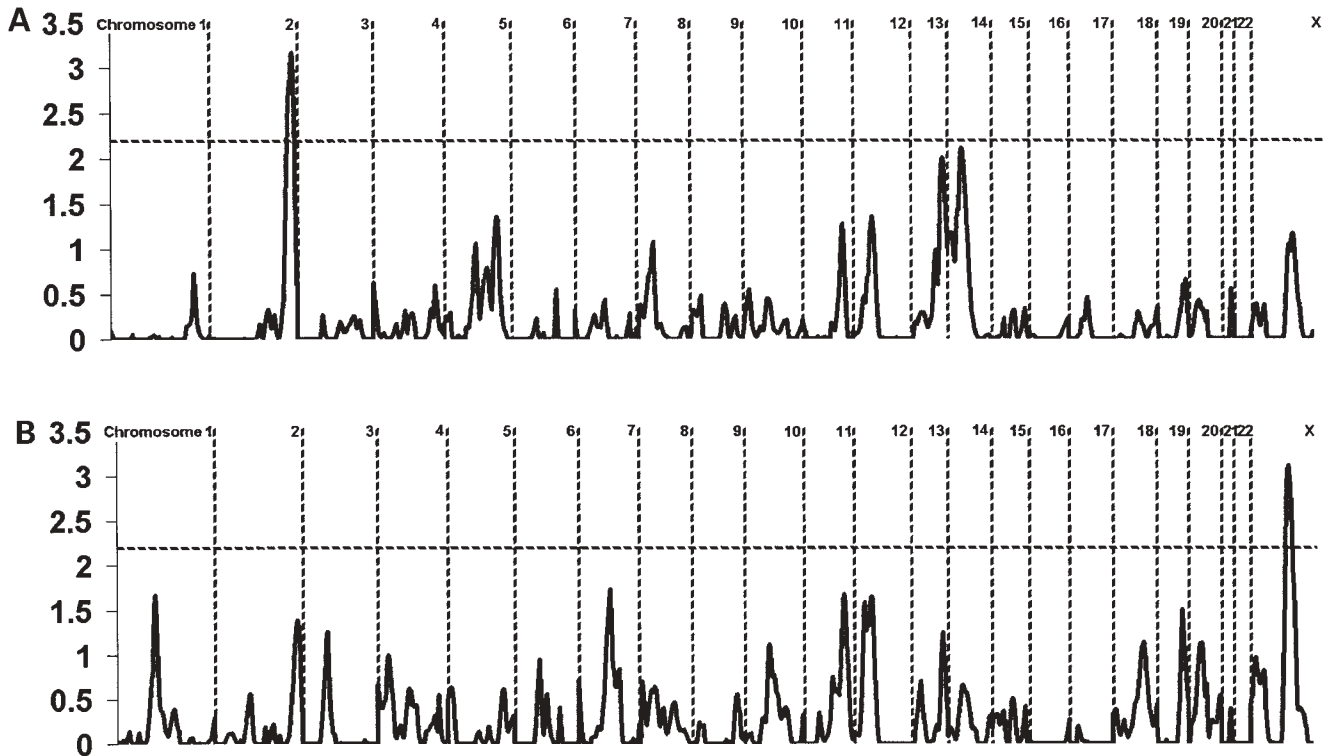


Figure 1. Multipoint LOD score graph summarizing the results of the genome screen for chromosomes 1–22 and X using only those families without *parkin* mutations. (A) Model I (narrow) definition of PD diagnosis; (B) model II (broad) definition of PD diagnosis.

previous PD linkage studies, we sought to reduce heterogeneity by genotyping all families using a marker in the *parkin* gene so as to prioritize families to be screened for likely mutations. This strategy allowed us to identify 39 families with *parkin* mutations, who were removed from subsequent genome screen analyses and later used to screen for epistatic interactions with the *parkin* gene.

The strongest evidence for linkage in our sample continues to be on the X chromosome, with most of the evidence derived from the 125 brother–brother pairs, who generated a LOD score of 2.5 when analyzed alone. Analyses of the 154 sister pairs and the 59 mixed gender sibships both produced LOD scores less than 0.50 in this region of the X chromosome. Two other PD family studies have also reported linkage to this region of Xq21–25 (42,51). A major PD susceptibility locus located on the X chromosome could explain the slightly higher incidence of the disease among males (52). An interesting candidate gene in this chromosomal region may be the locus for sex-linked dystonia parkinsonism (XDP), which has been reported at high incidence in Panay, Philippines (53).

The only other region in our study that exceeded Lander and Kruglyak's threshold for suggestive linkage ($\text{LOD} \geq 2.2$) (49) was on chromosome 2q36–37. The *Nurr1* gene on 2q has been implicated in idiopathic PD (39). The maximum LOD score on chromosome 2q in our study was located more than 50 cM from the *Nurr1* gene, and there was little evidence of linkage at the *Nurr1* locus ($\text{LOD} = 0.4$). Therefore, our finding on 2q36–37 probably represents linkage to a gene distinct from the *Nurr1* locus. Interestingly, the 18 Hispanic families provide

substantial evidence of linkage to chromosome 2q. Further analyses of additional markers in this region will be employed to determine if a common haplotype might be segregating in these families. None of the other PD genome-wide linkage studies (42,50,51) have reported evidence of linkage to chromosome 2q.

