Neuron, Vol. 44, 601–607, November 18, 2004, Copyright ©2004 by Cell Press

Mutations in *LRRK2* Cause Autosomal-Dominant Parkinsonism with Pleomorphic Pathology

Alexander Zimprich,[1,2,11] Saskia Biskup,[3,11] Petra Leitner,[1] Peter Lichtner,[3] Matthew Farrer,[4] Sarah Lincoln,[4] Jennifer Kachergus,[4] Mary Hulihan,[4] Ryan J. Uitti,[5] Donald B. Calne,[6] A. Jon Stoessl,[6] Ronald F. Pfeiffer,[7] Nadja Patenge,[1] Iria Carballo Carbajal,[1] Peter Vieregge,[8] Friedrich Asmus,[1] Bertram Müller-Myhsok,[9] Dennis W. Dickson,[4] Thomas Meitinger,[3,10,*] Tim M. Strom,[3,10] Zbigniew K. Wszolek,[5,*] and Thomas Gasser[1,*]

[1]Department of Neurodegenerative Disease
Hertie Institute for Clinical Brain Research
University of Tübingen
72076 Tübingen
Germany

[2]Neurological Department
Medical University of Vienna
1090 Vienna
Austria

[3]Institute of Human Genetics
GSF National Research Center
for Environment and Health
85764 Neuherberg
Germany

[4]Department of Neuroscience

[5]Department of Neurology
Mayo Clinic
Jacksonville, Florida 32224

[6]Pacific Parkinson's Research Centre
University of British Columbia
Vancouver, British Columbia V6T 2B5
Canada

[7]Department of Neurology
University of Tennessee Health Science Center
Memphis, Tennessee 38103

[8]Department of Neurology
Klinikum Lemgo
32657 Lemgo
Germany

[9]Computational Genetics Group
Max Planck Institute for Psychiatry
80804 Munich
Germany

[10]Institute of Human Genetics
Technical University Munich
81675 Munich
Germany

Summary

We have previously linked families with autosomal-dominant, late-onset parkinsonism to chromosome 12p11.2-q13.1 (PARK8). By high-resolution recombination mapping and candidate gene sequencing in 46 families, we have found six disease-segregating mutations (five missense and one putative splice site mutation) in a gene encoding a large, multifunctional protein, *LRRK2* (leucine-rich repeat kinase 2). It belongs to the ROCO protein family and includes a protein kinase domain of the MAPKKK class and several other major functional domains. Within affected carriers of families A and D, six post mortem diagnoses reveal brainstem dopaminergic degeneration accompanied by strikingly diverse pathologies. These include abnormalities consistent with Lewy body Parkinson's disease, diffuse Lewy body disease, nigral degeneration without distinctive histopathology, and progressive supranuclear palsy-like pathology. Clinical diagnoses of Parkinsonism with dementia or amyotrophy or both, with their associated pathologies, are also noted. Hence, *LRRK2* may be central to the pathogenesis of several major neurodegenerative disorders associated with parkinsonism.

*Correspondence: meitinger@gsf.de (T.M.); wszolek.zbigniew@mayo.edu (Z.K.W.); thomas.gasser@med.uni-tuebingen.de (T.G.)
[11]These authors contributed equally to this work.

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by a progressive loss of dopaminergic neurons of the substantia nigra, associated with the formation of fibrillar aggregates composed of α-synuclein and other proteins (Lewy bodies and Lewy neurites). In most cases, PD occurs as a sporadic disease of unknown etiology, but in rare instances, point mutations (Polymeropoulos et al., 1997) or multiplications (Singleton et al., 2003; Chartier-Harlin et al., 2004) of the α-synuclein gene can cause autosomal-dominant parkinsonism, which resembles sporadic disease in many aspects. Recessive forms of parkinsonism have been recognized that are caused by mutations in the genes for *parkin* (Kitada et al., 1998), *DJ-1* (Bonifati et al., 2003), and *PINK1* (Valente et al., 2004). Additional loci have been mapped on chromosomes 2p (Gasser et al., 1998), 12cen (Funayama et al., 2002), 1q (Hicks et al., 2002), and 2q (Pankratz et al., 2003).

As α-synuclein aggregation is a pathologic feature common to sporadic and dominantly inherited forms of PD, as well as other neurodegenerative diseases, including dementia with Lewy bodies (DLB) and multiple system atrophy (MSA), those diseases have been collectively called "α-synucleinopathies" (reviewed by Farrer et al., 1999). Other forms of parkinsonism are associated with the accumulation of filaments within neurons and glia, which are composed of the microtubule-associated protein tau. Mutations in this gene (MAPT) explain at least a subgroup of families with frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17; Hutton et al., 1998), while sporadic cases with tau pathology most commonly present as progressive supranuclear palsy (PSP) or corticobasal degeneration (CBD) (Dickson, 1999). Based on the putative central role of the tau protein in these diseases, they have been called "tauopathies" (reviewed in Arvanitakis and Wszolek, 2001; Ghetti et al., 2003).

