

Localization of a Novel Locus for Autosomal Recessive Early-Onset Parkinsonism, *PARK6*, on Human Chromosome 1p35-p36

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The cause of Parkinson disease (PD) is still unknown, but genetic factors have recently been implicated in the etiology of the disease. So far, four loci responsible for autosomal dominant PD have been identified. Autosomal recessive juvenile parkinsonism (ARJP) is a clinically and genetically distinct entity; typical PD features are associated with early onset, sustained response to levodopa, and early occurrence of levodopa-induced dyskinesias, which are often severe. To date, only one ARJP gene, *Parkin*, has been identified, and multiple mutations have been detected both in families with autosomal recessive parkinsonism and in sporadic cases. The *Parkin*-associated phenotype is broad, and some cases are indistinguishable from idiopathic PD. In $\geq 50\%$ of families with ARJP that have been analyzed, no mutations could be detected in the *Parkin* gene. We identified a large Sicilian family with four definitely affected members (the Marsala kindred). The phenotype was characterized by early-onset (range 32–48 years) parkinsonism, with slow progression and sustained response to levodopa. Linkage of the disease to the *Parkin* gene was excluded. A genomewide homozygosity screen was performed in the family. Linkage analysis and haplotype construction allowed identification of a single region of homozygosity shared by all the affected members, spanning 12.5 cM on the short arm of chromosome 1. This region contains a novel locus for autosomal recessive early-onset parkinsonism, *PARK6*. A maximum LOD score 4.01 at recombination fraction .00 was obtained for marker *D1S199*.

Introduction

Parkinson disease (PD [MIM 168600]) is a common neurodegenerative disorder that usually occurs later in life, presenting with bradykinesia, resting tremor, muscular rigidity, and postural instability, with sustained response to levodopa. The pathological hallmarks are the occurrence of Lewy bodies (cytoplasmic eosinophilic inclusions) and neuronal loss, which affects the substantia nigra pars compacta (SNpc) extensively and other regions of the brain less specifically (Fearnley and Lees 1991). A genetic contribution to the etiology of PD is now well established and appears to be particularly important in cases with early onset of the disease (Tanner et al. 1999). The gene α -synuclein (Polymeropoulos et al. 1997) has been proved in families with autosomal dominant PD, and a second gene, that for ubiquitin carboxy-terminal hydrolase L1, has been implicated in disease causation (Leroy et al. 1998). Moreover, two other

genetic loci have been mapped: *PARK3* on chromosome 2p13 and *PARK4* on chromosome 4p14-16.3 (Gasser et al. 1998; Farrer et al. 1999). However, autosomal dominant forms are rare and seem to account for only a small number of families. Autosomal recessive juvenile parkinsonism (ARJP [MIM 600116]) is a distinct clinical and genetic entity within familial PD; it is characterized by typical PD features associated with early age at onset (<40 years), slow progression of the disease, sustained response to levodopa, early levodopa-induced complications (fluctuations and dyskinesias) that are often severe, hyperreflexia, and mild dystonia mainly in the feet (Yamamura et al. 1973; Ishikawa and Tsuji 1996). Pathological studies have demonstrated a highly selective degeneration of dopaminergic neurons in the SNpc and the locus coeruleus and an absence of Lewy bodies (Takahashi et al. 1994). ARJP was initially described in Japanese patients (Yamamura et al. 1973). Recently, Matsumine and coworkers (1997) identified a novel gene, *Parkin*, located on chromosome 6q25.2-27 (Kitada et al. 1998). A wide variety of mutations have been detected in families with autosomal recessive parkinsonism and in sporadic cases of different ethnic origin (Hattori et al. 1998; Leroy et al. 1998; Abbas et al. 1999; Nisipeanu et al. 1999; Lücking et al. 2000). The inclusion criteria adopted in these studies were age at onset

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≤45 years (in at least one affected member of the family, for familial cases) and unaffected parents. It has been proved that, especially in non-Japanese cases, mutations in the *Parkin* gene are associated with a wide range in both age at onset (≤64 years) and clinical signs, and they can result in parkinsonism that is clinically indistinguishable from PD, making genotype-phenotype correlation uncertain (Abbas et al. 1999; Klein et al. 2000). Nevertheless, only ~50% of cases of autosomal recessive early-onset parkinsonism are related to the *Parkin* gene, and in most patients, especially sporadic cases, the contribution of genetic factors remains to be elucidated (Lücking et al. 2000). It is likely that a novel, as yet unidentified, gene is responsible for early-onset parkinsonism in some of these cases. We mapped a novel locus, named “*PARK6*,” to a 12.5-cM region on chromosome 1p35-36, in a large Italian family with autosomal recessive early-onset parkinsonism, confirming the genetic heterogeneity of this disorder.