More than half of the families with *parkin* mutations in our sample had only one identifiable mutation in one allele of the *parkin* gene. Since all affected family members were independently screened for *parkin* mutations, it is unlikely that a *parkin* mutation in the other allele was consistently missed. This raises the possibility that additional susceptibility alleles at other loci might be necessary for disease. To test this hypothesis, we performed a genome screen limited to the 23 families with a *parkin* mutation in only one of their two alleles. From these analyses, evidence of a potential epistatic interaction between the *parkin* gene and a locus on chromosome 10 ($\text{LOD} = 2.3$) was observed. In addition, review of the genome screen data using the full sample of 362 families generated a LOD score of 2.1 in this same region of chromosome 10 using the broad disease definition (model II). This same region on chromosome 10q was identified in previous analyses of model II using the initial sample of 182 families (43). Interestingly, analysis of the families with a strong family history of PD who also met criteria for model I ($\text{LOD} = 0.1$) as well as analyses of only the families meeting the more stringent model I definition ($\text{LOD} = 1.0$) both provided little evidence of linkage to this region. In contrast, analysis of the pedigrees with a weaker family history of PD

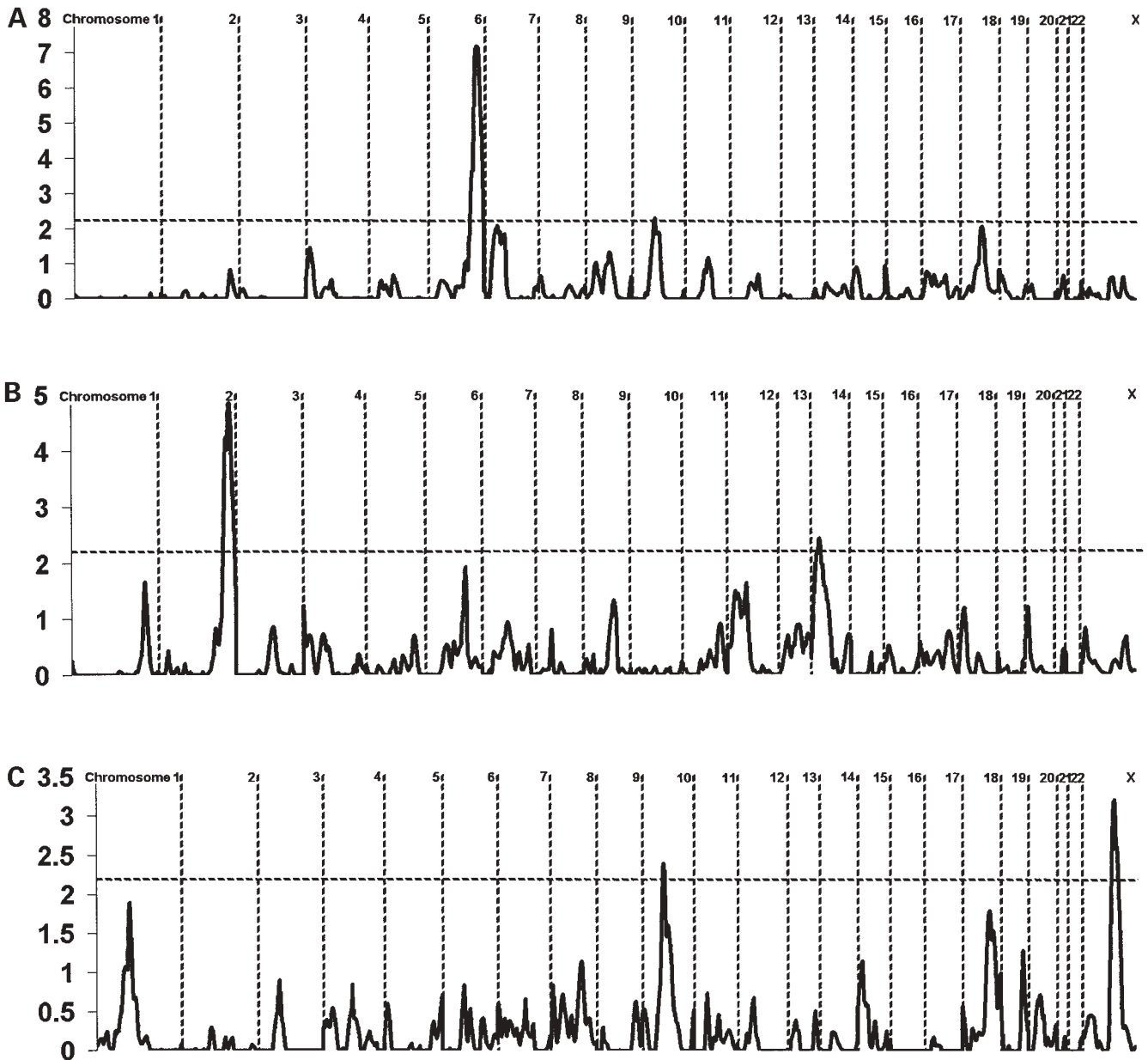


Figure 2. Multipoint LOD score graph summarizing the results of the genome screen for chromosomes 1–22 and X using: (A) 23 families with a single *parkin* mutation; (B) 85 families with a strong family history of PD; (C) 277 families with a weaker family history of PD.

also provided evidence of linkage to this chromosomal region (LOD = 2.4). These findings suggest a locus on chromosome 10q24 might be a susceptibility gene increasing the risk for a PD-like phenotype.

The region of chromosome 10 identified in this study was also one of the four regions reported by DeStefano *et al.* (50) as linked to PD in their sample of 113 affected sibling pairs. Furthermore, this region of chromosome 10 has also been linked to late-onset Alzheimer disease (54,55). The predominant theory behind the pathogenesis of PD and Alzheimer disease is the toxicity resulting from the aggregation of alpha-synuclein and a-beta, respectively (56). It has also been shown that the aggregation of alpha-synuclein (previously known as

the precursor protein of the non-amyloid beta-A4 protein) can lead to the aggregation of a-beta (57). Therefore, the coincident linkage of two different neurodegenerative diseases to the same region of chromosome 10 suggests that a gene in a common pathway may be a putative susceptibility gene.

