Neuron, Vol. 44, 595–600, November 18, 2004, Copyright ©2004 by Cell Press

Cloning of the Gene Containing Mutations that Cause *PARK8*-Linked Parkinson's Disease

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Summary

Parkinson's disease (PD; OMIM #168600) is the second most common neurodegenerative disorder in the Western world and presents as a progressive movement disorder. The hallmark pathological features of PD are loss of dopaminergic neurons from the substantia nigra and neuronal intracellular Lewy body inclusions. Parkinsonism is typically sporadic in nature; however, several rare familial forms are linked to genetic loci, and the identification of causal mutations has provided insight into the disease process. PARK8, identified in 2002 by Funayama and colleagues, appears to be a common cause of familial PD. We describe here the cloning of a novel gene that contains missense mutations segregating with *PARK8*-linked PD in five families from England and Spain. Because of the tremor observed in PD and because a number of the families are of Basque descent, we have named this protein dardarin, derived from the Basque word *dardara*, meaning tremor.

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Introduction

Parkinson's disease (PD) is a severe, progressive, neurodegenerative disorder characterized clinically by tremor, bradykinesia, rigidity, and postural instability. The disease is diagnosed postmortem by pallor of the substantia nigra pars compacta and the presence of brainstem Lewy bodies. While current therapies alleviate the motor symptoms of PD, they are largely palliative.

The identification of rare familial forms of parkinsonism and subsequent cloning of causal genetic mutations has had significant impact on our understanding of the molecular mechanisms underlying typical PD. The discovery of mutations in genes encoding α-synuclein (Polymeropoulos et al., 1997), parkin (Kitada et al., 1998), DJ-1 (Bonifati et al., 2003), and PINK1 (Valente et al., 2004) has provided a basis for much of the ongoing molecular work in the PD field and facilitates disease modeling and the design and testing of targeted therapeutics. Each new gene implicated in the etiology of PD sheds light on the disease process; hence, there is much interest in the identification of novel gene mutations.

In 2002, linkage of PD in a large family with apparently autosomal dominant disease was ascribed to a 13.6cM region at chromosome 12p11.2-q13.1 containing approximately 116 genes and given the name *PARK8* (Funayama et al., 2002). This linkage was independently replicated, and to date eight families have been convincingly associated with this locus (Funayama et al., 2002; N.K., S.J., N. Pavese, J. Holton, L.H. Eunson, M.G. Sweeney, W.P.G., J. Vaughan, J. Gayton, G. Lennox, T. Revesz, A.B.S., D.N., D. Brooks, A.L., P. Piccini, M.B. Davis, and N.W.W., unpublished; C.P.-R., A.S.P., A.L.d.M., I.M.C., A.M.G., J.F.M.-M., J.P.-T., unpublished; Zimprich et al., 2004).

While most *PARK8* families described possess a clinical phenotype of typical PD, the pathological findings range from pure nigral degeneration in the absence of Lewy body pathology as noted in the Sagamihara kindred (Funayama et al., 2002) to typical Lewy body PD in the British family, PL (N.K., S.J., N. Pavese, J. Holton,

Table 1. Markers Used to Fine-Map the Candidate Interval and Determine Interfamily Shared Haplotype and the Boundaries of This Haplotype