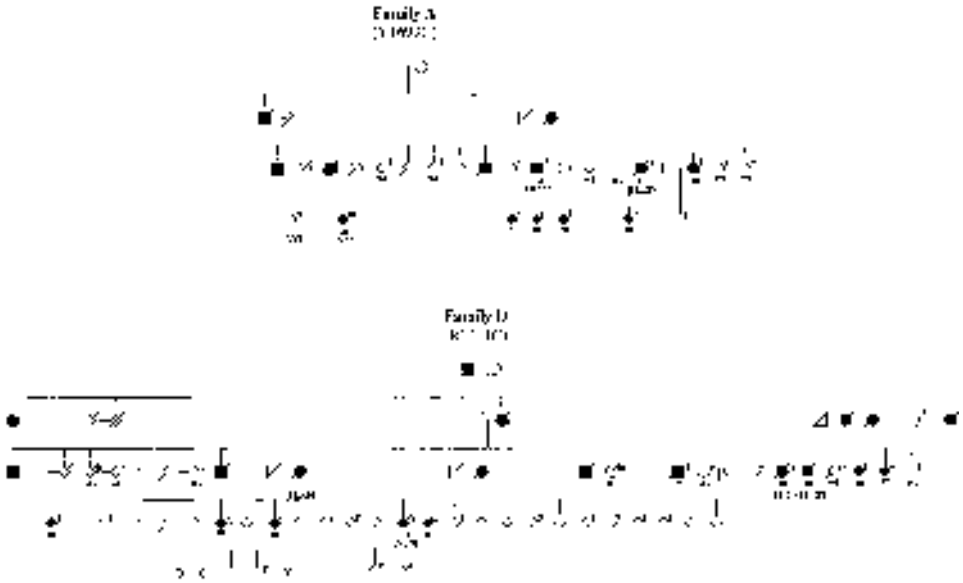

Figure 1. Pedigree Structure of the Two Largest Kindreds with LRRK2 Mutations

Shown are family A (German-Canadian) and family D (Western Nebraska). Blackened symbols denote affected family members. An asterisk denotes a genotyped individual, with "m" for mutation carriers and "wt" for wild-type *LRRK2*. To maintain confidentiality, genotypes of some unaffected individuals in families A and D are not shown. Additionally, the structure of the pedigrees has been altered, and the genders of individuals in some of the youngest generations have been disguised.

Recently, we have shown that two large families with autosomal-dominant late-onset parkinsonism (families A and D) are linked to the PARK8 locus on chromosome 12p11.2-q13.1 (OMIM# 607060) (Zimprich et al., 2004), originally mapped in a Japanese family by Funayama et al. (2002). High-resolution recombination mapping, candidate gene sequencing, and the identification of pathogenic mutations within linked families is reported in this article.

Results

Within our linked families, haplotype analysis of obligate recombinants initially refined a candidate region of 13 Mb, between flanking markers D12S1692 and D12S85. We subsequently sequenced a total of 29 genes in the region, in two patients from each family. Supplemental Table S1 (http://www.neuron.org/cgi/content/full/44/4/601/DC1/) lists the 29 genes in order of their genomic position. The primer pairs, sequence variants identified, and sequence chromatograms obtained are available upon request. Missense mutations were identified in a large gene, *LRRK2* (leucine-rich repeat kinase 2), in family A (Y1699C; 5096A>G) and in family D (R1441C; 4321C>T). Both mutations segregated with disease in these two families (Figure 1) and were not found in more than 1000 control individuals or 300 sporadic PD patients.

In family A (Wszolek et al., 1997), 16 individuals were genotyped (8 unaffecteds and 8 affecteds). All affecteds were heterozygous for the mutation, and all unaffecteds aged over 60 were wild-type. Individuals IV:1 and IV:2 (family A) were not included in our initial linkage analysis (Zimprich et al., 2004); both individuals have now been genotyped. Recalculation of the two-point LOD scores using the mutation as a marker gives maximum LOD scores of 3.78 at $\theta = 0$ and provides the statistical burden of proof for pathogenicity.

In the second family that was previously linked to the PARK8 locus, family D (Wszolek et al., 2004), 34 individuals were genotyped (10 affecteds and 24 unaffecteds). All affecteds were heterozygous for the 4321C>T (R1441C) mutation. Of the 24 clinically unaffected individuals who were genotyped, only two were older than 60 years of age and were mutation carriers. These individuals are considered to be at risk and likely presymptomatic, given that the average age of onset in this family is 65 years (Wszolek et al., 2004). To protect the confidentiality of family members, some pedigree symbols and genotypes have been omitted and gender has been disguised in the pedigrees (Figure 1).