We previously reported significant linkage under an autosomal dominant model of disease inheritance to chromosome 2q36–37 using kindreds with verified PD that had a stronger family history of PD (i.e. four or more affected family members or an affected parent). In our expanded sample reported herein, 85 families meet this rigorous criteria, and most of the linkage evidence to 2q36–37 continues to be derived from these individuals (LOD = 4.9; $P = 0.001$). This suggests that these

families might be segregating a Mendelian form of PD or a highly penetrant susceptibility gene. Interestingly, when these 85 families were employed in a genome screen to detect epistatic loci, only one region, on chromosome 14, produced suggestive linkage ($\text{LOD} \geq 2.2$). Modest evidence of linkage has also been reported for chromosome 14 by Hicks *et al.* (42) and Scott *et al.* (51).

If the putative gene on chromosome 2q represents a Mendelian form of PD, removal of these families may increase the power to detect susceptibility genes with smaller effects that increase the risk for common, idiopathic Parkinson disease. When we performed the genome screen using the remaining 277 families with weaker family history of PD, the maximum LOD score on the X chromosome increased to 3.2 and the LOD score on chromosome 10 increased to 2.4. Consistent with the results of the genome screen in the strong family history positive families, these analyses would suggest that the putative genes in these two regions do not directly interact with the chromosome 2q locus.

The genome screen study of Scott *et al.* (51) had its most significant linkage finding on chromosome 17, near the *tau* gene. The strength of the 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, they considered this potential phenotypic heterogeneity to be indicative of genotypic heterogeneity. Recent studies have also found that alpha-synuclein and *tau* proteins polymerize to amyloid fibrils, which subsequently form intraneuronal inclusions typically found in many neurodegenerative diseases (58). Unlike our previous report wherein we found minimal evidence of linkage to the *tau* gene, ($\text{LOD} = 0.0\text{--}0.2$) (43), in the larger sample of 362 families, we obtained a LOD score of 0.80 at the *tau* locus. Therefore, we sequenced the *tau* gene in five families from our sample that had a family-specific LOD score >0.25 under an autosomal dominant model with a marker near the gene. No mutations were identified.

The two chromosomal regions identified as having strong evidence of linkage in our previous sample continue to show strong evidence of linkage in this expanded sample of 425 affected sibling pairs. The findings on chromosomes 2 and X suggest the existence of loci that contribute to PD susceptibility. An important advantage of our study was the identification of families with *parkin* mutations prior to the genome screen analyses and the ability to stratify the sample using the *parkin* mutation positive families and the families with a stronger family history of PD that link strongly to chromosome 2q. This has reduced genetic heterogeneity in our sample and allowed us to detect possible epistatic interactions to regions on chromosomes 10 and 14. Genetic modeling that incorporates epistatic interactions will probably prove essential for the identification of the PD susceptibility loci in each chromosomal region. We continue to recruit families with multiple living members diagnosed with PD so as to narrow the chromosomal regions identified in these analyses and identify the putative PD genes. It is hoped that the elucidation of PD susceptibility genes may allow the early identification of individuals at high risk of PD and may lead to improved pharmacologic treatment for affected individuals.

MATERIALS AND METHODS

Subjects

Families consisting of at least one pair of living siblings diagnosed with PD were recruited through 59 Parkinson Study Group (PSG) sites located throughout North America. All study participants completed a uniform clinical evaluation (UPDRS) (59) and a Diagnostic Checklist (20,43). Responses on the Diagnostic Checklist were then used to classify study subjects as having verified PD (542 subjects) or non-verified PD (238 subjects). Thus, individuals classified as non-verified PD had clinical symptoms similar to PD, but when examined either failed to meet all inclusion criteria or fulfilled at least one of the exclusion criteria. Peripheral blood was obtained from all individuals after appropriate written informed consent approved by each individual institution's IRB was completed. The sample was primarily Caucasian (94%), although Hispanics (5%) also participated.

One of the advantages of our study was the use of the Diagnostic Checklist for the classification of disease status, since using stringent diagnostic criteria is essential for the successful identification of PD susceptibility genes. The high inter-rater and inter-site reliability of the diagnostic instrument provided further reassurance that error in diagnosis was kept to a minimum (60). Autopsies have been completed and a report generated for seven study participants. These included three individuals who, based on their clinical evaluation, had been classified as verified PD, with an autopsy confirming this diagnosis. However, three of the four individuals classified as non-verified PD also had pathological findings consistent with PD. Thus, it is likely that some of the individuals who are classified as non-verified PD based on the inclusion and criteria of the Diagnostic Checklist do in fact have PD, thus providing further rationale for using the broader disease definition in genetic analyses designed to identify PD susceptibility genes.

Parkin screening

As previously described, a marker in intron 7 of the *parkin* gene (D6S305) was genotyped in all study subjects. Families with positive LOD scores at this marker under either an autosomal dominant or autosomal recessive model of disease inheritance ($n = 124$) and families with an affected family member with an age at onset at or before 50 years ($n = 92$) were screened for *parkin* mutations using both direct sequencing and fluorescent dosage analysis (22). A total of 31 different *parkin* mutations were identified in 39 of the 173 families analyzed. Linkage analyses were performed with and without the families with a *parkin* mutation.

Genotyping

A genome screen using 400 dinucleotide markers from the ABI Prism Linkage Mapping Set (Applied Biosystems, Foster City, CA, USA) was completed as described previously (43). All genotypic data were evaluated for Mendelian inheritance of marker alleles with the program Pedcheck (61) and the marker genotypic data were used to verify the full sibling relationships among the subjects using the computer program RELATIVE

(62). Four half-sibling pairs were eliminated from further analyses due to significantly lower sharing of marker alleles identical by descent (IBD) than would be expected for full siblings.

