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Localization of a Gene for an Autosomal Recessive Form of Juvenile Parkinsonism to Chromosome 6q25.2-27

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Summary

An autosomal recessive form of juvenile Parkinsonism (AR-JP) (MIM 600116) is a levodopa-responsive Parkinsonism whose pathological finding is a highly selective degeneration of dopaminergic neurons in the zona compacta of the substantia nigra. By linkage analysis of diallelic polymorphism of the Mn-superoxide dismutase gene (SOD2), we found a family with AR-JP showing perfect segregation of the disease with the SOD2 locus. By extending the linkage analysis to 13 families with AR-JP, we discovered strong evidence for the localization of the AR-JP gene at chromosome 6q25.2-27, including the SOD2 locus, with the maximal cumulative pairwise LOD scores of 7.26 and 7.71 at D6S305 (θ = .03) and D6S253 (θ = .02), respectively. Observation of obligate recombination events, as well as multipoint linkage analysis, placed the AR-JP gene in a 17-cM interval between D6S437 and D6S264. Delineation of the AR-JP gene will be an important step toward our understanding of the molecular mechanism underlying selective degeneration of the nigral neurons.

Introduction

Parkinson disease (PD) is caused by a deficiency of dopamine in the nigrostriatal system resulting from a selective degeneration of dopaminergic neurons in the zona compacta of the substantia nigra in the midbrain (Gibb 1993). This disease affects >1% of the population >55 years of age (de Rijk et al. 1995) and causes characteristic clinical features consisting of rigidity, bradykinesia, tremor, and postural imbalance. Despite progress in research on the etiology and pathophysiology of PD (Mizuno et al. 1987, 1989; Schapira et al. 1989; Hattori 1991), the primary cause of this disease remains unknown.

A rare but distinct group of illnesses with autosomal recessively inherited Parkinsonism, with levodopa-response and selective degeneration of dopaminergic neurons in the zona compacta of the substantia nigra, has been reported, mainly in the Japanese literature, under different names, such as autosomal recessive early-onset Parkinsonism with diurnal fluctuation (AR-EPDF) (Yamamura et al. 1973, 1993), an autosomal recessive form of juvenile Parkinsonism (AR-JP) (MIM 600116) (Tanaka et al. 1991; Takahashi et al. 1994; Ishikawa and Tsuji 1996), or a familial form of juvenile Parkinsonism (Mizutani et al. 1988, 1993; Kondo et al. 1990; Takubo et al. 1996). Characteristic clinical features of AR-JP include insidious and juvenile-onset (<40 years of age in most patients) of typical Parkinsonism, with rigidity, resting and/or postural tremor, bradykinesia, postural imbalance, a slow and protracted course, superb response to levodopa, frequent and early occurrence of dopa-induced dyskinesia and wearing-off phenomenon, amelioration of symptoms after sleep (sleep benefit), and mild foot dystonia. Extensive reduction of tyrosine hydroxylase activity in the substantia nigra of the brain of affected individuals was reported and was quite similar to the finding in PD. Neuropathological features of AR-JP were the selective degeneration of dopaminergic neurons in the zona compacta of the substantia nigra, with reactive gliosis and no Lewy body formation and, to a lesser extent, neuronal loss in the locus coeruleus (Mizutani et al. 1988, 1993; Yamamura et al. 1993; Takahashi

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et al. 1994; Takubo et al. 1996). These pathological features are similar to those observed in PD, except for the absence of Lewy bodies in AR-JP.

To perform the candidate gene approach to delineate the molecular mechanisms of AR-JP, we were particularly interested in the manganese superoxide dismutase (MnSOD) gene (SOD2). MnSOD is an intramitochondrial enzyme that scavenges superoxide anions, which are generated from mitochondrial respiratory chains (Boveris 1977; Guidot et al. 1993). It is interesting that the impairment of the function of mitochondrial respiratory chains has been reported in PD (Mizuno et al. 1989; Schapira et al. 1989; Hattori et al. 1991), and it was shown recently that there might be a causal relationship between the inhibition of respiratory chain functions and the release of superoxide anions within mitochondria (Hasegawa et al. 1990; Guidot et al. 1993). Furthermore, MnSOD is inducible by the superoxide anions (Hassan 1988), and increased activity of MnSOD has been demonstrated in the substantia nigra of patients with PD (Saggu et al. 1989). These facts indicate the possible presence of oxidative stress within mitochondria in the PD brain and a possible role of MnSOD in modulating this process.

