Mihail 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 (PD) pedigree 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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Fig. 1. Multipoint exclusion map for chromosome 4q21-q23 markers. The multipoint lod scores (MLS) within the region are all less than -2.0 at \( \lambda = 2.5 \), excluding the entire candidate region identified by Polymeropoulos et al. (1). Arrows indicate chromosome markers.

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competitor DNA were reduced from 100 ng and 200 μg to 10 ng and 20 μg, respectively. The composition of buffer B was 1.2 M sorbitol and 10 mM KHPo4, at pH 7.5. After the second posthybridization wash in 40% formamide and 2× standard saline citrate (SSC), a 15-min wash at room temperature in 2× SSC and 0.1% Triton X-100 was done. The oligonucleotide probes were as follows: 5′-GCTGCGTGTGTAACGATGAAATCGGCCGCATGATGATATATCCTGTGTAATGCGGGGTTGTTAGAGGAATTCTGGGACCTGTTGTAATGCGAGACCTGATTGTGATGATACATGGGAAATGTTGGTGCATGATGACGAGAAGATTAACCTTGGGCTAGCTTTGTTGTGGTTGGTGGACT*CATCGGCGGTGT*GACGACCTAGT*CGATTCCAATT*CCTT*GC-3′. The probes were directly labeled with a Cy3 fluorochrome at amino-modified thymidine residues indicated by the asterisks (19). To detect poly(A) RNA, FISH was performed with T43 labeled with fluorescein isothiocyanate (20). In formamide-containing solutions, the concentration was reduced to 10% for poly(A) RNA detection. Images were taken with an Olympus IX70 inverted epifluorescence microscope and Oncor (Gaithersburg, MD) imaging software, version 2.0.5.

Strain K5552, which encodes an epitope-tagged version of Ash1p (Ash1p-myc9), was grown to midlogarithmic phase, fixed, and processed for simultaneous FISH and immunofluorescence. After FISH, immunofluorescence was performed as described previously (21) with the following alterations. Antibody to myc was diluted 1:5 into a solution of 1× phosphate-buffered saline, 0.1% bovine serum albumin, 20 mM vanadyl ribonucleoside complex, and ribonuclease inhibitor (40 U/ml). The secondary antibody, goat antibody to mouse immunoglobulin G, conjugated to dichlorotrianzyl amino fluorescein (Jackson Laboratories), was diluted 1:50 into the same solution.

Plasmid C3431 is a derivative of YEpplac195 (17) carrying a Sal I–Sac I ASH1 fragment. Plasmid pXMRS25 was constructed from pH218 (22) by insertion of an ASH1 fragment generated by the polymerase chain reaction (PCR). The PCR product contained the last five amino acid codons of ASH1 and extended 250 nucleotides beyond the stop codon. The ASH1 fragment was subcloned into the Sac I site of pH218 by the inclusion of a Sac I restriction site in the PCR primers. The primers for PCR were 5′-GGGC-CCGAGCTGGAATGAGAAATTCTGGGACCTGTTGTAATGCGAGACCTGATTGTGATGATACATGGGAAATGTTGGTGCATGATGACGAGAAGATTAACCTTGGGCTAGCTTTGTTGTGGTTGGTGGACT*CATCGGCGGTGT*GACGACCTAGT*CGATTCCAATT*CCTT*GC-3′ and 5′-GGCGGCGGCGGCGGCGGCCTGACGAGAGATTAACCTTGGGCTAGCTTTGTTGTGGTTGGTGGACT*CATCGGCGGTGT*GACGACCTAGT*CGATTCCAATT*CCTT*GC-3′. To verify that no mutations were introduced by PCR, the ASH1 region of plasmid pXMRS25 was confirmed by DNA sequencing.

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3. The families enrolled in this study were ascertained in the following manner. Each of the principal investigators 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
Polymopoulos et al. present results of a genome-wide screen for genetic linkage in a large family with 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 multi-generational 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 Polymopoulos et al. (1). No additional neurologic deficit was observed except for amytrophy in one affected family A. Multipoint analysis with eight polymorphic markers spanning the region from GATA10G07 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 region from founders in the pedigrees did not alter multipoint lod scores significantly.