Statistical analysis

Multipoint non-parametric linkage analysis was performed for both models of affection status using the maximum likelihood method implemented in the computer program Mapmaker/SIBS (49). Allele frequencies for all analyses were estimated from the full PD cohort of 1508 chromosomes. Analyses were performed employing all possible sibling pairs from families of size greater than two and with both dominance variance free to vary and fixed at zero. Holmans (63) has shown that analyzing affected sibling pair data under the assumption of dominance variance, when Holmans' 'possible triangle' is applied, appears to allow for a more sensitive test for putative genes acting in a recessive fashion than when dominance variance is fixed at zero.

Linkage analyses were initially performed using only the 194 families in which two or more affected individuals met the strict criteria for verified PD (model I). Subsequently, the genome screen was performed using the larger sample of 362 families in which all affected individuals were employed (model II). Thus, model II includes some families consisting of only individuals meeting the stricter disease definition, and other families in which some or all of the affected individuals meet only the broader disease classification. A genome screen using the same methodology was performed on the subset of the population with at least one mutant *parkin* allele, and was repeated for those with only one mutant allele. Since the linkage to chromosome 2q was shown to be due primarily to families with a more extensive family history of PD, a genome screen was also performed in this subset of 85 families, as well as in the 277 families with a weaker family history of PD. A strong family history was defined as a sibling pair with verified PD that had either an affected parent or a minimum of four affected individuals in the family.

All *P*-values were obtained empirically via simulations of the null hypothesis of no linkage. For each subset of the data, 2000 replicates were simulated with Allegro (64) using the same family structures, map distances and allele frequencies from the experimental data. Genome-wide significance of observed maximum LOD scores was obtained from sampling all points in the simulated data sets.

Tau screening

A marker near the gene encoding *tau* (D17S1868) was genotyped in all study subjects. The five families exceeding an autosomal dominant LOD score of 0.25 were screened for mutations in *MAPT* (the gene encoding *tau*). Exons 1–5, 7 and 9–13 were PCR amplified and directly sequenced. Primers from the intronic sequences surrounding all the exons were used so that the entire exon sequence and the splice signals could be analyzed (65). Standard amplification reactions were done with 50 ng of genomic DNA. The amplified products were gel purified using the Qiaquick gel purification kit (Qiagen, Valencia, CA, USA) and asymmetric amplification using the

DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA, USA) was performed. The amplified products were precipitated and resuspended in sample loading solution and loaded onto a CEQ 200XL DNA Analysis System (Beckman Coulter). Sequences were compared to the published *MAPT* sequence.

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PARKINSON STUDY GROUP INVESTIGATORS

Steering Committee: Lawrence Golbe, MD, UMDNJ Robert Wood Johnson Medical Center, New Brunswick, NJ; William Koller, MD, University of Miami, Miami, FL; Kelly Lyons, PhD, University of Miami, Miami, FL; Karen Marder, MD, Columbia-Presbyterian Medical Center, New York, NY; Frederick Marshall, MD, University of Rochester, Rochester, NY; David Oakes, PhD, University of Rochester, Rochester, NY; Alice Rudolph, PhD, University of Rochester, Rochester, NY; Clifford W. Shults, MD, University of California San Diego, San Diego, CA; Aileen Shinaman, JD, University of Rochester, Rochester, NY; Eric Siemers, MD, Eli Lilly & Company, Indianapolis, IN.

Participating investigators and coordinators: Joanne Wojcieszek, MD, Jo Belden, RN; Indiana University School of Medicine, Indianapolis, IN; Julie Carter, RN, MN, ANP, Richard Camicioli, MD, Pam Andrews, RN, Oregon Health and Science University, Portland, OR; Michel Panisset, MD, Jean Hall, RN, McGill Centre for Studies in Aging, Verdun, Quebec; Jean Hubble, MD, Magali Fernandez, MD, Carson Reider, PhD, Ohio State University, Columbus, OH; Ali Rajput, MD, Alex Rajput, MD, Theresa Shirley, RN, Saskatoon Health District Board, Saskatoon, Saskatchewan; Tilak Mendis, MD, David A Grimes, MD, Peggy Gray, RN, BSCN, Ottawa Civic Hospital, Ottawa, Ontario; Carmen Serrano Ramos, MD, Sandra Roque, RN, University of Puerto Rico School of Medicine, San Juan, Puerto Rico; Ronald Pfeiffer, MD, Brenda Pfeiffer, RN, BSN, University of Tennessee-Memphis, Memphis, TN; Lawrence Elmer, MD, PhD, Kathy Davis, RN, MSN, Medical College of Ohio, Toledo, OH; Joseph Friedman, MD, Hubert Fernandez, MD, Margaret Lannon, RN, MS, Brown University, Pawtucket, RI; Stephen Reich, MD, Becky Dunlop, RN, Johns Hopkins University, Baltimore, MD; Lauren Seeberger, MD, Christopher O'Brien, MD, Deborah Judd, RN, Colorado Neurological Institute, Englewood, CO; Robert Hauser, MD, Theresa Zesiewicz, MD, Holly Delgado, RN, University of South Florida, Tampa, FL; Cliff Shults, MD, Deborah Fontaine, RNC, GNP, University of California San Diego, San Diego, CA; Danna Jennings, MD, Kenneth Marek, MD, Susan Mendick, MPH, Institute for Neurodegenerative Disorders, New Haven, CT; Michael Aminoff, MD, FRCP, Mariann DiMinno, University of California San Francisco, San Francisco, CA; Peter Lewitt, MD, Maryan DeAngelis, RN, Clinical Neuroscience Center, West Bloomfield, MI; Rajesh Pahwa, MD, Stephanie Thomas, LPN, Kansas University