To estimate the prevalence of *LRRK2* mutations among PD families, one index patient each from 44 additional families with PD (32 with features consistent with autosomal-dominant PD and 12 affected sib-pairs) were subsequently sequenced. We identified two further missense and one putative splice site mutation, all in families with typical late-onset PD, compatible with a dominant transmission: (I1122V; 3364A>G) in family 21, (I2020T; 6059T>C) in family 32, and (L1114L; 3342A>G) in family 38, which is 6 bp away from the exon/intron border. Affected individuals in an additional family (469) were found to carry the same mutation as family D (R1441C; 4321C>T). These two families are not known to be related, nor do they share haplotypes for the closest flanking microsatellite repeat markers D12S2194, D12S1048, or three newly developed intragenic repeat markers within introns (see Experimental Procedures). This suggests the mutations are either extremely ancient or arose independently. Pedigree structures and clinical

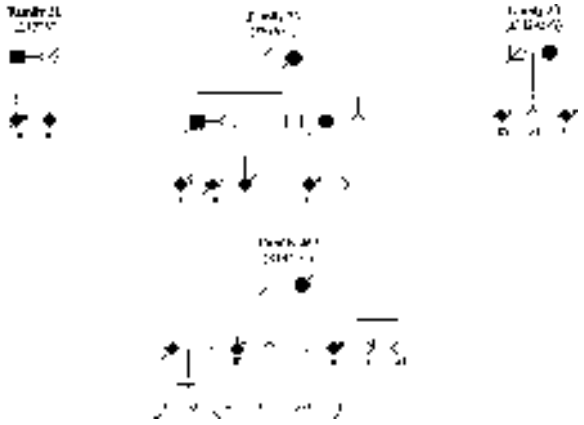

Figure 2. Pedigree Structures of Smaller Families with *LRRK2* Mutations

Blackened symbols denote affected family members. An asterisk denotes a genotyped individual, with "m" for mutation carriers and "wt" for wild-type *LRRK2*. To protect confidentiality, the genotypes of some unaffected individuals of family 469 are not shown. Additionally, the structure of the pedigrees has been altered, and the gender of individuals in the youngest generations has been disguised.

and pathological details of affected patients within these smaller families are illustrated in Figure 2. Clinical and genetic information for all families with *LRRK2* mutations is summarized in Table 1. While smaller families may not demonstrate significant evidence for linkage, the mutations appear to segregate with the disease in all of these families and none were found in controls. Three of the amino acid substitutions (R1441C, Y1699C, and I1122T) are highly conserved across species (Figure 3C).

LRRK2 was covered by several partial cDNA clones deposited in public data banks. From cross-species sequence alignments, it became clear that the clones were incomplete toward the 5′ end. To confirm whether *LRRK2* is indeed expressed in brain and to establish the complete cDNA sequence, we amplified the whole gene from human brain cDNA (Cat# 7400-1, Clontech, BD Bioscience, San Jose, CA) using overlapping primers predicted by homology searches (see Supplemental Data). The resulting gene spans a genomic region of 144 Kb, with 51 exons encoding 2527 amino acids (Figure 3B). The expression pattern of *LRRK2* was subsequently examined in several tissues. We hybridized human brain and multiple tissue Northern blots with a 1078 bp 3′ cDNA probe and found expression in most brain regions, albeit at very low levels. There appeared to be a major transcript of 9 kb and a minor transcript of ~8 kb, which may be explained by the alternative use of polyadenylation [poly(A)] sites. This would be in concordance with two mRNAs, deposited under the accession numbers AL832453 and AL834529, which make use of canonical poly(A) sites (aataaa) at positions 7962 and 8944, respectively. Multiple, very weak bands at lower sizes were found that suggested alternative splicing (data not shown).

Using real-time RT-PCR and RNA isolated from adult and fetal whole brain and from different brain regions, we assessed quantitative gene expression and alternative splicing. We designed primers to generate specific PCR products for exons 1–3, exons 12–13, and exons 32–34, respectively. Within the same tissue or brain region, transcript levels of all three assays showed no significant differences. We found low, but consistent, expression of full-length *LRRK2* in most brain regions, slightly higher in putamen, substantia nigra, and lung (Figure 4). Whether any of these transcripts result in translated, functional protein awaits the generation of specific antibodies. Of note, in adult human brain tissue, exon 6 was constitutively expressed within the full-length mRNA.

LRRK2 (also referred to as dardarin [see Paisán-Ruíz et al., 2004, published online October 22 and appearing in this issue of *Neuron*]) can be considered a member of the recently defined ROCO protein family (Bosgraaf

Table 1. Clinical and Pathologic Features of Kindreds with LRRK2 Mutations

Family	A	D	21	32	38	469
Mutation	Y1699C	R1441C	I1122V	I2020T	3342A>G	R1441C
Mean age at onset	53 (35–65)	65 (48–78)	51 (49–53)	54 (48–59)	73 (68–77)	56 (52–60)
Mean disease duration	13 (5–18)	13 (4–26)	18 (17,19)	20 (12–27)	11 (7,14)	23 (21–26)
Initial Signs	RT	B, RT	B	B	RT	RT
Bradykinesia	+	+	+	+	+	+
Rigidity	+	+	+	+	+	+
Resting tremor	+	+	+	+	+	+
Postural instability	+	+	+	+	-	+
Response to levodopa	+	+	+	+	+	+
Long-term complications	+	+	-	-	-	-
Other features	Two cases had amyotrophy and two cases had dementia	-	-	-	-	-
Pathologic features	Two cases had "nonspecific" SN degeneration with ubiquitin-positive neuronal inclusions. One had concurrent AD and one had mild motor neuron disease.	All cases had SN neuronal loss. One case had "nonspecific" SN degeneration with ubiquitin-positive neuronal inclusions. Two cases had Lewy bodies—one brainstem-type and one diffuse-type. One case had PSP-like changes.	NA	NA	NA	NA

Abbreviations: B, bradyskinesia; R, rigidity; RT, resting tremor; PI, postural instability; SN, substantia nigra; AD, Alzheimer's disease; PSP, progressive supranuclear palsy.