In this report, we searched for polymorphic mutations in the SOD2 gene and evaluated whether these polymorphisms cosegregate with AR-JP. Following the identification of a family with a concordant segregation of one of these markers with an AR-JP phenotype, we extended the linkage analysis to 13 families with AR-JP, and we here report the localization of the gene for AR-JP to chromosome 6q25.2-27, which harbors the SOD2 gene. The nucleotide sequence analysis of the coding regions of the SOD2 gene did not, however, reveal the causative mutations, suggesting the possibility that another, as yet unidentified, gene in this region is responsible for AR-JP.

Material and Methods

Clinical and Neuropathological Features of AR-JP Pedigrees

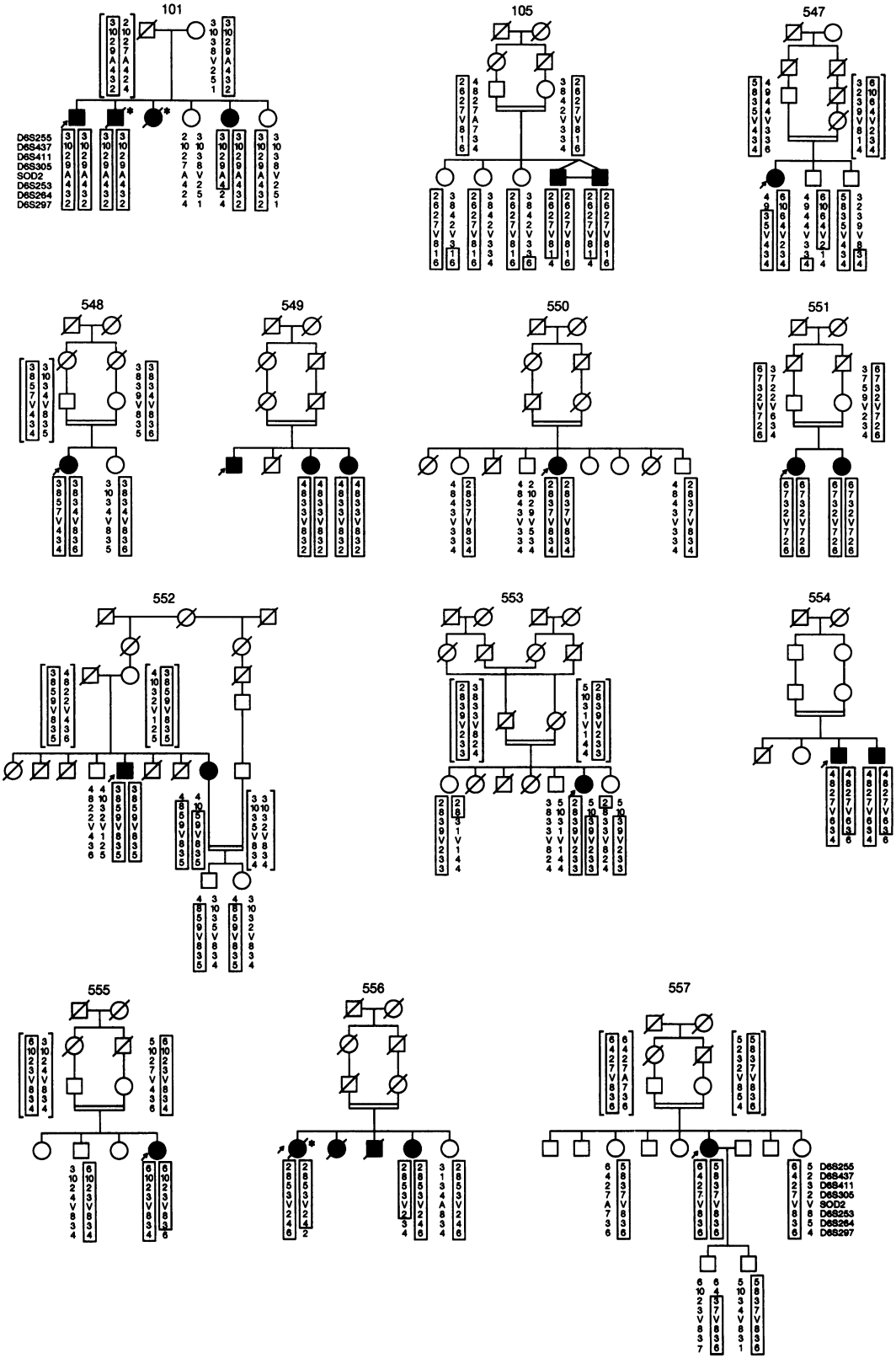
The affected individuals studied were born to consanguineous parents in 11 families and to nonconsanguineous parents in 2 families (fig. 1). After informed consent was obtained, blood samples were obtained from 50 family members (21 affected and 29 unaffected members).

