Genetic Complexity and Parkinson's Disease

Mihai H. Polymeropoulos et al. describe the genetic linkage of a large Parkinson's disease (PD) pedigree to chromosome 4q21-q23 (1). In this study, which affirms a long-hypothesized genetic component to the disease, linkage was detected in a single large family with the use of an autosomal dominant model with 99% penetrance of the disease trait. The clinical presentation in this family, however, may differ from typical idiopathic PD because of the apparent autosomal dominant transmission, early onset, rapid course, and less frequent occurrence of tremor as a significant symptom (2). Thus, it is unclear whether the putative PD locus identified by Polymeropoulos et al. (which they termed PD1) is responsible for the majority of familial idiopathic PD cases.

As part of an ongoing multicenter study of the genetics of idiopathic PD, we have ascertained 94 Caucasian families (a total of 213 affected relatives sampled: 108 affected sibpairs and 31 affected relative pairs) with at least two individuals in each family meeting clinical criteria for idiopathic PD (3). We have identified approximately 200 multiplex idiopathic PD families to ascertain for a genomic screen. The 94 families discussed here were those completely ascertained, with DNA sampled, at the time of the analysis. Linkage analysis of chromosome 4q21-q23 markers in these idiopathic PD families did not reveal evidence for linkage of an autosomal dominant, highly penetrant gene, as was described by Polymeropoulos et al. (1, 4). We determined two-point log odds (lod) scores, with the use of the model of Polymeropoulos et al. as well as a low penetrance "affecteds-only" autosomal dominant model. These lod scores were strongly negative for markers D4S2623, D4S2409, D4S3280, D4S1647, and D4S2623. Multipoint analysis of the genetic map of chromosome 4q21-q23 supported these findings for both models, excluding the entire candidate region. We found no evidence for heterogeneity of either the two-point (P > 0.20) or multipoint (in likelihood = 1) lod scores (5).

Because the power of the parametric lod score method suffers when the genetic model is misspecified, we also used nonparametric analyses of affected relative pairs (6). With the nonparametric lod score analysis, we found no significant evidence for linkage using either two-point or multipoint analysis; in this data set, the multipoint location scores (MLS) exclude the entire 27.5 cM region for recurrence risks to siblings as low as 0.25 (Fig. 1). Because the pedigree analyzed by Polymeropoulos et al. contained many younger onset cases (mean age at onset of the disease was 46), we repeated our analysis in the 22 families with at least one affected individual with an onset earlier than age 45; the analysis in the subset supported the results from the full sample (7).

The absence of linkage to chromosome 4q21-q23 in our dataset indicates that there is genetic heterogeneity in PD. It is possible that the region identified by Polymeropoulos et al. harbors a disease locus responsible only for a rare autosomal dominant form of PD. Such a situation would be analogous to the genetics of Alzheimer's disease (AD), where mutations in the amyloid precursor protein and the presenilin 1 and presenilin 2 genes) that cause autosomal dominant AD are responsible for less than 2% of all cases (8). Therefore, although the report by Polymeropoulos et al. is a first step in unraveling the genetic etiology of PD, other independent genetic effects likely remain to be discovered.

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3. The families enrolled in this study were ascertained in the following manner. Each of the principal investigations of the 12 study sites identified idiopathic PD patients with one or more first-degree relatives with PD. All 94 families included in the analysis were responsive to levodopa. Specifically excluded were patients with a history of encephalitis, neuroleptic therapy within the year before diagnosis, evidence of
normal pressure hydrocephalus, or a clinical course with atypical features, suggesting secondary Parkinsonism. All first-degree relatives of these patients who consented to participate in this study were subsequently examined and queried regarding the above exclusion criteria and atypical clinical features. Each of these individuals was assigned a status based on their history and the number of the following clinical signs that were present: resting tremor, bradykinesia, and rigidity. Individuals were coded as “affected” if their examination demonstrated at least two of the signs and had no other etiologies for parkinsonism or atypical clinical features, “unclear” if they had only one sign but may have had a history of atypical clinical features, and “at risk” if they had no signs. Mean age at onset of PD symptoms in unaffected individuals was 61.4 years (SD, 13.1 years). Mean age at examination in affected individuals was 71.5 years (SD, 10.2 years) and in unclear or at risk individuals was 68.4 years (SD, 14.5 years).