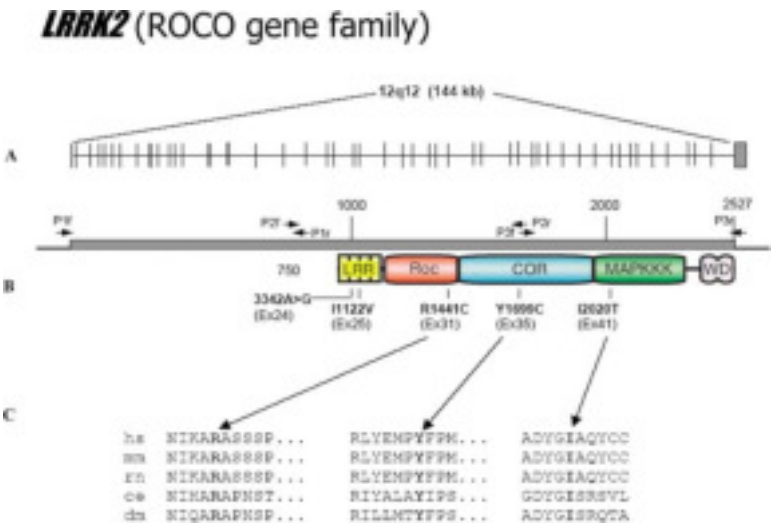

Figure 3. Structure of *LRRK2*

(A) Axon positions.

(B) Schematic drawing of *LRRK2*, with domains, primer positions for cDNA amplification, and positions of mutations.

(C) Protein alignment of three mutations conserved among the following: hs, *Homo sapiens*; mm, *Mus musculus*; rn, *Rattus norvegicus*; ce, *Caenorhabditis elegans*; and dm, *Drosophila melanogaster*.

and Van Haastert, 2003), as predicted in silico by the presence of two conserved domains: (1) a Roc (Ras in complex proteins) domain that belongs to the Ras/GTPase superfamily; and (2) a COR domain (C-terminal of Roc) that is also characteristic for this protein family. Ras/GTPase domains are involved in the reorganization of the actin cytoskeleton in response to external stimuli. They also have roles in cell transformation by Ras, as well as in cytokinesis, focal adhesion formation, and the stimulation of stress-activated kinase (Ridley, 2001). Three further conserved domains can be identified: (1) a leucine-rich repeat (LRR), consisting of 12 strands of a 22–28 amino acid motif, present in a tandem array; (2) a tyrosine kinase catalytic domain (TyrKc); and (3) a WD40 domain. Although proteins containing LRRs are associated with diverse functions, such as hormone receptor interactions, enzyme inhibition, cell adhesion, cellular trafficking, splicing, and substrate binding for ubiquitination, a common property involves protein-protein interaction (Kobe and Kajava, 2001). Enzymes with TyrKc domains belong to an extensive family of proteins that share a conserved catalytic core common to both serine/threonine and tyrosine protein kinases. They exert their function by catalyzing the transfer of the γ-phosphate of ATP to tyrosine residues on protein substrates (Hubbard and Till, 2000). Finally, WD40 domains have been previously implicated in signal transduction, pre-mRNA processing, and cytoskeleton assembly (Smith et al., 1999).

The sequence of this newly identified gene, therefore, suggests multiple functions. As we have found mutations in different functional domains, it is unclear which domain or domains are related to neurodegeneration. It is likely, however, that *LRRK2* may be central to a range of neurodegenerative processes based upon available evidence. First, *LRRK2* mutations appear to be a common cause of autosomal-dominant parkinsonism. In the present study, we identified six independent mutations in 34 families with autosomal-dominant PD. Second, affected individuals with *LRRK2* mutations exhibit strikingly variable pathologic changes (Wszolek et al., 2004), representing aspects of several of the major neurodegenerative diseases (Figure 5).