			BASQUE FAMILY ID			
IDENTIFIER	bp	CONSENSUS	UGM3	UGM4	UGM5	UGM6
D12S1698	30855986	-	122	126	118	124
D12S1621	31754700	-	191	191	191	191
rs1523118	37515966	-	T	T	C/T[a]	T
D12S331	37547321	-	177	177	177	177
rs11169992	37603474	-	C		C	C
rs10876410	37708557	-	T	A/T[a]	T	A
rs10876646	37887093	-	T	T	T	T
rs10747736	37912177	-	T	C	T	T
rs10747736	37912177	-	T		T	T
rs10876876[b]	38011263	-	A	-	A	A
rs11171789[b]	38024258	-	T	C	T	T
K543R[b]		-	G	A	G	G
rs10876886[b]	38035530	-	C	A/C[a]	C	C
rs11172282	38161804	-	G	C/G[a]	G	G
rs11172541	38229025	-	A	-	A	A
rs10877201	38298564	-	C	T/C[a]	C	C
rs4548690	38475137	-	T	C	T	T
rs7294916	38494630	T	T	T	T	T
rs4423249	38554997	T	T	T	T	T
rs515205	38689229	A	A	A/G[a]	A	A
rs937110	38815159	C	C	C/G[a]	C	C
SNP1	38815163	T	T	T	T	T
rs4768224	38947670	T	T	T	T	T
IVS13-54[c]	38943803	C	C/G[a]	C	C	C
IVS13+68[c]	38944038	G	G/A[a]	G	G	G
R1396G[c]	**38990503**	**C**	**C**	**C**	**C**	**C**
M1601T[c]	39011294	G	G	G	G	G
rs12423567	39063583	G	G	-	-	G
rs12423567	39063583	G	G	G	G	G
rs10784616	39117987	C	C	C	C	C
rs11612876	39256712	T	T	-	T	T/C[a]
rs10784800	39386364	C	C	C	C	C
rs10879192	39471471	C	C	C/T[a]	C	C
D12S1668	39489795	235	235	235	235	235
D12S1653	41093561	-	215	215	203	215

The black outline indicates the extent of the haplotype common between each Basque family.
[a] Phase not determined.
[b] Within KIF21A.
[c] Within DKFZp434H111.

L.H. Eunson, M.G. Sweeney, W.P.G., J. Vaughan, J. Gayton, G. Lennox, T. Revesz, A.B.S., D.N., D. Brooks, A.L., P. Piccini, M.B. Davis, and N.W.W., unpublished). A third family, the Western Nebraska kindred, with suggestive linkage to *PARK8*, showed a wide range of clinical and pathological phenotypes (Wszolek et al., 2004).

We have previously identified four families from the Basque region of Spain and a family from the United Kingdom that each showed positive linkage to the *PARK8* locus (N.K., S.J., N. Pavese, J. Holton, L.H. Eunson, M.G. Sweeney, W.P.G., J. Vaughan, J. Gayton, G. Lennox, T. Revesz, A.B.S., D.N., D. Brooks, A.L., P. Piccini, M.B. Davis, and N.W.W., unpublished; C.P.-R., A.S.P., A.L.d.M., I.M.C., A.M.G., J.F.M.-M., J.P.-T., unpublished). Using these families, we undertook a methodical assessment of candidate genes in an attempt to identify the underlying genetic lesion responsible for disease.

Results

In the course of sequencing the kinesin gene *KIF21A* as a candidate for *PARK8* disease, we identified a SNP, K543R (Basque allele frequency 0.01), segregating with disease in three apparently unrelated Basque families (UGM003, UGM005, and UGM006) (C.P.-R., A.S.P., A.L.d.M., I.M.C., A.M.G., J.F.M.-M., J.P.-T., unpublished) but absent from the fourth *PARK8*-linked Basque family we were studying, UGM004. Given the rarity of this variant, these data suggested that UGM003, UGM005, and UGM006 are ancestrally related and could be used to determine a minimal interkindred disease haplotype. In the first instance, we chose to type SNPs between D12S331 and D12S1668 in order to verify that there was indeed a common haplotype among the three families and in the hope that these data would limit this haplotype further. We identified a variant (rs10876410) that delimited the critical interval on the p arm of the disease haplotype shared by these three families (Table 1). However, more importantly, we demonstrated that all four Basque families we were studying shared a smaller disease haplotype, consisting of 15 SNPs and one microsatellite and delimited by flanking markers rs4548690 and D12S1653. This reduced the critical disease interval to 2.6 Mb, a region that contains only 11 genes and predicted transcripts (Figure 1).

