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 Data from one (previously unpublished) family of southern Bavarian origin showed positive lod scores with a maximum multipoint score of 1.5 (family K, Fig. 1).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 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.

T. Gasser, *Department of Neurology, Klinikum Großhadern, 81377 Munich, Germany;* **B. Müller-Myhsok,** *Bernhard-Nocht-Institute for Tropical Medicine, Hamburg 20359, Germany;* **Z. K. Wszolek,** *Section of Neurology, University of Nebraska, Omaha, NE 68198–2045, USA;* **A. Dürr,** *INSERM U289, Paris 75013, France;* **J. R. Vaughan,** *Institute of Neurology, The National Hospital Queen Square, London WC1N 3BG, UK;* **V. Bonifati** and **G. Meco,** *Dipartimento di Scienze Neurologiche, Università "La Sapienza," Rome 00142, Italy;* **B. Bereznai,** *Department of Neurology, Klinikum Großhadern;* **R. Oehlmann,** *Bernhard-Nocht-Institute for Tropical Medicine;* **Y. Agid** and **A. Brice,** *INSERUM U289;* **N. Wood,** *Institute of Neurology, The National Hospital Queen Square,* and the *European Consortium on Genetic Susceptibility in Parkinson's Disease (GSPD) (10)*

REFERENCES AND NOTES

1. M. H. Polymeropoulos *et al.*, *Science* **274**, 1197 (1996).
2. Z. K. Wszolek *et al.*, *Nervenarzt* **64**, 331 (1993).
3. M. A. Denson and Z. K. Wszolek, *Parkinsonism Related Disord.* **1**, 35 (1995).
4. Z. K. Wszolek *et al.*, *Neurology* **45**, 502 (1995).
5. V. Bonifati *et al.*, *Mov. Disord.* **11** (suppl. 1), 86 (1996), abstract.
6. P. Mazzetti *et al.*, *J. Neurol. Neurosurg. Psychiatry* **57**, 871 (1994).
7. G. V. Sawle *et al.*, *Ann. Neurol.* **32**, 609 (1992).
8. L. Kruglyak *et al.*, *Am. J. Hum. Genet.* **68**, 1347 (1996).
9. J. R. O'Connell and D. E. Weeks, *Nature Genet.* **11**, 402 (1995).
10. European Consortium on Genetic Susceptibility in Parkinson's disease: N. Wood and J. R. Vaughan, The National Hospital for Neurology and Neurosurgery, London, UK; A. Brice, A. Dürr, J. Tassin, Y. Agid, Institut National de la Santé et de la Recherche Médicale (INSERM U289), Paris, France, on behalf of the French Parkinson's Disease Genetics Network; M. Martinez, INSERM U358, and J. Feingold, INSERM U155, Paris, France; M. Breteler and B. Oostra, Erasmus University, Rotterdam, The Netherlands; V. Bonifati, E. Fabrizio, G. Meco, Università "La Sapienza," Rome, Italy; G. De Michele and G. Campanella, Universita Federico II, Naples, Italy.

30 December 1996; accepted 19 June 1997

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.

Mihael H. Polymeropoulos
Laboratory of Genetic Disease Research, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA
E-mail:mhp@aeolus.nhgri.nih.gov

REFERENCES AND NOTES

1. M. H. Polymeropoulos *et al.*, *Science* **274**, 1197 (1996).
2. ———, *ibid.*, **276**, 2045 (1997).

30 June 1997; accepted 1 July 1997

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.*

Roberto Pallini
Alessandro Consales
Liverana Lauretti
Eduardo Fernandez
Department of Neurosurgery, Catholic University School of Medicine, Rome 00168, Italy

REFERENCES

1. D. L. Choi-Lundberg *et al.*, *Science* **275**, 838 (1997).
2. G. Balercia, S. Chen, M. Bentivoglio, *J. Neurosci. Meth.* **45**, 87 (1992).