The common feature of the neuropathology of six af-

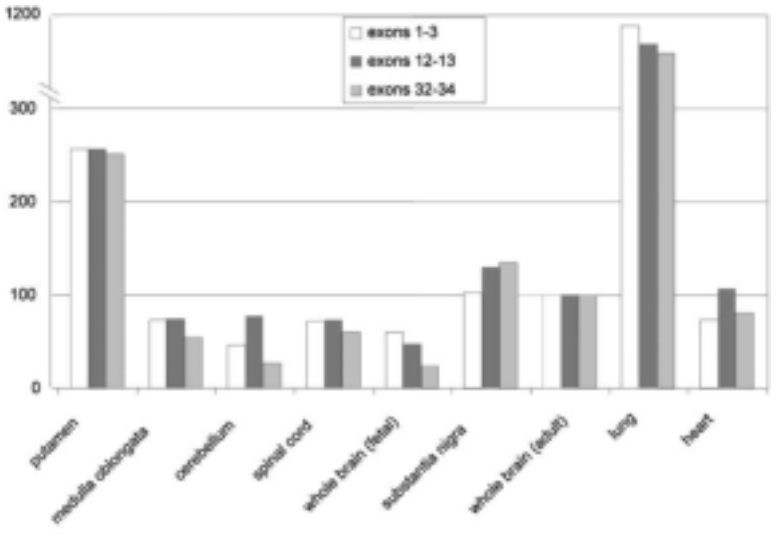

Figure 4. Transcript Levels of LRRK2 in Different Human Tissues

Quantitative RT-PCR assays show no differential expression of three regions of the *LRRK2* transcripts within the same tissue. Transcript levels are given as ratios of *LRRK2*/h-PBGD, a low-copy housekeeping gene, normalized to adult whole brain expression as 100%. Note the comparably high expression levels of *LRRK2* transcripts in putamen and substantia nigra, brain regions vulnerable to neurodegeneration in PD.

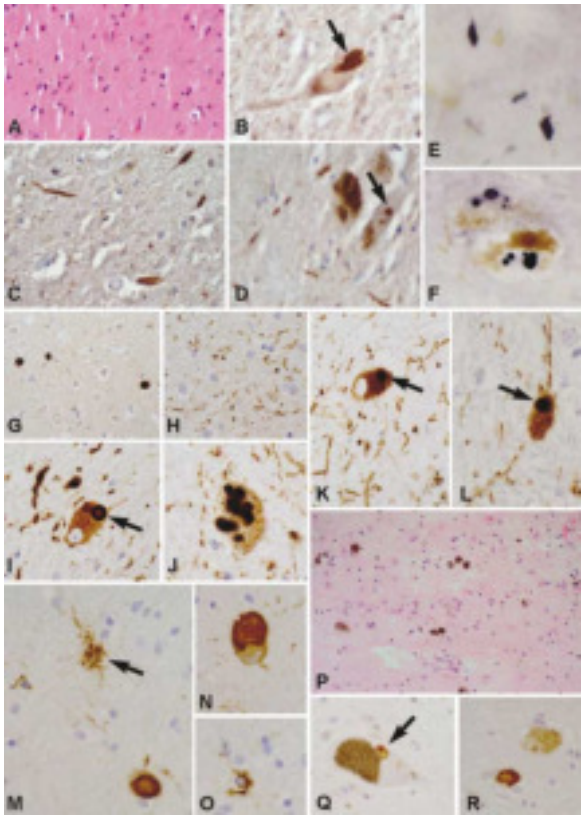

Figure 5. Substantia Nigra Pathology in Families A and D

Summary of substantia nigra pathology in six autopsies from family A (III:29, [A]–[D]; II:27, [E] and [F]) and family D (III:20, [G]–[J]; III:14, [K] and [L]; III:21, [M]–[O]; and IV:20, [P]–[R]). Three cases had nonspecific neuronal loss and gliosis in the substantia nigra (hematoxylin and eosin stain) (A and P) with ubiquitin-immunoreactive neuronal lesions. Ubiquitin immunohistochemistry revealed a range of lesions, including irregular cytoplasmic inclusions (arrow in B) (B and D), curvilinear dystrophic neurites (C and E), granular and foamy axonal spheroids (R), and multiple nuclear inclusions (arrows in D and Q) (D, F, and Q). α-synuclein immunohistochemistry was positive in two individuals, showing many cortical Lewy bodies (G) and cortical Lewy neurites (H) in one case, as well as typical brainstem-type Lewy bodies (arrow in I, K, and L) or pleomorphic Lewy bodies (J) in the substantia nigra and brainstem nuclei. One case had tau-immunoreactive lesions similar to those seen in PSP, including globose neurofibrillary tangles (M and N), tufted astrocytes (arrow in M), and oligodendroglial coiled bodies (M and O).

fected individuals from families A and D who have come to autopsy is neuronal loss and gliosis in the substantia nigra, which is the likely pathologic substrate of parkinsonism. In three of the individuals (two from family A and one from family D), there was nonspecific loss of dopaminergic neurons, with ubiquitin-immunoreactive cytoplasmic and nuclear inclusions. The latter were similar to Marinesco bodies, while the neuronal cytoplasmic inclusions appear to be novel and await further characterization. Two individuals (both from family D) had Lewy body pathology. In one individual, who died prematurely following a neurosurgical ablation procedure, Lewy bodies were relatively restricted to brainstem nuclei as in typical PD. In the other individual, there was widespread α-synuclein pathology, including Lewy bodies and Lewy neurites, in a pattern typical of diffuse Lewy body disease. In the final individual, there were tau-immunoreactive neuronal and glial lesions that morphologically resembled lesions of PSP; however, the density and distribution of the lesions was not diagnostic of PSP. Alzheimer type pathology, which might be expected given the advanced age at autopsy, was detected in several individuals, and one met neuropathologic criteria for Alzheimer's disease. Several individuals in family A had clinical features of motor neuron disease, and there was mild decrease of anterior horn cells, gliosis, and axonal spheroids in these cases (Wszolek et al., 1997).