Details on the clinical features of the families, except for families 105 and 555, have already been published (Mizutani et al. 1988, 1993; Kondo et al. 1990; Tanaka et al. 1991; Takahashi et al. 1994; Ishikawa and Tsuji 1996; Takubo et al. 1996). Neuropathological findings have been reported regarding family 101 (Mizutani et al. 1988; Mizutani et al. 1993; Takubo et al. 1996) and family 556 (Takahashi et al. 1994) (marked with an asterisk [*] in figs. 1 and 2). The age at onset was from 8 years to 43 years, with the mean of 24.6 years, and in the majority it was <40 years of the age, except for the affected individual in family 557, who had onset at age 43 years. Cardinal features of the Parkinsonism were rigidity, resting tremor and/or fine postural tremor, bradykinesia, and postural instability. Response to levodopa was dramatic. Early and marked drug-induced dyskinesia with development of motor fluctuations were frequent problems in nearly all of the patients studied. In the off periods, bradykinesia, postural instability, and freezing of gait were occasionally manifested markedly. Sleep benefit of the Parkinsonian symptoms was present in almost all of the patients. Some dystonic features in one or more extremities were frequent but were always associated with the cardinal symptoms of Parkinsonism. Insidious onset and a definitely slow progressive course of the disease were characteristic. Mental function and ocular movements were normal, and no cerebellar signs were seen. Cranial computed tomography scans and magnetic resonance images were also normal.

Autopsy reports of two affected individuals in family 101, marked with an asterisk in figures 1 and 2 (Mizutani et al. 1988, 1993; Takubo et al. 1996), and of an affected individual in family 556, marked with an asterisk in figure 1 (Takahashi et al. 1994), showed selective and severe degeneration of the nigral neurons, particularly in the ventrolateral and medial areas of the zona compacta of the substantia nigra, with reactive gliosis; no Lewy bodies were found. Mild neuronal loss with depigmentation was observed in the locus coeruleus.

Analysis of the Genomic Sequences of the Human SOD2 Gene

Genomic DNA was extracted from peripheral whole blood cells according to standard procedures (Sambrook et al. 1989) and from formalin-fixed and paraffin-embedded tissues as described by Goelz et al. (1985). Nucleotide sequences of coding exons (exons 1–5) and adjacent introns were determined by direct sequencing of PCR products obtained from genomic DNA. For exons 1 and 2 of the SOD2 gene, we first amplified genomic DNA by nested PCR using oligonucleotides (forward primer 5′-ATGTTGAGCCGGGCAGTG-3′ and the two reverse primers 5′-TACTTCTCCTCGGTGACG-3′ and 5′-GCGTGGTGCTTGCTGTG-3′) that were designed on the basis of the human cDNA sequences (Beck et al. 1987; Heckl et al. 1988), and we determined exon-intron boundaries and nucleotide sequences of exons 1 and 2, as well as intron 1 (H. Matsumine, unpublished data [accession no. D83493]). The primers for exons 1 and 2, and their junctional sequences, were then designed on the basis of the nucleotide sequences determined above (exon 1: 5′-CAGCAGATCGGCGGC-


101
D6S255
D6S437
D6S411
D6S305
SOD2
D6S253
D6S264
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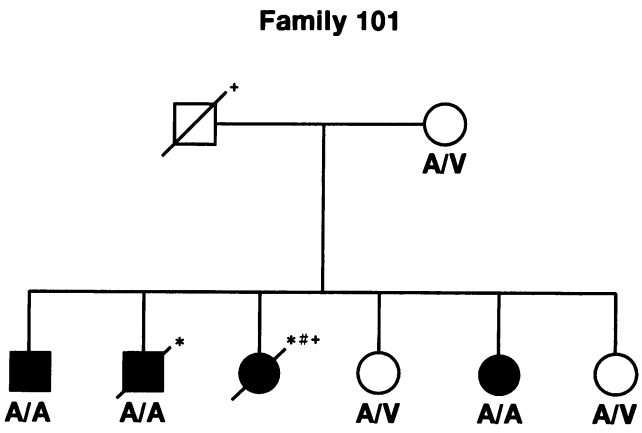