Subjects and Methods

Subjects

A large Italian family (the Marsala kindred) comprising 122 members was investigated. The methodology and the clinical characterization of the family have been reported elsewhere (Valente et al. 1999). We examined 35 subjects, including 30 family members and 5 spouses. After informed consent was obtained, venous-blood samples were taken from 27 family members and the 5 spouses, for DNA analysis.

DNA and Linkage Analysis

DNA was extracted from leukocytes, by standard techniques. Exclusion of linkage between the disease and *PARK2* on chromosome 6q has been reported elsewhere (Valente et al. 1999). A simulation study performed with the program SLINK (Ott 1989) revealed a maximum expected LOD score of 4.2 at recombination fraction (θ) .00. The family was then considered suitable for a genome-wide homozygosity mapping (Lander and Botstein 1987). In the first screen, only the four definitely affected individuals were genotyped. We analyzed 400 highly polymorphic fluorescent microsatellite markers spanning the 22 autosomes and having an average distance of 10 cM (Linkage Mapping Set version 2; Applied Biosystems). Microsatellite markers were amplified from genomic DNA by the PCR technique, as specified by the manufacturers, and were electrophoresed on a denaturing acrylamide gel by a 377 XL DNA sequencer (Applied Biosystems). DNA fragment-size analysis was performed semiautomatically by GENESCAN and GENOTYPER software (Applied Biosystems), to determine genotypes.

When all the affected individuals were shown to be

homozygous for the same allele at a given marker, the surrounding region was saturated with closely spaced microsatellite markers. All the available family members were then genotyped, and haplotypes were constructed manually. Phase was assigned on the basis of the minimum number of recombinants.

Two-point LOD scores were generated using the FASTLINK version of the MLINK program (Cottingham et al. 1993; Dwarkadas et al. 1994), an assumption of equal recombination rate for the two sexes, autosomal recessive inheritance, reduced penetrance (.90), gene frequency .001, and equal allele frequencies for each marker. Loops were broken by the automated loop-breaking procedure described by Becker and co-workers (1998). Marker order and genetic distances were based on the chromosome 1 genetic map of the Center for Medical Genetics, Marshfield Medical Research Foundation, and on the chromosome 1 physical map of the National Center for Biotechnology Information, available at The Human Genome website.

Results

Clinical Analysis

A simplified pedigree of the family is shown in figure 1. The age at onset in affected subjects was 32–48 years. The patients presented with the typical parkinsonian phenotype, including slow progression of the disease, sustained response to levodopa, and occurrence of levodopa-associated dyskinesias of variable severity. Foot dystonia and sleep benefit were not observed. No pathological data are available.

Both the high frequency of consanguineous marriages within the family and the presence of affected members of both sexes in only one generation strongly suggest an autosomal recessive mode of inheritance. Moreover, genealogic records permitted us to trace the origin of the family to a common ancestral couple seven generations back (18th century). A summary of the clinical presentation in the family and of the Hoehn and Yahr (1967) stage (on-state) are given in table 1.