Discussion

Although only a small proportion of PD cases are caused by gene mutations with high penetrance, identification of these genes has had a major impact on the understanding of the molecular mechanisms leading to dopaminergic neurodegeneration. Those mechanisms include the aggregation of proteins such as α-synuclein and tau, deficits in ubiquitin-proteasome protein degradation, or mitochondrial dysfunction and oxidative stress. Until now, it was assumed that these represented separate molecular pathways, all eventually leading to neuronal death, but originating from different initial insults. This view will need to be revised given the present findings.

LRRK2 appears to be central to the etiology of not just one but several neurodegenerative diseases, including those associated with α-synuclein and tau pathology and clinical parkinsonism. Previously, it was shown that α-synuclein and tau pathologies may be closely linked. For example, tau-aggregations have been found in the brains of patients carrying pathogenic A53T mutation in the gene for α-synuclein (Duda et al., 2002). Tau has also been shown to colocalize with a subset of Lewy bodies in sporadic PD (Ishizawa et al., 2003). Moreover, tau and α-synuclein have been shown to reciprocally promote fibril formation in vitro (Giasson et al., 2003). The major pathogenic protein aggregating in AD, Aβ, has been shown to promote fibrillization of tau and enhanced formation of neurofibrillary tangles in transgenic animal models (Gotz et al., 2001; Lewis et al., 2001). Interestingly, a genomic region overlapping the PARK8 locus has been identified in a linkage study of familial Alzheimer disease (Scott et al., 2000). Evidence for linkage was derived in a large part from families with at least one member with autopsy-proven diffuse Lewy body disease. Whether this linkage result reflects variants in the *LRRK2* gene remains to be determined.

We hypothesize that the function(s) of LRRK2 are crucial to the initiation and development of several neurodegenerative disorders. Potentially, LRRK2 may be responsible for the phosphorylation of both α-synuclein and tau; its kinase activity could be a key event in the accumulation and aggregation of these unfolded proteins within degenerating neurons.

Experimental Procedures

Genetic Analyses

DNA Extraction

Genomic DNA from peripheral blood lymphocytes was extracted using standard protocols. Blood was collected after obtaining in-

formed consent from all participating family members. All human studies are approved by institutional review boards of participating institutions.

Sequence Analysis

Genomic sequences and annotations were obtained from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) and University of California, Santa Cruz (UCSC) (http://genome.ucsc.edu/). Primers for mutation screening were designed using Primer3 software integrated into script to allow for automated primer design (http://ihg.gsf.de/ihg/ExonPrimer.html). We amplified exon sequences and exon-intron boundaries with intronic primers and sequenced them directly by BigDye Terminator Cycle sequencing kit (Applied Biosystems). Primer sequences are provided in the Supplemental Data.

Between markers D12S1692 and D12S85, a total of 29 genes or RNAs were sequenced. These sequences are listed in Supplemental Table S1. In the gene *PPHLN1* (Periphilin), we detected an amino acid change (K142E) in one small family of two affecteds. We cannot determine whether this alteration is, in fact, pathogenic, because no further nucleotide change was found, even after extensive mutation analysis in all of the other families investigated.

Haplotype Analysis

Haplotypes were constructed by hand using the repeat markers previously used for linkage analysis (Zimprich et al., 2004). Intragenic markers for haplotype analysis for family 469 and family D were established by searching the whole gene for repeat polymorphisms using a "tandem repeat finding program" (http://c3.biomath.mssm.edu/trf.html). Polymorphic repeats were found in intron 5 (caa), intron 20 (atct), and intron 29 (ac). Primer sequences for intragenic markers are available from the authors on request.

Linkage Analysis

Two point LOD scores were calculated using the MLINK program (V 5.10) in its FASTLINK implementation (V4.1P). Phenocopy rate was set at 0.01 and penetrance for the heterozygous state and homozygous mutation carriers at 0.90. The allele frequency of the disease-causing allele was set at 0.001, as was the frequency of the mutation used as the marker.

Screening of Mutations and Polymorphisms

Mutation screening was performed using an ABI 7900 Allelic Detection system. PCR was performed by using Taqman Universal PCR Master Mix and Assays-on-Demand MGB primer and dye-labeled probe sets (Applied Biosystems). For each mutation and polymorphism, at least 1200 control chromosomes from a mixed European descent were screened. In addition, we screened 300 patients with sporadic PD. Genotyping was performed on a MALDI-TOF mass-spectrometer (Sequenom MassArray system) using the homogeneous mass-extension (hME) process for producing primer extension products (Tang et al., 1999).