Medical Center, Kansas City, KS; Daniel Truong, MD, Mayank Pathak, MD, An Tran, RN, Parkinson's and Movement Disorder Institute, Fountain Valley, CA; Robert Rodnitzky, MD, Judith Dobson, RN, University of Iowa, Iowa City, IA; William Koller, MD, William Weiner, MD, Kelly Lyons, PhD, University of Miami, Miami, FL; Roger Kurlan, MD, Debra Berry, MSN, NP University of Rochester, Rochester, NY; John Bertoni, MD, PhD, Carolyn Peterson, RN, Creighton University, Omaha, NE; Wayne Martin, MD, Marguerite Wieler, BSC, PT, University of Alberta, Edmonton, Alberta; Paul Tuite, MD, Robyn Schacherer, RN, University of Minnesota Fairview University Medical Center, Minneapolis, MN; Karen Marder, MD, Juliette Harris, MS PhD, Columbia-Presbyterian Medical Center, New York, NY; Joseph Jankovic, MD, Christine Hunter, RN, Baylor College of Medicine, Houston, TX; Anthony Lang, MD, Galit Kleimer-Fisman, MD, Anette Nieves, MD, Julie So, RN, Toronto Western Hospital, Toronto, Ontario; Stewart Factor, DO, Sharon Evans, LPN, Albany Medical College, Albany, NY; Bala Manyam, MD, Brian Wulbrecht, Scott and White Hospital/Texas A&M University, Temple, TX; Francis Walker, MD, Victoria Hunt, RN, Wake Forest University School of Medicine, Winston-Salem, NC; Mark F. Gordon, MD, Joanna Hamman, Long Island Jewish Medical Center, New Hyde Park, NY; Un Jung Kang, MD, Joan Young, University of Chicago, Chicago, IL; Karen Blindauer, MD, Jeannine Petit, CNRN, GNPC, Medical College of Wisconsin, Milwaukee, WI; Jayaraman Rao, MD, Maureen Cook, RN, BSN, LSU Medical Center, New Orleans, LA; Mark Stacy, MD, Kelli Williamson, RN, Barrow Neurological Institute, Phoenix, AZ; Rachel Saunders Pullman, MD, Karyn Boyar, RN.C, MS, FNP, Beth Israel Medical Center, New York, NY; Maureen Leehey, MD, Theresa Derian, RN, University of Colorado Health Sciences Center, Denver, CO; Paul Gordon, MD, Joan Werner, University of New Mexico, Albuquerque, NM; Brad Racette, MD, Laura Good, RN, Washington University, St Louis, MO; David Simon, MD, Lisa Scollins, RN, Beth Israel Deaconess Medical Center, Boston, MA; Arif Dalvi, MD, Donna Schwieterman, MA, CCRC, University of Cincinnati Medical Center, Cincinnati, OH; Richard Dewey, MD, Melinda Meacham, RN, BSN, UT Southwestern Medical Center, Dallas, TX; James Sutton, MD, Brad Hutchinson, California Medical Clinic for Movement Disorders, Oxnard, CA; Mandar Jog, MD, PhD, Cheryl Horn, London Health Sciences Center, London, Ontario; Kapil Sethi, MD, Joan Carpenter, RN, Medical College of Georgia, Augusta, GA; Paul Atchison, MD, Susan Rolli, RN, University of Alabama Birmingham, Birmingham, AL; Lewis Sudarsky, MD, Claire Corwin, PAC, BS, Brigham and Women's Hospital, Boston, MA; Miodrag Velickovic, MD, Sabrina Phipps, Mount Sinai Medical Center, New York, NY; Tanya Simuni, MD, Annette Kaczmarek, Northwestern University Medical School, Chicago, IL; Neal Hermanowicz, MD, Shari Niswonger, RN, University of California Irvine, Irvine, CA; Andrew Feigin, MD, Barbara Shannon, RN, North Shore University Hospital, Manhasset, NY; Vincent Calabrese, MD, Peggy Roberge, RN, Hunter Homes McGuire Veterans Medical Center, Richmond, VA; Lisa Shulman, MD, Kelly Dustin, RN, University of Maryland School of Medicine, Baltimore, MD; Todd Ajax, MD, Janet Manner, RN, McFarland Clinic, Mary Greely Hospital,

Ames, IA; G.David Podskalny, MD, Lisa Giffin, LPN, UMDNJ School of Osteopathic Medicine, Stratford, NJ; Ryan Uitti, MD, Margaret Foster Turk, RN, Mayo Clinic Jacksonville, Jacksonville, FL.

Biostatistics and clinical trials coordination centers staff: David Oakes PhD, Arthur Watts BS, Antai Wang MA, Tori Ross, MA, Susan Bennett, AAS, Elaine Julian-Baros, Susan Daigneault, University of Rochester, Rochester, NY.