Figure 2 Concordant segregation of the SOD2 polymorphism of Ala-9Val with AR-JP in family 101. Affected individuals are represented by black squares (males) and circles (females). Autopsy studies were performed in two affected individuals marked with an asterisk (*). Neuropathological findings in both cases were selective neurodegeneration of the zona compacta of the substantia nigra, with reactive gliosis (Mizutani et al. 1988; Mizutani et al. 1993; Takubo et al. 1996). Tyrosine hydroxylase activity in the substantia nigra and striatum, which was measured in an affected individual marked with a pound sign (#), was markedly decreased (Kondo et al. 1990). DNA was not available in the two individuals marked with a plus symbol (+). −9Ala allele and −9Val allele are designated as A and V, respectively. It should be noted that, despite the absence of consanguinity in their parents, the three affected individuals were homozygous for the rare −9Ala allele, while others were heterozygous for the −9Ala allele. The maximal pairwise LOD score of 1.34 was obtained at this SOD2 polymorphism ($\theta = 0$). (see Results)

ATCAG-3′ and 5′-TGCGGCCACTGTCGCCATTG-3′ and exon 2: 5′-CCGGGCTGTGCTTTCTCGT-3′ and 5′-CCCGCTCAGCCTGGAACCT-3′). For the amplification of exons 3–5, primer pairs were designed on the basis of the reported genomic sequences (Hartman et al. 1988; Church et al. 1992). For exon 3, we first amplified the genomic fragment with primers 5′-ATGTGGTTTGCACTTTTAAC-3′ and 5′-TACTGTGCAAGAAGTTGTAG-3′. Then, the 5′ portion of exon 3 was amplified by secondary nested PCR using primers 5′-ATGTGGTTTGCACTTTTAAC-3′ and 5′-GTTCTCCACCACCGTTAG-3′, whereas the 3′ portion of exon 3 was amplified by primers 5′-CACTTTTAACTTTTAAG-3′ and 5′-TGCAAGAAGTTGTAGATA-3′. Exons 4 and 5 were amplified by the following primers: exon 4, 5′-TGTCGCATTCTGATGTTGTC-3′ and 5′-AAACATTTTTCTTACACAAG-3′, and exon 5, 5′-GACTGAAACTGTGGTTGG-3′ and 5′-TGAAATAAGTGACTAAGCA-3′.

The amplifications were performed in a total volume of 50 μl, containing 100 ng of genomic DNA, 50 pmol of each primer, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl at pH 8.3, 0.001% gelatin, 0.1 mM dNTPs, and 0.25 units of *Taq* DNA polymerase (Perkin Elmer). Annealing temperatures for PCRs were 62°C for exon 1 and exon 2, 47°C for exon 3, 53°C for exon 4, and 50°C for exon 5. The PCR products were purified into single-stranded DNA by using streptavidine-coated magnetic beads. The nucleotide sequences were analyzed by the dideoxy nucleotide chain termination method using T7 polymerase (Pharmacia Autoread sequencing kit) or BcaBEST DNA polymerase (Pharmacia BcaBEST sequencing kit). The results were analyzed with a Pharmacia ALF2 fluorescence automated sequence analyzer.

The polymorphism of Ala-9Val in exon 2 was analyzed by PCR-SSCP with primers 5′-CAGCCCAGCCTGCGTAGACG-3′ and 5′-GCGTTGATGTGAGGTTCCAG-3′, which are located in intron 1 and exon 2, respectively. The latter primer was FITC labeled, and PCR was performed in a volume of 10 μl with 1 pmol of each primer. PCR conditions were denaturation at 94°C for 4 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 59°C for 1 min, and extension at 72°C for 1 min, followed by extension at 72°C for 7 min. PCR products were run at 15°C at 30 W in 1 × TBE through a 5% nondenaturing polyacrylamide gel containing 10% glycerol. Fragment analysis was performed with a Pharmacia ALF2 fluorescence automated sequence analyzer.

DNA Typing for Microsatellite Markers

One of the primer pairs for the microsatellite markers (Ziegler et al. 1993; Gyapay et al. 1994; Volz et al. 1994) was fluorescently labeled. PCR was performed in 10 μl reaction solution, containing 100 ng genomic DNA, 0.1 μM of each primer, 0.2 mM dNTP, 10 mM Tris-HCl at pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, and 0.5 U of AmpliTaq DNA polymerase. After an initial denaturing step for 5 min at 95°C, amplification was performed for 35 cycles at 94°C for 0.5 min, 50°C for 0.5 min, and 72°C for 0.5 min, fol-