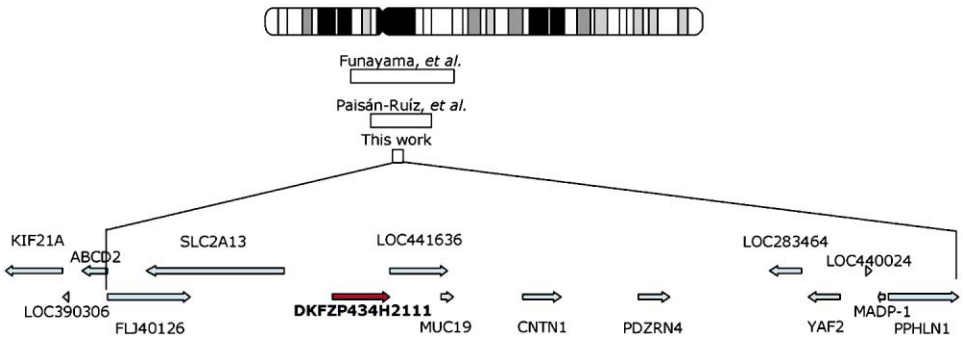

Figure 1. Ideogram of PARK8 Candidate Region

(A) Ideogram of chromosome 12 showing the linked areas defined by Funayama et al. in 2002, the refined area reported here, and the region shared by all four Basque families.

(B) Schematic representation of the known genes and predicted transcripts in the area shared by the Basque families. In red, the putative transcript where mutations in *PARK8*-linked families were identified.

We undertook a systematic sequence analysis of these genes and predicted open reading frames in affected members of the four Basque families and in a fifth large family from the UK, kindred PL. We identified segregating mutations within a putative kinase domain-containing transcript, DKFZp434H2111, which we chose to call *PARK8*. *PARK8* is a 9 kb predicted transcript, which contains a 7449 bp open reading frame encoding a 2482 amino acid protein that includes a leucine-rich repeat, a kinase domain, a RAS domain, and a WD40 domain (Figure 2). We identified a variant R1396G that segregates with disease in all four Basque families and a variant Y1654C in the same gene that segregates with disease in family PL (Figures 2 and 3). We failed to identify either of these variants in 1300 chromosomes from North American controls and 160 chromosomes from Basque controls.

In order to assess the prevalence of these two mutations in PD patients, we examined 137 Basque PD patients (30 with a family history and 107 sporadic cases) and 94 PD patients from North America with a positive family history for disease. We did not identify the Y1654C mutation in any patients. We identified 11 Spanish PD patients, 10 Basque, who carried the R1396G mutation; of these, 6 had a positive family history for PD.

We proceeded to verify that this gene is expressed in brain. Analysis of the multitissue Northern blots probed with a sequenced PCR product spanning both mutation sites indicated the presence of a 9 kb transcript (Figure 4) in all tissue types examined. These data also show that this gene is expressed throughout the adult human brain but at a lower level than in heart and liver. We successfully amplified overlapping cDNA fragments across the predicted 9 kb transcript (Figure 5).

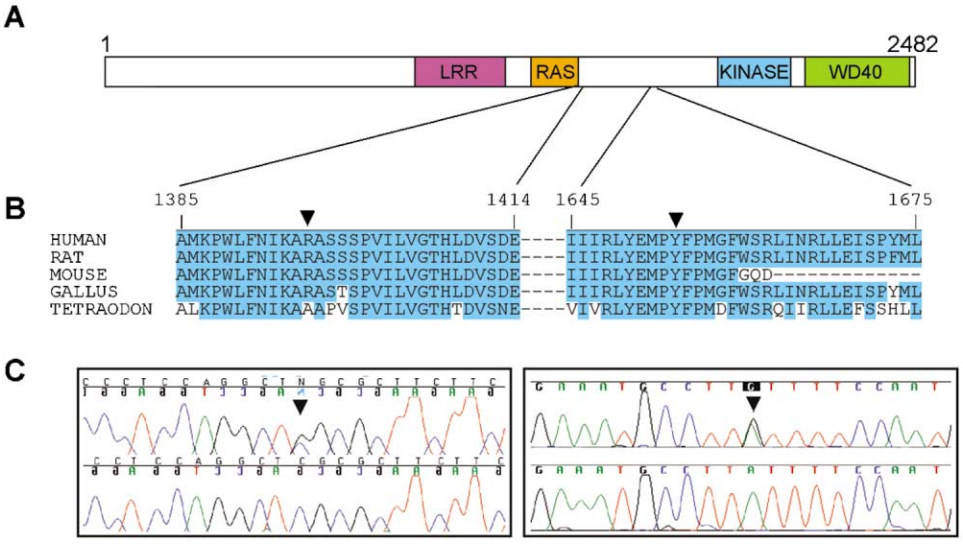

Figure 2. Mutations in Dardarin that Are Linked to PARK8 PD

(A) Schematic of dardarin structure.