**Table 1.** Demographic and clinical characteristics in 13 families with inherited parkinsonism.

<table>
<thead>
<tr>
<th>Family</th>
<th>Number of affected relatives</th>
<th>AFFECTED relatives examined</th>
<th>Age at onset* (years)</th>
<th>L-Dopa response</th>
<th>Atypical features</th>
<th>Reference†</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>2</td>
<td>51 (35 to 60)</td>
<td>Positive</td>
<td>Amyotrophy and dementia in some</td>
<td>(2, 3)</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>8</td>
<td>62 (51 to 82)</td>
<td>Positive</td>
<td>Dementia in some</td>
<td>(2, 3)</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>11</td>
<td>60 (55 to 66)</td>
<td>Positive</td>
<td>None</td>
<td>(3, 4)</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>18</td>
<td>63 (48 to 78)</td>
<td>Positive</td>
<td>Dementia in some</td>
<td>(5)</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>11</td>
<td>56 (48 to 74)</td>
<td>Positive</td>
<td>None</td>
<td>(2)</td>
</tr>
<tr>
<td>IT-1</td>
<td>6</td>
<td>5</td>
<td>54 (36 to 89)</td>
<td>Positive</td>
<td>None</td>
<td>(2)</td>
</tr>
<tr>
<td>K</td>
<td>7</td>
<td>4</td>
<td>45 (37 to 63)</td>
<td>Positive</td>
<td>None</td>
<td>–</td>
</tr>
<tr>
<td>FR-041</td>
<td>8</td>
<td>3</td>
<td>63 (60 to 65)</td>
<td>Positive</td>
<td>None</td>
<td>(2)</td>
</tr>
<tr>
<td>FR-722</td>
<td>9</td>
<td>8</td>
<td>59 (53 to 64)</td>
<td>Positive</td>
<td>None</td>
<td>(6)</td>
</tr>
<tr>
<td>FR-727</td>
<td>10</td>
<td>4</td>
<td>41 (31 to 52)</td>
<td>Positive</td>
<td>None</td>
<td>–</td>
</tr>
<tr>
<td>FR-755</td>
<td>11</td>
<td>4</td>
<td>39 (29 to 52)</td>
<td>Positive</td>
<td>None</td>
<td>–</td>
</tr>
<tr>
<td>UK-A</td>
<td>12</td>
<td>14</td>
<td>53 (42 to 70)</td>
<td>Positive</td>
<td>None</td>
<td>(3, 4)</td>
</tr>
<tr>
<td>UK-B</td>
<td>13</td>
<td>5</td>
<td>37 (31 to 41)</td>
<td>Positive</td>
<td>None</td>
<td>(7)</td>
</tr>
</tbody>
</table>

*Mean and range. †Unpublished (–).

**Fig. 1.** Multipoint linkage analysis of the PD1-region on chromosome 4q21-q23 in seven families with familial parkinsonism. Polymorphic DNA fragments were amplified by PCR with the use of published primer sequences and a standard protocol. Multipoint analysis was performed using GENEHUNTER (8), and two-point analysis was done using VITESSE (9). An autosomal dominant model with an age-dependent penetrance was assumed. As was done by Polymopoulos et al. (1), unaffected individuals were set to be unaffected only when they were older than the mean age of onset in the respective families; all other unaffected individuals were treated as unknown. Frequency of disease allele was set to 0.001. Marker allele frequencies were set to be equal for all alleles. Estimating marker allele frequencies from founders in the pedigrees did not alter multipoint lod scores significantly.
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 recombinations (no allele shared by all affected) 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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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.

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Experiments in a Parkinson’s Rat Model

Derek L. Choi-Lundberg et al. present evidence (1) that a replication-defective adenoviral (Ad) vector that encodes human glial cell line-derived neurotrophic 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.

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