Figure 1 Haplotype analysis of the AR-JP families. DNA markers are listed from the top to the bottom (D6S255, D6S437, D6S411, D6S305, *SOD2*, D6S253, D6S264, and D6S297). D6S411, D6S305, and SOD2 are 0 cM apart (Ziegler et al. 1993; Gyapay et al. 1994; Volz et al. 1994). Filled circles (women) and squares (men) represent affected individuals. Haplotypes are assigned in such a way as to minimize the number of crossovers in each family. Haplotypes segregating with the disease are shown in boxes. Inferred haplotypes assuming minimal recombination events are shown in brackets. The critical chromosomal region for AR-JP is defined by the two polymorphic loci, D6S437 and D6S264, which revealed obligatory recombination events in affected individuals in families 101 and 556 for D6S264 and in family 552 for D6S437. Neuropathological findings have been reported in three affected individuals marked with an asterisk (*) (Mizutani et al. 1988, 1993; Takahashi et al. 1994; Takubo et al. 1996) (see Material and Methods).

lowed by a final extension for 5 min at 72°C. The PCR products were analyzed using the Pharmacia ALF2 fluorescence automated sequence analyzer.

Linkage Analysis

All of the family members were examined by neurologists, and the diagnosis of AR-JP was made according to the clinical features described above. We selected D6S311, D6S441, D6S255, D6S415, D6S437, D6S411, D6S305, D6S253, D6S264, and D6S297, which flank the SOD2 gene, for the linkage analysis. Recent mapping data of the long arm of chromosome 6 indicated that D6S305, D6S411, and the SOD2 gene are 0 cM apart from each other; D6S255 is located 7 cM centromeric to the SOD2 gene, and D6S253 is located 3 cM telomeric to the SOD2 gene (Ziegler et al. 1993; Gyapay et al. 1994; Volz et al. 1994).

Pairwise and multipoint LOD scores were calculated using the computer programs Linkage (version 5.2) (Lathrop and Lalouel 1985; Lathrop et al. 1985) and the FASTLINK package (Cottingham et al. 1993; Schäffer et al. 1994), a faster version of Linkage. Most of the runs were performed using FASTLINK, version 2.3P, sequentially. Some of the long multipoint runs on family 547 were done using version 3.0P in parallel (Butler and Lusk 1994; Gupta et al. 1995). Version 3.0P has better algorithms for looped pedigrees, and we were able to use a fast 4-processor Silicon Graphics, Inc., multiprocessor at Rice University. To reduce computation time, the calculation of multipoint LOD score was performed by dividing the marker loci into three subgroups: (1) D6S441–D6S415–D6S437, (2) D6S437–D6S305–D6S253–D6S264, and (3) D6S264–D6S297. Calculation assumed a disease allele frequency of .003, which was estimated in a previous report (Tanaka et al. 1991), and no sex difference. The allele frequency for each marker was determined by the analysis of 84 normal unrelated Japanese individuals. Five liability classes were set taking cumulative age-dependent penetrance into account (I: 0–12 years of age, 0.032; II: 13–23 years of age, 0.33; III: 24–34 years of age, 0.62; IV: 35–44 years of age, 0.89; and V: >45 years of age, 1.00) (Tanaka et al. 1991). Recombination frequencies were assumed to be equal between men and women. Multipoint linkage analysis was performed between the AR-JP allele and the most informative 6q markers: D6S441, D6S415, D6S437, D6S305, D6S253, D6S264, and D6S297. Although D6S255 and D6S415, which were both located at the same genetic map distance from D6S437, were equally informative, D6S415 was used for the calculation. Their relative map orders and physical map distances were based on published data (Ziegler et al. 1993; Gyapay et al. 1994; Volz et al. 1994). Multipoint linkage analysis was also performed, with the HOMOZ package in the MAPMAKER linkage program (Kruglyak et al. 1995), which is a software package for rapid multipoint likelihood calculation by homozygosity mapping (Lander and Botstein 1987). The admixture test (A-test) for genetic heterogeneity was carried out using the HOMOG computer program (Ott et al. 1991).