(B) Mutations R1396G and Y1654C are indicated by black arrowheads. Human dardarin protein aligned with other orthologs from rat (XP_235581), mouse (BAC28700), chicken (XP_425418), and tetraodon (CAG05593). Amino acid number is based on the human protein.

(C) Chromatograms showing mutant sequence (top) and wild-type sequence (bottom) of R1396G (left) and Y1654C (right).

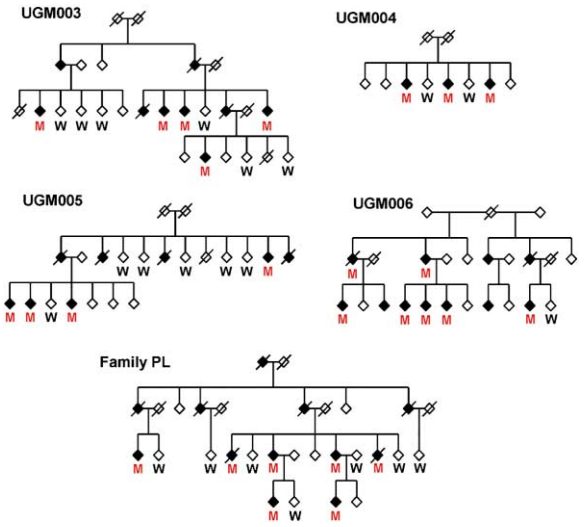

Figure 3. PARK8-Linked Pedigrees

UGM003, UGM004, UGM005, and UGM006 originate from the Basque region; kindred PL was collected in the United Kingdom. WT indicates a mutation-negative subject, M indicates a subject carrying a heterozygous mutation (R1396G UGM003, UGM004, UGM005, and UGM006; Y1654C Family PL). Gender has been masked to protect the anonymity of the families. Currently unaffected mutation carriers have not been shown; in all instances these individuals have not yet reached the maximum age at onset observed.

Discussion

We provide here data that implicates mutations in the novel protein dardarin as a cause of *PARK8* PD. This is supported by four pieces of genetic evidence; first, we have identified two missense mutations segregating with disease in five families previously linked to PARK8, a total of 26 mutation-positive affected family members; second, with the exception of LOC441636 (which is similar to submaxillary apomucin and thus a gene unlikely to play a role in neurodegeration), we have sequenced every known gene and predicted transcript in the candidate region, as defined by the minimal interfamily haplotype shared by the Basque kindreds (Table 1), and the gene encoding dardarin was the only gene that contained variants not identified in the general population; third, these mutations are not present in 1300 North American control and 160 Basque control chromosomes; and fourth, we identified the 1396G mutation in 8% of a series of 137 apparently unrelated Basque PD cases. As is the case in most dominant genetic diseases, the mutations we describe here alter residues that are conserved across species, with the exception of the R1396 residue, which is an alanine in *Tetraodon nigroviridis*.

We have identified seven affected mutation-positive family members in kindred PL and seven unaffected members who do not carry the mutation. These data support the view that this variant is 100% penetrant in this family. In the Basque pedigrees excluding all unaffected family members below the maximum observed age at onset of 79 years, the penetrance of this mutation is also 100%. These data contrast with the 70% penetrance originally observed in the Sagamihara kindred (Funayama et al., 2002).

The data presented here suggest that R1396G is a relatively common cause of PD in the Basque population, accounting for approximately 8% of disease in the series we examined. Furthermore, our data indicate that this mutation can result in a late-onset disease, which can reduce the apparent familiality of this form, as mutation carriers may die before manifesting or being diagnosed with the signs and symptoms of PD. This is highlighted by the fact that five of the identified 1396G-positive PD cases presented in the absence of a family history for disease. Although the proportion of cases carrying this mutation may be high because of a founder effect in a relatively isolated population, it is reasonable to suspect that other sporadic cases in the general population will also harbor *PARK8* mutations.

The phenotype noted in the Basque and British families is remarkably similar. The mean age at onset is around 65 years and there is a benign course with excellent response to low doses of L-Dopa; furthermore, the majority of patients in the British and Basque families present with unilateral leg or hand tremor and the absence of cognitive impairment.