Homozygosity Mapping and Linkage Analysis

Linkage to *PARK2* has been excluded by a previous study (Valente et al. 1999). Given the autosomal recessive pattern of inheritance, we expected homozygosity at the level of the disease locus in affected individuals, owing to identity by descent from a common progenitor (Lander and Botstein 1987). In the four definitely affected family members, 400 microsatellite markers covering all autosomes were analyzed. These individuals were homozygous for the same allele at seven marker loci, located on chromosomes 1, 8, 10, 14, and 19. The regions surrounding these seven loci and all regions sur-

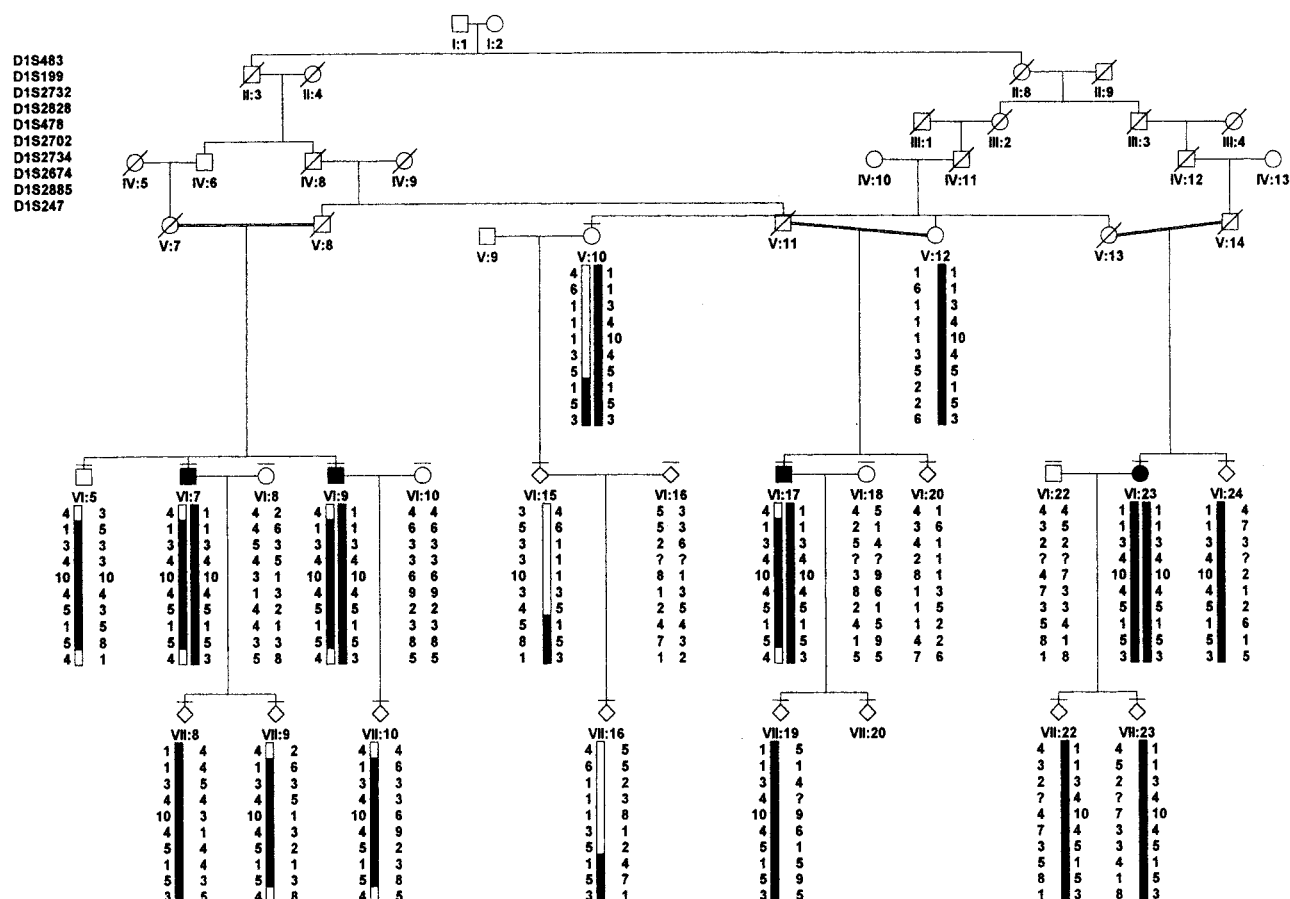


Figure 1 Simplified pedigree of the family, and haplotypes of marker loci spanning the linked region on chromosome 1p35-36. Black symbols denote definitely affected individuals, and deceased members are denoted by a diagonal bar. A short thin horizontal bar above a symbol indicates a family member who was examined clinically. The black bar denotes the disease-associated haplotype. To protect patient confidentiality, a diamond symbol has been used to mask the sex of some unaffected individuals.