17 March 1997; accepted 19 June 1997

Response: In our study (*1*), we injected FG into the striatum in order to retrogradely label a subpopulation of dopaminergic (DA) neurons in the SN. We injected 6-OHDA into the same site 7 days later, so that the FG-labeled DA neurons would be those most susceptible to the lesion. This research design allowed us to identify DA neurons that project specifically to this site without relying on their phenotype. Relevant to this rationale are studies showing that DA neurons, as identified solely by tyrosine hydroxylase (TH), "disappear" when lesions are induced with 6-OHDA and then "reappear" after injection of GDNF protein (*2*). In our study, the reduction of FG^+ neurons in the SN in control groups after treatment with 6-OHDA confirmed that these cells died and did not merely lose phenotypic marker expression. Microglia and other small cells were labeled with FG, which suggests either that these were cells that had phagocytosed degenerating FG^+ neuronal debris or were shrunken, degenerating DA neurons. Rats treated with an adenoviral vector (Ad) that encodes GDNF had significantly more large FG^+ neurons in the SN 42 days after injection of 6-OHDA than were found in control rats—an average of 79% (Ad GDNF) as opposed to 31% (controls) [see figure 3 in (*1*)]—which we interpreted as protection of these FG^+ DA neurons by Ad GDNF.

Pallini *et al.* offer an alternative explanation that FG^+ DA neurons degenerated and released FG, and that increased concentrations of GDNF promoted the uptake of FG by neighboring DA neurons. Because only a subpopulation of DA neurons in the SN were labeled with FG, it is possible that FG released by dying neurons could have been taken up by neighboring unlabeled neurons. However, the observed anatomical distribution of FG^+ neurons argues against this possibility. On the unlesioned side, FG^+ DA neurons were located in the ventral and medial SN through the rostrocaudal extent of the SN. In the anterior portion of the SN, nearly every DA neuron in the ventral SN was FG^+ [figure 2C in (*1*)]. The distribution of FG^+ neurons was unchanged in the Ad GDNF group [figure 2D in (*1*)]. If neurons had degenerated with subsequent uptake by other DA neurons, then the FG labeling would have been in more dorsal neurons, with some FG^+ neurons remaining in the ventral portion of the SN (some FG^+ neurons survived in the ventral SN in control groups [figure 2, E through G in (*1*)]. However, this distribution was not observed, which suggests that FG^+ neurons did not degenerate in rats treated with Ad GDNF. Another observation refuting this possibility is that almost no small FG^+ cells were present in sections of the SN in Ad GDNF–treated rats: If FG^+ DA neurons had degenerated, some microglia would likely have phagocytosed FG^+ DA neuronal debris.

Pallini *et al.* also suggest that injection of FG, after the degeneration of SN neurons had occurred, would demonstrate the protective effect of Ad GDNF. However, we did not observe any obvious differences among the various treatment groups in the size of the 6-OHDA lesion in the striatum, as indicated by the density of TH fiber staining (*1*). This suggests that Ad GDNF delivered near the SN did not protect DA nerve terminals from striatal 6-OHDA, as was also reported after injection of GDNF protein near the SN (*3*). Injection of FG after 6-OHDA would likely lead to little retrograde transport to the SN as DA nerve terminals would have been destroyed. If the striatal lesion volume had been reduced by GDNF treatment, this could have been caused by protection of nerve terminals or induction of sprouting into the denervated area. These possibilities might be distinguished by labeling with FG before the lesion, with subsequent injection of another tracer after the lesion. Consequently, if FG had been injected at the end of the experiment as suggested by Pallini *et al.*, this would have labeled only those DA neurons whose fibers had spouted or remained in the lesion site, defeating the original purpose of the labeling.

Derek L. Choi-Lundberg
Department of Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642, USA
Martha C. Bohn
Department of Pediatrics, Children's Memorial Institute for Education and Research, Northwestern University Medical School, Chicago, IL 60614, USA

REFERENCES

1. D. L. Choi-Lundberg *et al.*, *Science* **275**, 838 (1997).
2. K. E. Bowenkamp *et al.*, *J. Comp. Neurol.* **355**, 479 (1995).
3. H. Sauer, C. Rosenblad, A. Björklund, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 8935 (1995).

29 April 1997; accepted 19 June 1997