Our analyses suggest that *PARK8* is expressed throughout the adult brain. Although this gene appears expressed at higher levels in other tissues, this is not unusual for genes that cause neurodegenerative diseases (Li et al., 1995). Based on Northern blot data, the major mutation-containing transcript is the 9 kb species, although our data indicate that there may be some additional alternative splicing in this gene. Three other pieces of evidence support the presence of a 9 kb transcript: first, there are numerous overlapping ESTs and cDNA clones extending across the predicted mRNA and these clones and ESTs overlap by multiple exons; second,

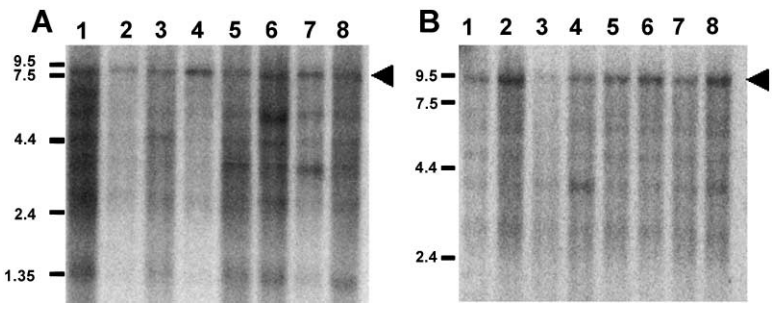

Figure 4. Northern Blot Analysis of Dardarin

Multiple tissue Northern blots (BD Biosciences) probed for *dardarin* with a 1 kb probe amplified across the two exons that contain mutations. Blot A: lane 1, heart; lane 2, brain (whole); lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, pancreas. Blot B is a multiregion brain blot: lane 1, cerebellum; lane 2, cerebral cortex; lane 3, medulla; lane 4, spinal cord; lane 5, occipital pole; lane 6, frontal lobe; lane 7, temporal lobe; lane 8, putamen. A major transcript of 9 kb was identified (black arrowhead) in addition to minor smaller transcripts.

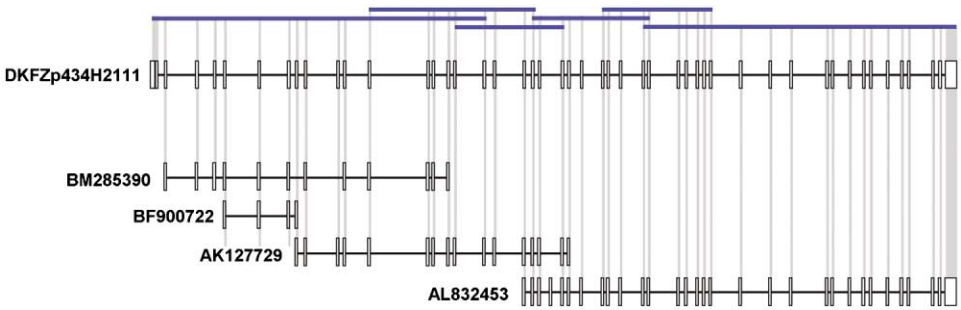

Figure 5. Predicted Transcript Based on Alignment of Multiple Human Multiexon ESTs and Fully Sequenced Human cDNA Clones

The most informative overlapping clones and ESTs are shown. Tiled blue lines represent overlapping fragments successfully amplified from cDNA isolated from M17 neuroblastoma cells and from a brain marathon-ready cDNA library (BD Biosciences).

orthologous proteins with identical domain structures and of equivalent length are predicted in *Mus musculus* (Caenepeel et al., 2004), *Rattus norvegicus* (XP_235581), and *Tetraodon nigroviridis* (CAG05593); and third, we have successfully amplified overlapping cDNA fragments across the transcript.