REFERENCES

- de Rijk, M.C., Tzourio, C., Breteler, M.M., Dartigues, J.F., Amaducci, L., Lopez-Pousa, S., Manubens-Bertran, J.M., Alperovitch, A. and Rocca, W.A. (1997) Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON Collaborative Study. European Community Concerted Action on the Epidemiology of Parkinson's disease. *J. Neurol. Neurosurg. Psychiat.*, **62**, 10–15.
- Gasser, T. (2001) Genetics of Parkinson's disease. *J. Neurol.*, **248**, 833–840.
- Fearnley, J.M. and Lees, A.J. (1991) Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain*, **114** (Pt 5), 2283–2301.
- Braak, H. and Braak, E. (2000) Pathoanatomy of Parkinson's disease. *J. Neurol.*, **247** (Suppl. 2), I13–10.
- Kuopio, A., Marttila, R.J., Helenius, H. and Rinne, U.K. (2001) Familial occurrence of Parkinson's disease in a community-based case-control study. *Parkinsonism Relat. Disord.*, **7**, 297–303.
- Payami, H., Larsen, K., Bernard, S. and Nutt, J. (1994) Increased risk of Parkinson's disease in parents and siblings of patients. *Ann. Neurol.*, **36**, 659–661.
- Autere, J.M., Moilanen, J.S., Myllyla, V.V. and Majamaa, K. (2000) Familial aggregation of Parkinson's disease in a Finnish population. *J. Neurol. Neurosurg. Psychiat.*, **69**, 107–109.
- Sveinbjornsdottir, S., Hicks, A.A., Jonsson, T., Petursson, H., Guggmundsson, G., Frigge, M.L., Kong, A., Gulcher, J.R. and Stefansson, K. (2000) Familial aggregation of Parkinson's disease in Iceland. *New Engl. J. Med.*, **343**, 1765–1770.
- Preux, P.M., Condet, A., Anglade, C., Druet-Cabanac, M., Debrock, C., Macharia, W., Couratier, P., Boutros-Toni, F. and Dumas, M. (2000) Parkinson's disease and environmental factors. Matched case-control study in the Limousin region, France. *Neuroepidemiology*, **19**, 333–337.
- Elbaz, A., Grigoletto, F., Baldereschi, M., Breteler, M.M., Manubens-Bertran, J.M., Lopez-Pousa, S., Dartigues, J.F., Alperovitch, A., Tzourio, C. and Rocca, W.A. (1999) Familial aggregation of Parkinson's disease: a population-based case-control study in Europe. EUROPARKINSON Study Group. *Neurology*, **52**, 1876–1882.
- Uitti, R.J., Shintoh, H., Hayward, M., Schulzer, M., Mak, E. and Calne, D.B. (1997) 'Familial Parkinson's disease'—a case-control study of families. *Can. J. Neurol. Sci.*, **24**, 127–132.
- De Michele, G., Filla, A., Marconi, R., Volpe, G., D'Alessio, A., Scala, R., Ambrosio, G. and Campanella, G. (1995) A genetic study of Parkinson's disease. *J. Neural Transm. Suppl.*, **45**, 21–25.
- Vierregge, P. (1994) Genetic factors in the etiology of idiopathic Parkinson's disease. *J. Neural Transm. Park. Dis. Dement. Sect.*, **8**, 1–37.
- Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R. *et al.* (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science*, **276**, 2045–2047.
- Pastor, P., Munoz, E., Ezquerro, M., Obach, V., Marti, M.J., Valldeoriola, F., Tolosa, E. and Oliva, R. (2001) Analysis of the coding and the 5' flanking regions of the alpha-synuclein gene in patients with Parkinson's disease. *Mov. Disord.*, **16**, 1115–1119.
- Chan, P., Jiang, X., Forno, L.S., Di Monte, D.A., Tanner, C.M. and Langston, J.W. (1998) Absence of mutations in the coding region of the alpha-synuclein gene in pathologically proven Parkinson's disease. *Neurology*, **50**, 1136–1137.
- Farrer, M., Wavrant-De Vrieze, F., Crook, R., Boles, L., Perez-Tur, J., Hardy, J., Johnson, W.G., Steele, J., Maraganore, D., Gwinn, K. *et al.* (1998) Low frequency of alpha-synuclein mutations in familial Parkinson's disease. *Ann. Neurol.*, **43**, 394–397.