Results

Identification of Polymorphisms in the Human SOD2 Gene and the Linkage Analysis

To perform the candidate gene approach to delineate the genetic mechanism of AR-JP, we searched for polymorphisms of the SOD2 gene. By nucleotide sequence analysis of the SOD2 gene, we identified four polymorphisms in the SOD2 gene. The polymorphisms identified in the present study include one in exon 2 substituting alanine for valine (GCT/GTT) at the position −9 (Ala-9Val) in the mitochondrial targeting sequence, a diallelic intronic polymorphism of G/A at the ninth position of intron 1, a Ser3Arg polymorphism (AGC/AGA) in exon 2, and a neutral polymorphism (GGA/GGG) at the third letter of codon 157Gly in exon 5. The exon-intron boundaries that we determined were identical with those reported previously (Hartman et al. 1988; Church et al. 1992), except for the boundary between exon 1 and exon 2, which was different by 1 nt from that reported by Hartman et al. (1988) but was identical with that reported by J. W. Grant (unpublished data [accession no. L34217])

Since the Ala-9Val among these polymorphisms was informative in the AR-JP pedigrees, we analyzed this polymorphism as the first step to investigate the possibility that SOD2 was a candidate locus for AR-JP. SSCP analysis for this mutation in 124 Japanese normal individuals gave the allele frequencies of .113 and .887 for −9 alanine and −9 valine, respectively. In family 101, the only family where this polymorphism was informative, we found that all three affected individuals were homozygous for the rare −9 alanine allele and that the phenotypically normal mother, as well as the two unaffected siblings, were heterozygous for the −9 alanine and −9 valine. It should be noted that all the affected individuals are homozygous for the rare allele, despite the absence of known consanguineous marriage in their parents (fig. 2). These genotypes were confirmed by direct nucleotide sequence analysis of the PCR products obtained from each individual. Preliminary analysis in this family with six additional microsatellite markers (D6S255, D6S411, D6S305, D6S253, D6S264, and D6S297), which are located in the vicinity of the SOD2 gene (Ziegler et al. 1993; Gyapay et al. 1994; Volz et al. 1994), revealed cosegregation with the disease with an obligate recombination at D6S264 in an affected individual (fig. 1). The highest pairwise LOD scores, of 1.45 and 1.34, were obtained at D6S305 ($\theta = 0$) and at the SOD2 gene ($\theta = 0$) for this family, respectively. The

findings raised the possibility that the gene for AR-JP is linked to the SOD2 gene and the adjacent regions on the long arm of chromosome 6.

Linkage of AR-JP to Chromosome 6q25.2-27

We extended our analysis to include 13 families with AR-JP by use of 10 microsatellite markers (D6S311, D6S441, D6S255, D6S415, D6S437, D6S411, D6S305, D6S253, D6S264, and D6S297), as well as the SOD2 gene polymorphism, which encompass a 35-cM region of the long arm of chromosome 6q24-27 (fig. 3). The maximal pairwise cumulative LOD scores, 7.26 and 7.71, were obtained at D6S305 (θ = .03) and D6S253 (θ = .02), respectively (table 1). Additional markers in this region also gave positive scores (table 1), including D6S411 (Z_{max} = 4.44 at θ = .05) and SOD2 (Z_{max} = 1.69 at θ = 0). Calculation of pairwise LOD scores by use of another disease allele frequency of .001 gave essentially the same results. We found no evidence for locus heterogeneity among our AR-JP families by testing with the HOMOG computer program.