***Amplification of* LRRK2**

The complete coding sequence of *LRRK2* was amplified from human brain cDNA by using Marathon-Ready cDNA (BD Biosciences Clontech). Primers were set to amplify three overlapping fragments from exons 1–21 (P1f, P1r), from exons 20–35 (p2f, p2r), and from exons 34–51 (p3f, p3r) (Figure 3). Sequence information was derived from published mRNA of DKFZp434H211. PCR products were run on agarose gel to check length and integrity. All oligonucleotides are available upon request.

Northern Blot Analysis

Northern blot analysis was performed according to the manufacturer's protocols (BD Biosciences). For hybridization, we used a *LRRK2* cDNA fragment (bp 6577–7655) corresponding to exon 45-3′UTR.

LightCycler Experiments

mRNAs from different human tissues were purchased from BD Biosciences (Clontech BD Sciences, Palo Alto, CA) and were reverse transcribed with the Transciptor First Strand cDNA Synthesis Kit (Roche Applied Sciences, Mannheim, Germany) according to the manufacturer's protocol. For real-time amplification of *LRRK2*, three specific PCR products spanning exons 1–3, exons 12–13, and exons 32–34 were quantified using the LightCycler Instrument (Roche Applied Sciences). Fluorescence-labeled hybridization probes providing maximum specificity were used for product detection. Calculation of sample concentrations were performed using the fit-point algorithm. The porphobilinogen deaminase (h-PBGD) gene, a low-copy housekeeping gene, was used as an external standard and quantified using the h-PBGD Housekeeping Gene Set (Roche-Applied Science). Relative transcript levels were calculated as ratios of *LRRK2*/PBGD normalized to adult whole brain adult expression as 100%.

Acknowledgments

The three corresponding authors are equally responsible senior authors. Drs. Gasser and Meitinger led the genetic investigations, and Dr. Wszolek was responsible for the longitudinal clinical study of the large families. We gratefully acknowledge the participation of all patients and family members. The work was funded in part by the German National Genome Network (NGFN; Grant number 01GS0116, German Ministry for Education and Research), the Competence Network Parkinson's disease (German Ministry for Education and Research, Grant number 01GI0201), the National Institutes of Health, the National Institute of Neurologic Disorders and Stroke, the Morris K. Udall Parkinson's Disease Research Center of Excellence (grant awarded to the Mayo Clinic Jacksonville, P50-NS40256-06), the Canadian Institutes for Health Research, and the Pacific Parkinson's Research Institute. Dr. Y. Tsuboi, Mrs. E. Wszolek, S. Calne, B. Pfeiffer, A. Strongosky, and J. Searcy helped to collect clinical material. Drs. P. McGeer, K. Berry, and R. McComb provided the pathology material for reevaluation, and Dr. Y. Tsuboi assisted in neuropathologic studies.

Received: October 22, 2004
Revised: October 28, 2004
Accepted: November 1, 2004
Published: November 17, 2004

References

Arvanitakis, Z., and Wszolek, Z.K. (2001). Recent advances in the understanding of tau protein and movement disorders. Curr. Opin. Neurol. *14*, 491–497.

Bonifati, V., Rizzu, P., van Baren, M.J., Schaap, O., Breedveld, G.J., Kreiger, E., Dekker, M.C., Squitieri, F., Ibanez, P., Joose, M., et al. (2003). Mutations in the DJ-1 gene associated with autosomal recessive early-onset Parkinsonism. Science *299*, 256–259.

Bosgraaf, L., and Van Haastert, P.J. (2003). Roc, a Ras/GTPase domain in complex proteins. Biochim. Biophys. Acta *1643*, 5–10.

Chartier-Harlin, M.C., Kachergus, J., Roumie, R.C., Mouroux, V., Douay, X., Lincoln, S., Levecque, C., Larvor, L., Andrieux, J., Hulihan, M., et al. (2004). Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet *364*, 1167–1169.

Dickson, D.W. (1999). Neuropathologic differentiation of progressive supranuclear palsy and corticobasal degeneration. J. Neurol. *246* (*Suppl 2*), 6–15.

Duda, J.E., Giasson, B.I., Mabon, M.E., Miller, D.C., Golbe, L.I., Lee, V.M., and Trojanowski, J.Q. (2002). Concurrence of alpha-synuclein and tau brain pathology in the Contursi kindred. Acta Neuropathol. (Berl.) *104*, 7–11.

Farrer, M., Gwinn-Hardy, K., Hutton, M., and Hardy, J. (1999). The genetics of disorders with synuclein pathology and Parkinsonism. Hum. Mol. Genet. *8*, 81–85.

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.

Gasser, T., Müller-Myhsok, B., Wszolek, Z.K., Oehlmann, R., Calne, D.B., Bonifati, V., Bereznai, 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.

Ghetti, B., Hutton, M., and Wszolek, Z.K. (2003). Frontotemporal dementia and parkinsonism linked to chromosome 17 associated with tau gene mutations (FTDP-17). In Neurodegeneration: The Molecular Pathology of Dementia and Movement Disorders, D.W. Dickson, ed. (Basel, Switzerland: ISN Neuropath Press), pp. 86–102.