18. Vaughan, J.R., Farrer, M.J., Wszolek, Z.K., Gasser, T., Durr, A., Agid, Y., Bonifati, V., DeMichele, G., Volpe, G., Lincoln, S. *et al.* (1998) Sequencing of the alpha-synuclein gene in a large series of cases of familial Parkinson's disease fails to reveal any further mutations. The European Consortium on Genetic Susceptibility in Parkinson's Disease (GSPD). *Hum. Mol. Genet.*, **7**, 751–753.
19. Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y. and Shimizu, N. (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*, **392**, 605–608.
20. Foroud, T., Uniacke, S.K., Liu, L., Pankratz, N., Rudolph, A., Halter, C., Shults, C., Marder, K., Conneally, P.M., Nichols, W.C. and the Parkinson Study Group (2003) Heterozygosity for a mutation in the parkin gene leads to later onset Parkinson disease. *Neurology*, **60**, 796–801.
21. Oliveira, S.A., Scott, W.K., Martin, E.R., Nance, M.A., Watts, R.L., Hubble, J.P., Koller, W.C., Pahwa, R., Stern, M.B., Hiner, B.C. *et al.* (2003) Parkin mutations and susceptibility alleles in late-onset Parkinson's disease. *Ann. Neurol.*, **53**, 624–629.
22. Nichols, W.C., Pankratz, N., Uniacke, S.K., Pauculo, M.W., Halter, C., Rudolph, A., Conneally, P.M. and Foroud, T. (2002) Linkage stratification and mutation analysis at the parkin locus identifies mutation positive Parkinson's disease families. *J. Med. Genet.*, **39**, 489–492.
23. Kann, M., Jacobs, H., Mohrmann, K., Schumacher, K., Hedrich, K., Garrels, J., Wieggers, K., Schwinger, E., Pramstaller, P.P., Brakefield, X.O. *et al.* (2002) Role of parkin mutations in 111 community-based patients with early-onset parkinsonism. *Ann. Neurol.*, **51**, 621–625.
24. West, A., Periquet, M., Lincoln, S., Lucking, C.B., Nicholl, D., Bonifati, V., Rawal, N., Gasser, T., Lohmann, E., Deleuze, J.F. *et al.* (2002) Complex relationship between Parkin mutations and Parkinson disease. *Am. J. Med. Genet.*, **114**, 584–591.
25. Hedrich, K., Marder, K., Harris, J., Kann, M., Lynch, T., Meija-Santana, H., Pramstaller, P.P., Schwinger, E., Bressman, S.B., Fahn, S. *et al.* (2002) Evaluation of 50 probands with early-onset Parkinson's disease for Parkin mutations. *Neurology*, **58**, 1239–1246.
26. Lucking, C.B., Durr, A., Bonifati, V., Vaughan, J., De Michele, G., Gasser, T., Harhangi, B.S., Meco, G., Deneffe, P., Wood, N.W. *et al.* (2000) Association between early-onset Parkinson's disease and mutations in the parkin gene. French Parkinson's Disease Genetics Study Group. *New Engl. J. Med.*, **342**, 1560–1567.
27. Abbas, N., Lucking, C.B., Ricard, S., Durr, A., Bonifati, V., De Michele, G., Bouley, S., Vaughan, J.R., Gasser, T., Marconi, R. *et al.* (1999) A wide variety of mutations in the parkin gene are responsible for autosomal recessive parkinsonism in Europe. French Parkinson's Disease Genetics Study Group and the European Consortium on Genetic Susceptibility in Parkinson's Disease. *Hum. Mol. Genet.*, **8**, 567–574.
28. Lucking, C.B., Abbas, N., Durr, A., Bonifati, V., Bonnet, A.M., de Broucker, T., De Michele, G., Wood, N.W., Agid, Y. and Brice, A. (1998) Homozygous deletions in parkin gene in European and North African families with autosomal recessive juvenile parkinsonism. The European Consortium on Genetic Susceptibility in Parkinson's Disease and the French Parkinson's Disease Genetics Study Group. *Lancet*, **352**, 1355–1356.
29. Wintermeyer, P., Kruger, R., Kuhn, W., Muller, T., Voitalla, D., Berg, D., Becker, G., Leroy, E., Polymeropoulos, M., Berger, K. *et al.* (2000) Mutation analysis and association studies of the UCHL1 gene in German Parkinson's disease patients. *Neuroreport*, **11**, 2079–2082.
30. Zhang, J., Hattori, N., Giladi, N. and Mizuno, Y. (2000) Failure to find mutations in ubiquitin carboxy-terminal hydrolase L1 gene in familial Parkinson's disease. *Parkinsonism Relat. Disord.*, **6**, 199–200.
31. Harhangi, B.S., Farrer, M.J., Lincoln, S., Bonifati, V., Meco, G., De Michele, G., Brice, A., Durr, A., Martinez, M., Gasser, T. *et al.* (1999) The Ile93Met mutation in the ubiquitin carboxy-terminal-hydrolase-L1 gene is not observed in European cases with familial Parkinson's disease. *Neurosci. Lett.*, **270**, 1–4.
32. Bonifati, V., Rizzu, P., van Baren, M.J., Schaap, O., Breedveld, G.J., Krieger, E., Dekker, M.C., Squitieri, F., Ibanez, P., Joosse, M. *et al.* (2003) Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science*, **299**, 256–259.
33. Gasser, T., Muller-Myhsok, B., Wszolek, Z.K., Oehlmann, R., Calne, D.B., Bonifati, V., Bereznoi, B., Fabrizio, E., Vieregge, P. and Horstmann, R.D. (1998) A susceptibility locus for Parkinson's disease maps to chromosome 2p13. *Nat. Genet.*, **18**, 262–265.
34. Farrer, M., Gwinn-Hardy, K., Muentner, M., DeVrieze, F.W., Crook, R., Perez-Tur, J., Lincoln, S., Maraganore, D., Adler, C., Newman, S. *et al.* (1999) A chromosome 4p haplotype segregating with Parkinson's disease and postural tremor. *Hum. Mol. Genet.*, **8**, 81–85.
35. Valente, E.M., Bentivoglio, A.R., Dixon, P.H., Ferraris, A., Ialongo, T., Frontali, M., Albanese, A. and Wood, N.W. (2001) Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35–p36. *Am. J. Hum. Genet.*, **68**, 895–900.
36. Funayama, M., Hasegawa, K., Kowa, H., Saito, M., Tsuji, S. and Obata, F. (2002) A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2–q13.1. *Ann. Neurol.*, **51**, 296–301.
37. Xu, P.Y., Liang, R., Jankovic, J., Hunter, C., Zeng, Y.X., Ashizawa, T., Lai, D., and Le, W.D. (2002) Association of homozygous 7048G7049 variant in the intron six of Nurr1 gene with Parkinson's disease. *Neurology*, **58**, 881–884.
38. Zheng, K., Heydari, B. and Simon, D.K. (2003) A Common NURR1 Polymorphism associated with Parkinson disease and diffuse Lewy body disease. *Arch. Neurol.*, **60**, 722–725.
39. Le, W.D., Xu, P., Jankovic, J., Jiang, H., Appel, S.H., Smith, R.G. and Vassilatis, D.K. (2003) Mutations in NR4A2 associated with familial Parkinson disease. *Nat. Genet.*, **33**, 85–89.
40. van der Walt, J.M., Nicodemus, K.K., Martin, E.R., Scott, W.K., Nance, M.A., Watts, R.L., Hubble, J.P., Haines, J.L., Koller, W.C., Lyons, K. *et al.* (2003) Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. *Am. J. Hum. Genet.*, **72**, 804–811.
41. Marx, F.P., Holzmann, C., Strauss, K.M., Li, L., Eberhardt, O., Gerhardt, E., Cookson, M.R., Hernandez, D., Farrer, M.J., Kachergus, J. *et al.* (2003) Identification and functional characterization of a novel R621C mutation in the synphilin-1 gene in Parkinson's disease. *Hum. Mol. Genet.*, **12**, 1223–1231.
42. Hicks, A.A., Petursson, H., Jonsson, T., Stefansson, H., Johannsdottir, H.S., Sainz, J., Frigge, M.L., Kong, A., Gulcher, J.R., Stefansson, K. *et al.* (2002) A susceptibility gene for late-onset idiopathic Parkinson's disease. *Ann. Neurol.*, **52**, 549–555.
43. Pankratz, N., Nichols, W.C., Uniacke, S.K., Halter, C., Rudolph, A., Shults, C., Conneally, P.M. and Foroud, T. (2002) Genome screen to identify susceptibility genes for Parkinson disease in a sample without parkin mutations. *Am. J. Hum. Genet.*, **71**, 124–135.
44. Pankratz, N., Nichols, W.C., Uniacke, S.K., Halter, C., Rudolph, A., Shults, C., Conneally, P.M. and Foroud, T. (2003) Significant linkage of Parkinson disease to chromosome 2q36–36. *Am. J. Hum. Genet.*, **72**, 1053.
45. Klein, C., Pramstaller, P.P., Kis, B., Page, C.C., Kann, M., Leung, J., Woodward, H., Castellan, C.C., Scherer, M., Vieregge, P. *et al.* (2000) Parkin deletions in a family with adult-onset, tremor-dominant parkinsonism: expanding the phenotype. *Ann. Neurol.*, **48**, 65–71.
46. Lucking, C.B., Bonifati, V., Periquet, M., Vanacore, N., Brice, A. and Meco, G. (2001) Pseudo-dominant inheritance and exon 2 triplication in afamily with parkin gene mutations. *Neurology*, **57**, 924–927.
47. Farrer, M., Chan, P., Chen, R., Tan, L., Lincoln, S., Hernandez, D., Forno, L., Gwinn-Hardy, K., Petrucelli, L., Hussey, J. *et al.* (2001) Lewy bodies and parkinsonism in families with parkin mutations. *Ann. Neurol.*, **50**, 293–300.
48. Hedrich, K., Kann, M., Lanthaler, A.J., Dalski, A., Eskelson, C., Landt, O., Schwinger, E., Vieregge, P., Lang, A.E., Brakefield, X.O. *et al.* (2001) The importance of gene dosage studies: mutational analysis of the parkin gene in early-onset parkinsonism. *Hum. Mol. Genet.*, **10**, 1649–1656.
49. Lander, E. and Kruglyak, L. (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.*, **11**, 241–247.
50. DeStefano, A.L., Golbe, L.I., Mark, M.H., Lazzarini, A.M., Maher, N.E., Saint-Hilaire, M., Feldman, R.G., Guttman, M., Watts, R.L., Suchowersky, O. *et al.* (2001) Genome-wide scan for Parkinson's disease: the GenePD Study. *Neurology*, **57**, 1124–1126.
51. Scott, W.K., Nance, M.A., Watts, R.L., Hubble, J.P., Koller, W.C., Lyons, K., Pahwa, R., Stern, M.B., Colcher, A., Hiner, B.C. *et al.* (2001) Complete genomic screen in Parkinson disease: evidence for multiple genes. *JAMA*, **286**, 2239–2244.
52. Tanner, C.M. (1992) Epidemiology of Parkinson's disease. *Neurol. Clin.*, **10**, 317–329.
53. Lee, L.V., Munoz, E.L., Tan, K.T. and Reyes, M.T. (2001) Sex linked recessive dystonia parkinsonism of Panay, Philippines (XDP). *Mol. Pathol.*, **54**, 362–368.