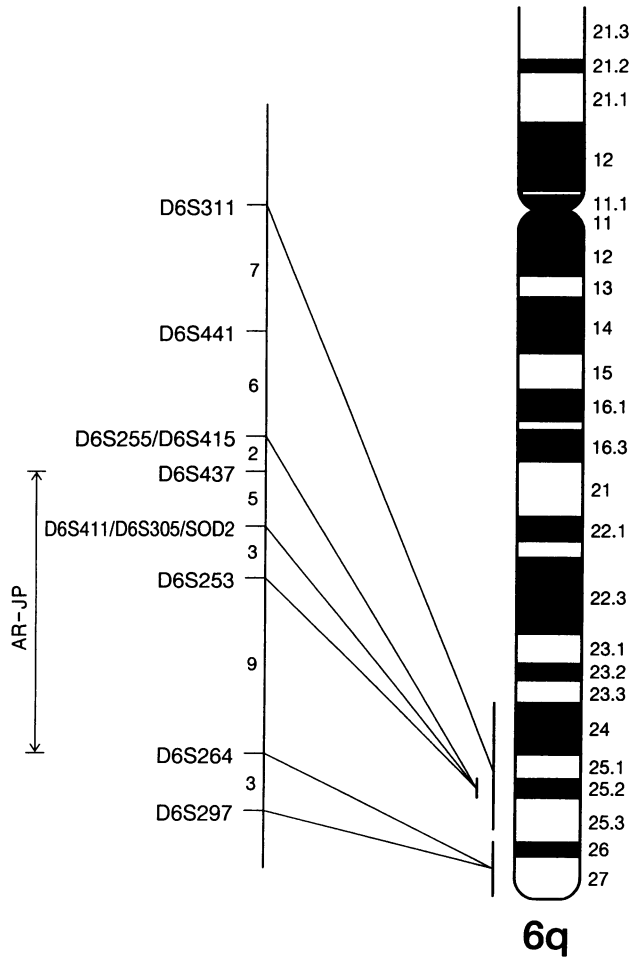

Figure 3 Genetic regional maps of chromosome 6q. We selected 10 microsatellite markers (D6S311, D6S441, D6S255, D6S415, D6S437, D6S411, D6S305, D6S253, D6S264, and D6S297) and the SOD2 polymorphism for analysis. The genetic map was derived from the Genome Data Base and published map information (Ziegler et al. 1993; Gyapay et al. 1994; Volz et al. 1994). The genetic distances between the markers were shown in centimorgans (Kosambi). The most likely location for the AR-JP locus on chromosome 6q is given.

Multipoint linkage analysis, shown in figure 4, indicates that the AR-JP gene is most likely located in the interval between D6S437 and D6S264, with the highest maximal LOD score of 9.44 obtained 0.9 cM telomeric to D6S253. Homozygosity mapping by MAPMAKER/HOMOZ program, using the markers D6S311, D6S411, D6S415, D6S305, D6S253, D6S264, and D6S297, gave the highest maximal LOD score of 14.1 at 2 cM telomeric to D6S253.

The haplotypes were constructed on the assumption of the most parsimonious linkage phase. Direct inspection of the reconstructed haplotypes clearly showed cosegregation between the chromosome 6q markers and the AR-JP phenotype (fig. 1). Obligatory recombination events for the telomeric border of the AR-JP region were observed at D6S264 in families 101 and 556, and that for the centromeric border was observed at D6S437 in family 552, thus defining the critical chromosomal region for AR-JP in a 17-cM interval between D6S437 and D6S264 (fig. 3). No linkage disequilibrium, nor any commonly shared haplotype for AR-JP chromosomes, was observed with the markers used in this study.

The analysis of the Ala/Val polymorphism in the mitochondrial targeting signal peptide of the SOD2 gene in the 13 AR-JP families revealed that patients were homozygous for the −9 Ala allele in family 101, while patients were homozygous for the −9 Val allele in the other 12 families. The allele frequencies for −9 Val and −9 Ala alleles in the founder chromosomes in the 15 AR-JP chromosomes were .87 and .13, respectively, which are comparable to those in 248 normal chromosomes (−9 Val, .887; −9 Ala, .113). There was no statistical difference in the allele frequency between AR-JP and normal chromosomes as determined by the χ^2 test.

Nucleotide Sequence Analysis of SOD2 Gene in Affected Individuals with AR-JP

Nucleotide sequences of all of the coding exons and their exon-intron boundaries of the SOD2 gene were determined for at least one affected individual from each pedigree by direct nucleotide sequence analysis as described in Material and Methods. We did not find any disease-specific mutations in these regions of the SOD2 gene of AR-JP-affected individuals.

Discussion

In this report, we have mapped the AR-JP gene to chromosome 6q25.2-27, which includes the SOD2 gene.

Table 1

Pairwise LOD Scores between Chromosome 6q Markers and AR-JP

	LOD SCORE AT θ =								
MARKER	0	.01	.05	.1	.15	.2	.3	Z_{max}	θ
D6S311	−∞	−7.26	−2.47	−.82	−.16	.12	.19	.20	.26
D6S441	−∞	−5.29	−1.39	−.10	.36	.50	.42	.51	.22
D6S255	−∞	−.76	2.24	2.85	2.73	2.36	1.39	2.86	.11
D6S415	−∞	2.19	3.02	2.86	2.45	1.97	1.07	3.03	.06
D6S437	−∞	−1.88	.20	.76	.84	.75	.44	.84	.14
D6S411	2.72	3.77	4.44	4.14	3.56	2.90	1.60	4.44	.05
D6S305	6.55	7.10	7.12	6.34	5.36	4.32	2.36	7.26	.03
SOD2[a]	1.69	1.66	1.53	1.34	1.12	.91	.48	1.69	0
D6S253	7.28	7.64	7.47	6.61	5.59	4.55	2.60	7.71	.02
D6S264	−∞	1.35	2.30	2.42	2.21	1.88	1.14	2.44	.08
D6S297	−∞	−1.24	1.53	2.22	2.24	2.00	1.24	2.27	.13

[a] Manganese superoxide dismutase gene.