rounding noninformative markers were saturated with closely spaced microsatellite markers. All available family members were genotyped, and haplotypes were constructed. A combination of negative LOD scores and the detection of different haplotypes carried by the affected individuals allowed exclusion of all the autosomes, except for a region on the short arm of chromosome 1. All markers spanning this candidate interval produced positive LOD scores, with a maximum LOD score of 4.01 ($\theta = .00$), between the disease and marker *D1S199* (table 2). Calculation of pairwise LOD scores under different penetrance values and under the assumption of affected individuals-only analysis did not result in a significant change (data not shown). All affected individuals in the family shared a region of homozygosity, between *D1S199* and *D1S2885* (fig. 1), allowing the identification of a 12.5-cM interval containing a novel gene (i.e., *PARK6*) for autosomal recessive parkinsonism. The upper and lower extents of the region are determined by recombinations occurring between markers

D1S483 and *D1S199* (telomeric end) and between markers *D1S2885* and *D1S247* (centromeric end), respectively, which were detected in subjects VI:7, VI:9, and VI:17.

Discussion

We have identified, by homozygosity mapping, a second locus for autosomal recessive early-onset parkinsonism, a locus named "*PARK6*," on the short arm of chromosome 1 in a family from Sicily. The phenotype is characterized by early-onset parkinsonism with slow progression, sustained response to levodopa, and frequent levodopa-induced fluctuations and dyskinesias. Other signs often reported in ARJP, such as dystonia at onset and sleep benefit, are absent. The clinical picture in this family overlaps with that described in *Parkin*-positive families of non-Japanese origin (Abbas et al. 1999). Lücking and coworkers screened 73 families with onset age ≤ 45 years in at least one affected member and

Table 1**Clinical Phenotype in Four Individuals with PD**

PATIENT CHARACTERISTICS	STATUS OF AFFECTED FAMILY MEMBER ^a			
	VI:7	VI:9	VI:17	VI:23
Sex	M	M	M	F
Age at onset (years)	45	48	32	38
Asymmetry at onset?	Yes	No	Yes	Yes
Disease duration (years)	29	20	20	7
Levodopa-induced dyskinesias	++	++	+++	+
Phenotype at examination:				
Resting tremor	81	±	69	68
Bradykinesia	0	77	0	0
Rigidity	0	0	0	0
Postural instability	0	0	0	-
Hoehn and Yahr (1967) stage (on-state)	II.5	II.5	III	II
Daily medication:				
Levodopa (mg)	300	625	800	500
Pergolide (mg)	4	...	3	...

^a + = Mild; ++ = moderate; +++ = severe; ± = uncertain.

100 sporadic cases with onset age ≤ 45 years (Lücking 2000). They found *Parkin* mutations in approximately half of the families, but in only 18/100 sporadic cases, most (14/18) with onset age ≤ 20 years. The typical ARJP features—such as dystonia, hyperreflexia, sustained response to levodopa, and early levodopa-induced dyskinesias—were more frequent in the *Parkin*-positive patients than in the *Parkin*-negative group. However, these signs were less frequent than in previous reports, and none could be used to specifically distinguish between the two groups. The age at onset in the *Parkin*-positive group was, on average, lower than that in the *Parkin*-negative group, but mutations were identified in patients with onset age ≥ 45 years (≤ 64 years) and with clinical features difficult to distinguish from those of PD (Klein et al. 2000; Lücking et al. 2000).

The identification of several families that have autosomal recessive parkinsonism but that do not have *Parkin* mutations and are clinically indistinguishable from the *Parkin*-positive families strongly suggests the existence of at least one other, possibly similar, gene responsible for autosomal recessive parkinsonism. The identification of a novel locus for autosomal recessive early-onset parkinsonism in a family with a phenotype similar to that reported for *Parkin* is a crucial step toward the identification of another gene that could account for at least a subset of the *Parkin*-negative cases. It is noteworthy that the disease in this family is more characteristic of sporadic, Lewy-body PD, since it has both a later onset age than is seen in *Parkin*-positive patients and fewer atypical features. Still, the absence of autptic confirmation does not allow us to assign a definite diagnosis of PD. The term “early-onset parkin-

sonism” is preferred in the case of families, such as those studied in the present article, for which information on the occurrence of Lewy bodies and on the pattern of neuronal loss is missing.