54. Myers, A., Holmans, P., Marshall, H., Kwon, J., Meyer, D., Ramic, D., Shears, S., Booth, J., DeVrieze, F.W., Crook, R. *et al.* (2000) Susceptibility locus for Alzheimer's disease on chromosome 10. *Science*, **290**, 2304–2305.
55. Ertekin-Taner, N., Graff-Radford, N., Younkin, L.H., Eckman, C., Baker, M., Adamson, J., Ronald, J., Blangero, J., Hutton, M. and Younkin, S.G. (2000) Linkage of plasma Aβ₄₂ to a quantitative locus on chromosome 10 in late-onset Alzheimer's disease pedigrees. *Science*, **290**, 2303–2304.
56. Mouradian, M.M. (2002) Recent advances in the genetics and pathogenesis of Parkinson disease. *Neurology*, **58**, 179–185.
57. Yoshimoto, M., Iwai, A., Kang, D., Otero, D.A., Xia, Y. and Saitoh, T. (1995) NACP, the precursor protein of the non-amyloid β/A4 protein (A β) component of Alzheimer disease amyloid, binds A β and stimulates A β aggregation. *Proc. Natl Acad. Sci. USA*, **92**, 9141–9145.
58. Giasson, B.I., Forman, M.S., Higuchi, M., Golbe, L.I., Graves, C.L., Kotzbauer, P.T., Trojanowski, J.Q. and Lee, V.M. (2003) Initiation and synergistic fibrillization of tau and alpha-synuclein. *Science*, **300**, 636–640.
59. Lang, A.E. and Fahn, S. (1989) In Munsat, T.L. (ed.), *Quantification of Neurologic Deficit*. Butterworth, Boston, MA, pp. 285–309.
60. Siemers, E.R., Hubble, J., Tuite, P., Comella, C., Kompoliti, K., Oakes, D., Wojcieszek, J., Foroud, T. and Conneally, P.M. (1998) Clinical diagnostic criteria for idiopathic Parkinson's disease. *Mov. Disord.*, **13**, 862.
61. O'Connell, J.R. and Weeks, D.E. (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am. J. Hum. Genet.*, **63**, 259–266.
62. Goring, H.H. and Ott, J. (1997) Relationship estimation in affected sib pair analysis of late-onset diseases. *Eur. J. Hum. Genet.*, **5**, 69–77.
63. Holmans, P. (1993) Asymptotic properties of affected-sib-pair linkage analysis. *Am. J. Hum. Genet.*, **52**, 362–374.
64. Gudbjartsson, D.F., Jonasson, K., Frigge, M.L. and Kong, A. (2000) Allegro, a new computer program for multipoint linkage analysis. *Nat. Genet.*, **25**, 12–13.
65. Murrell, J.R., Spillantini, M.G., Zolo, P., Guazzelli, M., Smith, M.J., Hasegawa, M., Redi, F., Crowther, R.A., Pietrini, P., Ghetti, B. *et al.* (1999) Tau gene mutation G389R causes a tauopathy with abundant pick body-like inclusions and axonal deposits. *J. Neuropathol. Exp. Neurol.*, **58**, 1207–1226.