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.

Gotz, J., Chen, F., van Dorpe, J., and Nitsch, R.M. (2001). Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. Science *293*, 1491–1495.

Hicks, A.A., Petursson, H., Jonsson, T., Stefansson, H., Johannsdottir, H.S., Sainz, J., Frigge, M.L., Kong, A., Gulcher, J.R., Stefansson, K., and Sveinbjomsdottir, S. (2002). A susceptibility gene for late-onset idiopathic Parkinson's disease. Ann. Neurol. *52*, 549–555.

Hubbard, S.R., and Till, J.H. (2000). Protein tyrosine kinase structure and function. Annu. Rev. Biochem. *69*, 373–398.

Hutton, M., Lendon, C.L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A., et al. (1998). Association of missense and 5′-splice-site mutations in *tau* with the inherited dementia (FTDP-17). Nature *393*, 702–705.

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.

Ishizawa, T., Mattila, P., Davies, P., Wang, D., and Dickson, D.W. (2003). Colocalization of tau and alpha-synuclein epitopes in Lewy bodies. J. Neuropathol. Exp. Neurol. *62*, 389–397.

Kobe, B., and Kajava, A.V. (2001). The leucine-rich repeat as a protein recognition motif. Curr. Opin. Struct. Biol. *11*, 725–732.

Lewis, J., Dickson, D.W., Lin, W.-L., Chisholm, L., Corral, A., Jones, A.G., Yen, S.-H., Sahara, N., Skipper, L., Yager, D., et al. (2001). Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science *293*, 1487–1491.

Paisán-Ruíz, C., Jain, S., Evans, E.W., Gilks, W.P., Simón, J., van der Brug, M., López de Munain, A., Aparicio, S., Gil, A.M., Khan, N., et al. (2004). Cloning of the gene containing mutations that cause *PARK8*-linked Parkinson's disease. Neuron *44*, this issue, 595–600. Published online October 22, 2004. 10.1016/S0896627304006890

Pankratz, N., Nichols, W.C., Uniacke, S.K., Halter, C., Rudolp, A., Shults, C., Conneally, P.M., and Foroud, T. (2003). Significant linkage of Parkinson disease to chromosome 2q36–37. Am. J. Hum. Genet. *72*, 1053–1057.

Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boye, R.R., et al. (1997). Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science *276*, 2045–2047.

Ridley, A.J. (2001). Rho family proteins: coordinating cell responses. Trends Cell Biol. *11*, 471–477.

Scott, W.K., Grubber, J.M., Conneally, P.M., Small, G.W., Hulette, C.M., Rosenberg, C.K., Saunders, A.M., Roses, A.D., Haines, J.L., and Pericak-Vance, M.A. (2000). Fine mapping of the chromosome 12 late-onset Alzheimer disease locus: potential genetic and phenotypic heterogeneity. Am. J. Hum. Genet. *66*, 922–932.

Singleton, A.B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., et al. (2003). α-synuclein locus triplication causes Parkinson's disease. Science *302*, 841.

Smith, T.F., Gaitatzes, C., Saxena, K., and Neer, E.J. (1999). The WD repeat: a common architecture for diverse functions. Trends Biochem. Sci. *24*, 181–185.

Tang, K., Fu, D.J., Julien, D., Braun, A., Cantor, C.R., and Koster, H. (1999). Chip-based genotyping by mass spectrometry. Proc. Natl. Acad. Sci. USA *96*, 10016–10020.

Valente, E.M., Abou-Sleiman, P.M., Caputo, V., Muqit, M.M., Harvey, K., Gispert, S., Ali, Z., Del Turco, D., Bentivoglio, A.R., Healy, D.G., et al. (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science *304*, 1120–1122.

Wszolek, Z., Vieregge, P., Uitti, R.J., Gasser, T., Yasuhara, O., McGeer, P., Berry, K., Calne, D., Vingerhoets, F.J.G., Klein, C., and Pfeiffer, R.F. (1997). German-Canadian family (family A) with parkinsonism, amyotrophy, and dementia—longitudinal observations. Parkinsonism Relat. Disord. *3*, 125–139.

Wszolek, Z.K., Pfeiffer, R.F., Tsuboi, Y., Uitti, R.J., McComb, R.D., Stoessl, A.J., Strongosky, A.J., Zimprich, A., Muller-Myhsok, B., Farrer, M.J., et al. (2004). Autosomal dominant parkinsonism associated with variable synuclein and tau pathology. Neurology *62*, 1619–1622.

Zimprich, A., Muller-Myhsok, B., Farrer, M., Leitner, P., Sharma, M., Hulihan, M., Lockhart, P., Strongosky, A., Kachergus, J., Calne, D.B., et al. (2004). The PARK8 locus in autosomal dominant Parkinsonism: confirmation of linkage and further delineation of the disease-containing interval. Am. J. Hum. Genet. *74*, 11–19.

Accession Numbers

The GenBank accession numbers are AY792511 for the human gene (*LRRK2*) and AY792512 for the mouse gene (*Lrrk2*).