4. Microsatellite markers spanning the region defined by Polymeropoulos et al. (1) for the analysis; the resulting genetic map was: D4S2361-7.7cM-D4S2409-5.3cM-D4S2380-4cM-D4S1647-10.5cM-D4S2623 (Cooperative Human Linkage Center, database is online at www.chic.org). Two-point and multipoint lod scores were calculated with the use of the VITESSE software package (J. R. O’Connell and D. E. Weeks, Nature Genet. 11, 402 (1995)), we assumed, as did Polymeropoulos et al., autosomal dominant inheritance, a disease allele frequency of 0.001, and 99% penetrance. A low penetrance, “affecteds only” analysis was also performed. Allele frequencies were estimated from 75 unrelated Caucasian controls.

5. Heterogeneity analysis of two-point and multipoint lod scores was performed using the admixture test, performed. Allele frequencies were estimated from 75 unrelated Caucasian controls.

6. Two-point affected-relative-pair analysis was performed using the Simian software package (S. Davis, M. Schroeder, L. R. Goldin, D. E. Weeks, Am. J. Hum. Genet. 58, 867 (1996)). Multipoint affected sibpair exclusion mapping was performed with the use of ASPEX (available from N. Risch, Stanford University, and based on N. Risch, Am. J. Hum. Genet. 46, 229 (1990)] with the use of all five microsatellite markers. Estimates of the recurrence risk to siblings (\(\lambda_s\)) range from 3 to 150, with the majority of studies supporting a \(\lambda_s\) between 10 and 20 [R. C. Duvoisin, Adv. Neurol. 60, 306 (1993); K. Kondo and K. Watanabe, ibid., p. 348]. Exclusion mapping with the recurrence risk set at the low end of this range determined that the region can be excluded for \(\lambda_s \geq 2.5\).

7. W. K. Scott et al., unpublished data.


9. The Deane Laboratory Parkinson’s Disease Research Group is a multicenter study of the genetics of idiopathic Parkinson Disease coordinated by the Center for Human Genetics, Duke University Medical Center. The collaborating sites and principal investigators are: University of Minnesota, M. Nance; Ohio State University, J. Hubides; University of Kansas Medical Center, W. Koller; University of Pennsylvania Graduate Neurological Center, M. Stern and A. Colcher; Emory University School of Medicine, R. L. Watts; Rush Presbyterian–St. Luke’s Hospital, C. Goetz and E. F. Pappert; Carolina Neurological Clinic, F. H. Allen Jr.; Bishop’s College of Medicine, J. Jankovic and W. Ondo; Marshall Clinic, B. C. Hiner; University of California, San Francisco, M. Aminoff and G. Dowling; University of California, Los Angeles, G. W. Small.

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Polymorphooupolous et al. present results of a genome-wide screen for genetic linkage in a large family with autosomal-dominantly inherited L-Dopa-responsive parkinsonism with Lewy-body pathology (1). They convincingly demonstrate linkage with polymorphic markers on chromosome 4q21-4q23, with a maximum two-point lod score of 6.00 for marker D4S2380. The locus was termed PD1. The role of the PD1 locus in other families with inherited parkinsonism and in sporadic PD remains to be investigated.

We have examined polymorphic markers closely linked to PD1 in 13 multigenetic families with inherited parkinsonism (Table 1). Affected members in all families exhibited at least two of the three cardinal clinical signs of PD (akinesia, rigidity, and resting tremor), as well as asymmetry at onset and a marked improvement on L-Dopa treatment. Rigorous exclusion criteria were applied (supranuclear ophthalmoplegia, cerebellar or pyramidal signs, and severe autonomic or postural disturbance within 2 years of onset). The wide range of age at onset and spectrum of clinical features, including the presence of dementia in addition to parkinsonism in some affected individuals, was similar to that observed in the family studied by Polymorphooupolous et al. (1). No additional neurologic deficit was inherited except for amyotrophy in one affected of family A. Multipoint analysis with eight polymorphic markers spanning the region from GATA 10G07 to D4S2623 excluded the entire 17 cM region likely to contain PD1 in five of the families (families A, B, C, D, and IT-1). In one additional family (G), the major portion of the critical region was also excluded, with lod scores between -1.9 and -2 for the remainder of
the interval (Fig. 1). Data from one (previously unpublished) family of southern Bavarian origin showed positive lod scores with a maximum multipoint score of 1.5 (family K, Fig. 1). This lod score is close to the theoretical maximum in this relatively small family.