Although the function of dardarin is unknown, the structure suggests that this protein is probably a cytoplasmic kinase. Indeed, using a bioinformatic approach, *PARK8*, along with a similar predicted gene, *LRRK1,* was previously mapped as a tyrosine kinase-like (TKL) protein (Manning et al., 2002). Because phosphorylation of proteins has been implicated in the pathogenesis of neurodegenerative disease, it is particularly tempting to hypothesize a role for dardarin in the phosphorylation of proteins central to PD, such as α-synuclein and Tau. Taking this into account, the description of myriad *PARK8* pathological phenotypes ranging from pure nigral degeneration to pathologies with varied protein deposition, including synucleinopathy and tauopathy, is intriguing and these data raise the possibility that dardarin may present a link between these key molecules. Some care must be exercised here; no conclusions about these described pathologies can be made until each examined case is shown to possess mutations and the prevailing pathology is shown to be above and beyond that observed in normal aging. In three common neurodegenerative diseases, i.e., Alzheimer's disease, Huntington's disease, and PD, dominant mutations occur in genes that encode the predominant deposited protein species (Chartier-Harlin et al., 1991; Huntington Group, 1993; Polymeropoulos et al., 1997; Singleton et al., 2004). The exceptions to this rule are presenilins 1 and 2 (Rogaev et al., 1995; Sherrington et al., 1995), where mutations occur in proteins that are involved in the processing of the deposited species. Analogy might suggest that dardarin is involved in the processing, expression, or clearance of α-synuclein.

In summary, we present here compelling evidence implicating mutations in a novel gene containing a leucine-rich repeat, kinase domain, RAS domain, and WD40 domain as the first genetic cause of late-onset familial and sporadic PD. We have named this gene *PARK8* and the protein dardarin. These data will impact not only patients and their families but will open novel avenues of research aimed at identifying and ultimately halting the molecular events that lead to PD.

Experimental Procedures

Candidate Gene Sequencing

Genomic primer design for all genes was performed using ExonPrimer (http://ihg.gsf.de/ihg/ExonPrimer.html). Each exon and at least 50 bp of flanking intronic sequence was PCR amplified using primer pairs listed (available upon request) from genomic DNA. PCR amplification was carried out using Qiagen *taq* polymerase, and 10 pmol of both forward and reverse primers as per the manufacturer's instructions (Qiagen Inc, CA). In the first instance, DNA from two affected, disease haplotye-carrying members of each family was used. Any variants of interest were then assessed for segregation in the remaining family members. Each product was sequenced using forward and reverse primers with Applied Biosystems BigDye terminator v3.1 sequencing chemistry as per the manufacturer's directions. The resulting reactions were run on an ABI3100 genetic analyzer and analyzed with Sequencher software (Genecodes, VA).

SNP Analyses

In order to assess the extent of the interkindred disease haplotype, SNPs were selected from dbSNP between markers D12S331 and D12S1668. SNPs were selected from dbSNP, choosing those verified variants that were likely to be informative. SNPs were genotyped by PCR amplification and direct sequencing. All available family members of the Basque kindreds were typed for these SNPs in order to maximize our ability to obtain phase.

Mutation Assay

Mutations R1396G and Y1654C were assessed in 650 North American control subjects, 80 Basque control subjects, 94 North American PD cases with a positive family history of PD, and 137 Spanish PD cases with and without a family history of PD. To identify the R1396G mutation, a genomic fragment was amplified using primer pair DKFZp434 30F 5′-TCAACAGGAATGTGAGCAGG-3′ and DKFZp434 30R 5′-CCCACAATTTTAAGTGAGTTGC-3′. The resulting 386 bp fragment was digested with *Bst*UI at 37°C overnight and electrophoresed on a 2% agarose gel containing ethidium bromide. The products were assessed by UV visualization. The presence of the 1396G mutation resulted in an uncut full-length product of 386 bp, whereas the wild-type fragment cuts to produce 72 bp and 314 bp bands. A mutation-positive assay result was confirmed by sequencing as described above. There was no restriction assay readily available for the 1654C mutation so the genomic fragment was PCR amplified as described above from each sample using primer pair DKFZp434 34F 5′-CATTTGCTCAACAAGGTTGG-3′ and DKFZp434 34R 5′-ATG CCATCTCCCTAATTTCTC-3′ and sequenced as described above.