It is interesting that most of the affected individuals showed homozygosity across the candidate regions, not only in pedigrees with consanguinity (pedigrees 105, 549, 550, 551, 553, 554, 555, and 556) but also in pedigrees without apparent consanguinity (pedigrees 101 and 552). Homozygosity in nonconsanguineous families 101 and 552 indicates that the mother and father were distantly related, and thus the same ancestral mutant allele is segregating within each family. In fact, both parents in each family originated from the same restricted geographic area. On the other hand, affected individuals from consanguineous marriages in pedigrees 547, 548, and 557 did not show apparent homozygosity. Several explanations could be possible for the absence of homozygosity in these pedigrees. First, the markers used in this study, which were placed at a 3–9-cM interval, may not be sufficiently tightly placed to detect such homozygosity. Second, these pedigrees may not be linked to this region, although a heterogeneity test by the HOMOG program provided no evidence for locus heterogeneity. Finally, the affected individuals may be a compound heterozygote with two independent mutant alleles. This is less likely, especially under the influence of consanguinity, but cannot be excluded until mutational studies of the AR-JP gene are possible.

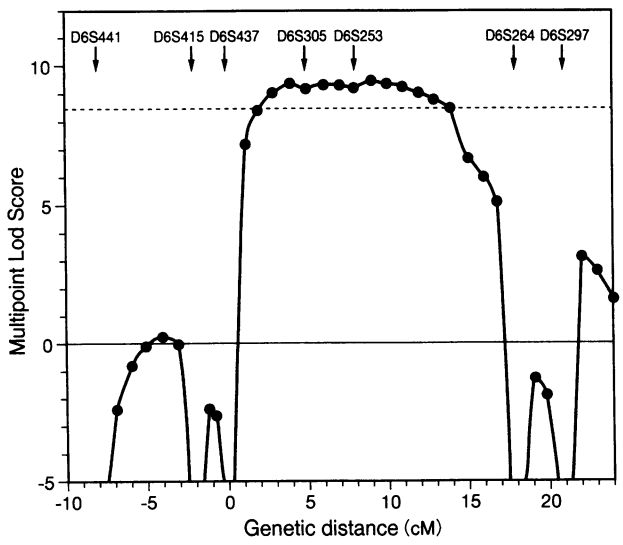

Figure 4 Multipoint linkage map of the AR-JP locus on chromosome 25.2–27. Multipoint LOD scores obtained using D6S441, D6S415, D6S437, D6S305, D6S253, D6S264, and D6S297 are shown. The dotted line indicates the highest maximal LOD score (−1).

In our dataset, we did not observe any linkage disequilibrium or commonly shared haplotype. This might indicate either the presence of multiple independent mutations in the AR-JP gene or the decay of allelic disequilibrium because of a long period of evolution of the chromosomal region around the AR-JP gene. Alternatively, the present markers might not be placed densely enough to detect the disequilibrium. Further analyses using more tightly spaced markers and a larger number of AR-JP pedigrees are necessary to elucidate the mechanism.

Although we started the present study using the SOD2 gene as a candidate locus for AR-JP, we did not observe any causative mutations in the exons and the splicing junctions of the SOD2 gene. This suggests the possibility that another, as yet unidentified, gene in the candidate region may be responsible for AR-JP. Nevertheless, we could not neglect the possibility of mutations in other areas of the SOD2 gene, such as in the regulatory motifs, untranslated exons, and intron sequences (Campuzano et al. 1996), because we could not detect any critical recombination events at the SOD2 gene locus (D6S305,

D6S411, and SOD2). A more extensive structural analysis of the SOD2 gene, as well as studies on the mRNA, protein, and enzymal activity of MnSOD, will be required to conclude whether or not the SOD2 gene is a causative gene for AR-JP.

In addition to the SOD2 gene, genes for VIP (vasoactive intestinal peptide), IGFR2 (insulin-like growth factor II receptor), and PLG (plasminogen) are located in the 17-cM region between D6S437 and D6S264 (Ziegler et al. 1993; Gyapay et al. 1994; Volz et al. 1994). These genes should also be thoroughly investigated as the candidate genes for AR-JP.

In conclusion, we presented here the first report of the genetic localization of the gene for an autosomal recessive form of levodopa-responsive Parkinsonism. Our identification of the AR-JP locus indicates that loss-of-function mutation in a single gene could cause selective degeneration of dopaminergic neurons in the substantia nigra. Given the chromosomal localization of the gene for AR-JP, we can now apply a standard approach of positional cloning for identification of the AR-JP gene. The delineation of its function will open the way to understanding what is needed to maintain the survival of nigral neurons and why the nigral neurons primarily undergo degeneration.

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