In six families (FR-041, FR-722, FR-727, FR-755, UK-A, and UK-B), only the two polymorphic markers most closely linked to PD1 (D4S1647 and D4S2380) have been analysed. Obligate reconbinations (no allele shared by all affecteds) were observed in five of these families either for each of the markers individually (three families), or for the haplotype of both markers (two families), again strongly arguing against linkage with the PD1 locus. In one family (FR-041), a positive pairwise lod score was obtained for D4S2380 (0.29 at Theta = 0). Positive lod scores in families K and FR-041 may reflect true linkage, but they may also be a result of random fluctuations, because the relatively small size of these families precludes definite proof of linkage.

We conclude that mutations at the PD1 locus are probably a rare cause of autosomal-dominant parkinsonism. The role of the PD-1 gene in sporadic PD is still to be determined.

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**Technical Comments**

**Experiments in a Parkinson’s Rat Model**

Derek L. Choi-Lundberg et al. present evidence (1) that a replication-defective adeno-viral (Ad) vector that encodes human glial cell–line derived neurotoxic factor (GDNF) protects dopaminergic neurons in substantia nigra (SN) in rats from progressive degeneration induced by the neurotoxin 6-hydroxydopamine (6-OHDA) that has been injected into the striatum. These results are important because of possible applications of Ad vector–mediated GDNF gene therapy in patients with Parkinson’s disease. The experimental design used by Choi-Lundberg et al., however, raises some concerns.

Choi-Lundberg et al. (1) injected 6-OHDA into the striatum of rats 7 days after labeling SN neurons with the retrograde fluorescent tracer fluorogold (FG). Thus, the neurotoxin acted mainly on SN neurons that were loaded with FG. Because of neuronal death and membrane disruption, the fluorescent tracer diffused in the extracellular space, from where it might have been incorporated by other cells. That such an uptake of tracer really occurred in the experiment by Choi-Lundberg et al. is demonstrated by figure 2, C through G, in their report, showing that microglia and other non-neuronal cells in the SN have been labeled with FG. Similar to non-neuronal cells, SN neurons that survived the neurotoxin might have incorporated the tracer through their cell membranes (2).

To conclude, the finding (1) of a reduced loss of FG-labeled neurons in the SN of GDNF-treated rats does not necessarily imply a neuroprotective action of GDNF. A control in which the injection of FG is made after the complete or nearly complete degeneration of the SN neurons would seem to be necessary to definitely support the conclusions made by Choi-Lundberg et al.

**REFERENCES**


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Response: Scott et al. and Gasser et al. are discussing genetic studies of families with PD that are designed to examine whether a locus that we previously reported (1) on chromosome 4q21-q23 is operating in their sample. The results of Scott et al. in 94 Caucasian families do not demonstrate linkage even when the 22 families with earlier onset are examined separately. Similarly, Gasser et al. exclude linkage in 13 multigenerational families with Parkinson’s disease, with the exception of one family for which they achieved a maximum multipoint lod score of 1.5 for genetic markers in the 4q21-q23 region. Cumulatively, these comments suggest that the chromosome 4 locus will not account for the majority of familial Parkinson's disease and will be expected to operate only in a small percentage of families with the illness.

We have recently demonstrated that a mutation in the alpha synuclein gene is responsible for the phenotype in four families with early onset Parkinson’s disease (2). Because the mutation was not detected in 50 individuals with sporadic PD, or in two other families with late onset of the illness, we concluded that mutations in the alpha synuclein gene will not account for the majority of the genetic factors of PD, but rather for a proportion of those families with an early onset autosomal dominant form of the illness. These results are in agreement with the observation of Scott et al. and Gasser et al., and suggest that the understanding of genetic complexity of Parkinson’s disease is just beginning to take shape.

**REFERENCES**


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