Northern Blot

A 1011 bp probe that covered both mutation containing exons was generated from cDNA template using 20 pmol of primers CDNAex29F 5′-GAGCATTGTACCTTGCTGTC-3′ and CDNAEx34R 5′-GACAATAAGCTTCAGGAGACC-3′ and the expand high-fidelity PCR system as per the manufacturer's instructions (Roche Diagnostics, CA). The resulting product was gel excised, labeled with

[α-^{32}P]dCTP, and column purified using standard protocols. The labeled probe was hybridized to multitissue Northern blots (BD Biosciences, CA) as per the manufacturer's instructions. Following medium stringency washing, the blots were exposed to phosphor screens overnight and an image captured the next day on a Storm phosphorimager.

cDNA Amplification

Total RNA was extracted from an M17 neuroblastoma cell line, and first strand synthesis of cDNA was performed with oligo dT priming using Superscript III as per the manufacturer's instructions (Invitrogen, CA). The resulting cDNA and cDNA from a marathon RACE ready adult human brain library (BD Biosciences) was used as template to amplify overlapping fragments of the predicted 9 kb transcript. Overlapping primers within each exon were designed using Generunner (http://www.generunner.com), and PCR was carried out using the Qiagen *taq* polymerase kit and 10 pmol of both forward and reverse primers as per the manufacturer's instructions (Qiagen).

Acknowledgments

We would like to thank the patients and their families for participating in this research. We would like to thank Graham Lennox for aiding the family collection. A.B.S. would like to thank Drs. John Hardy and Mark Cookson for critical discussion of this manuscript. Thanks to the Reta Lila Weston Trust for Medical Research for funding S.J., A.L., and R.d.S. Thanks to the Parkinson's Disease Society for funding N.W.W. and D.N. Work at the laboratory of J.P.-T. was funded by a grant from the Fundación Ramón Areces (DI-TER PARKINSON) and from the Spanish Ministerio de Educación y Ciencia (GEN2001-4851-C06). C.P.-R. is a recipient of a fellowship from the Spanish Ministerio de Educación y Ciencia. J.S. is a recipient of a predoctoral I3P fellowship from the CSIC. J.P.-T. is a member of the Grupos de Excelencia network of the Generalitat Valenciana (GRUPOS03/015).

Received: October 15, 2004
Revised: October 20, 2004
Accepted: October 20, 2004
Published online: October 22, 2004

References

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.

Caenepeel, S., Charydczak, G., Sudarsanam, S., Hunter, T., and Manning, G. (2004). The mouse kinome: discovery and comparative genomics of all mouse protein kinases. Proc. Natl. Acad. Sci. USA *101*, 11707–11712.

Chartier-Harlin, M.C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., Goate, A., Rossor, M., Roques, P., and Hardy, J. (1991). Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. Nature *353*, 844–846.

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.

The Huntington's Disease Collaborative Research Group. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell *72*, 971–983.

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.

Li, J., Ma, J., and Potter, H. (1995). Identification and expression analysis of a potential familial Alzheimer disease gene on chromosome 1 related to AD3. Proc. Natl. Acad. Sci. USA *92*, 12180–12184.

Manning, G., Whyte, D.B., Martinez, R., Hunter, T., and Sudarsanam, S. (2002). The protein kinase complement of the human genome. Science *298*, 1912–1934.

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.

Rogaev, E.I., Sherrington, R., Rogaeva, E.A., Levesque, G., Ikeda, M., Liang, Y., Chi, H., Lin, C., Holman, K., and Tsuda, T. (1995). Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. Nature *376*, 775–778.

Sherrington, R., Rogaev, E.I., Liang, Y., Rogaeva, E.A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., and Holman, K. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature *375*, 754–760.

Singleton, A., Myers, A., and Hardy, J. (2004). The law of mass action applied to neurodegenerative disease: a hypothesis concerning the etiology and pathogenesis of complex diseases. Hum. Mol. Genet. *13*, R123–R126.

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*, 1158–1160.

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. Erratum: Am. J. Hum. Genet. *75*, 534.

Note Added in Proof

The HUGO Gene Nomenclature Committee (http://www.gene.ucl.ac.uk/nomenclature/) has updated the name of this gene to Leucine Rich Repeat Kinase 2, gene symbol *LRRK2*. The gene name *PARK8* will be removed from usage upon publication of this manuscript. The protein name will remain dardarin.