The role of this novel locus needs to be tested in other autosomal recessive *Parkin*-negative families and in cases of early-onset sporadic parkinsonism, since a sporadic presentation may underlie an autosomal recessive mode of inheritance. Until the *PARK6* gene has been identified, a screen will be possible only by a search of regions of homozygosity within the candidate interval. Homozygosity mapping is a powerful technique to map genes responsible for autosomal recessive diseases in either consanguineous or nonconsanguineous pedigrees (Wang et al. 1997). However, this method is not completely accurate in determining the frequency of a gene.

It is worth noting that individual V:10 shares with the affected individuals a smaller region of homozygosity, from *D1S2674* to *D1S247*. At the latest examination, this individual was 84 years old and did not show any signs of PD. It is likely that the gene is located outside the region of homozygosity shared by the unaffected person. In this case, the linked interval would be reduced to a 9-cM region between *D1S483* and *D1S2674*. Alternatively, the identification of a definitely unaffected family member partially sharing the region of homozygosity can be explained if a reduced penetrance of the underlying gene is assumed. This would be in agreement with the reduced (.9) penetrance of the *Parkin* gene, and, in fact, we used this penetrance value for linkage calculations in this family. This issue will be clarified by detection of recombinations in affected individuals from other families.

The *Parkin* protein appears to be involved in protein degradation, as a ubiquitin-protein ligase interacting with the ubiquitin-conjugating enzyme UbcH7 (Shimura et al. 2000). The protein is abundant in the melanin-containing neurons of SNpc in normal individuals and in cases of sporadic parkinsonism, where it is also detected in association with α -synuclein within Lewy

Table 2**Pairwise LOD Scores for PD and Markers on Chromosome 1p**

MARKER	LOD SCORES AT $\theta =$						
	.0	.01	.05	.1	.2	.3	.4
<i>D1S483</i>	.18	.53	.83	.83	.59	.31	.10
<i>D1S199</i>	4.01	3.92	3.55	3.08	2.14	1.24	.48
<i>D1S2732</i>	2.65	2.60	2.39	2.10	1.46	.83	.32
<i>D1S2828</i>	3.49	3.42	3.12	2.72	1.89	1.08	.41
<i>D1S478</i>	3.45	3.36	3.03	2.61	1.78	1.00	.38
<i>D1S2702</i>	3.36	3.16	2.84	2.44	1.65	.93	.36
<i>D1S2734</i>	3.62	3.55	3.26	2.87	2.03	1.19	.46
<i>D1S2674</i>	3.29	3.21	2.87	2.44	1.60	.87	.32
<i>D1S2885</i>	3.94	3.85	3.49	3.03	2.11	1.23	.49
<i>D1S247</i>	1.01	1.35	1.59	1.48	1.03	.58	.19

bodies. Conversely, mutations in the *Parkin* gene completely abolish the expression of the Parkin protein, which is not detectable in any region of patients' brains (Shimura et al. 1999). The mechanism of selective neuronal cell death remains to be clarified; however, it is likely that the absence of Parkin protein results in accumulation of still-unidentified proteins leading to selective neuronal degeneration.

The work of the human genome-sequencing project has resulted in availability of a draft sequence of much of the genome, including the 12.5-cM region that we have identified on chromosome 1. This region, which is equivalent to ~10.5 Mb on physical maps of the region, contains a large number of genes, both predicted and already known. Included in this group are a number of homeobox genes and the genes for endothelin-converting enzyme 1 and a G-protein-coupled receptor. However, none of them either presents similarities with *Parkin* or represents an obvious candidate for PD. The identification of other families with linkage to chromosome 1p will help in refinement of the locus position on the genetic map, which is an essential step toward the identification of the gene and its function.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, <http://research.marshfieldclinic.org/genetics/> (for the chromosome 1 genetic map)
Human Genome, The, <http://www.ncbi.nlm.nih.gov/genome/guide/human/> (for the chromosome 1 physical map)
Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for PD [MIM 168600] and ARJP [MIM 600116])

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