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A chromosome 4p haplotype segregating with Parkinson's disease and postural tremor

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We investigated a large family with levodopa-responsive, Lewy body parkinsonism in which the disease segregates as an apparent autosomal dominant trait. After performing a genome screen, we identified a chromosome 4p haplotype that segregates with the disease. However, this haplotype also occurs in individuals in the pedigree who do not have clinical Lewy body parkinsonism but rather suffer from postural tremor, consistent with essential tremor. These data demonstrate a new locus for Lewy body parkinsonism and suggest that in some circumstances postural tremor can be an alternative phenotype of the same pathogenic mutation as Lewy body parkinsonism.

INTRODUCTION

The most common cause of parkinsonism is Parkinson's disease (PD), first described as 'paralysis agitans' in 1817, a clinical syndrome of resting tremor, rigidity, bradykinesia and postural instability (1). It was not until 100 years later, however, that loss of dopamine-containing cells in the substantia nigra was described (2). The presence of Lewy bodies was noted thereafter in regions including the substantia nigra, locus ceruleus, nucleus basalis and hypothalamus (3). The etiology of the disease remains unknown, although recent work has clearly shown that heredity is a major factor (4). Of greatest importance has been the demonstration of a genetic linkage on chromosome 4q followed by the identification of α-synuclein gene mutations (5,6). Still more recently, a locus for PD on chromosome 2p has been reported (7) and mutations in autosomal recessive, juvenile parkinsonism described (8).

We have carried out linkage analysis on a large well-documented family with parkinsonism (9–11). The pedigree shown in Figure 1 includes six generations and consists of two branches, (A) and (B) that have been described separately (9–11) but recently linked through genealogical investigation. The pedigrees have been documented as having family members with early-onset, levodopa-responsive, Lewy body parkinsonism or parkinsonism-dementia (9–11). All patients in the present generation (1009, 1019 and 1023) were clinically evaluated and exhibit young onset, early weight loss, rapidly progressive dopa-responsive parkinsonism; 1009 subsequently has died and had Lewy body disease demonstrated at autopsy (D.Dickson, personal communication). In generation II, four individuals (2009, 2013, 2016 and 2030) were clinically affected with parkinsonism. These individuals also had autopsy-proven Lewy body disease (11). In the third generation, clinical notes were available for 3000, 3004 and 3022, although none was examined post-mortem. Affected family members meet the criteria for probable or definite PD (12) (Fig. 1, shaded symbols). Other family members who do not have parkinsonism have been documented to have postural tremor (Fig. 1, half-shaded symbols) (11). Essential tremor is tremor that occurs posturally, with activation, and in which there are no other features of neurological dysfunction (such as parkinsonism) (13). All family members noted to have postural tremor meet at least the criteria for possible essential tremor, four meet the criteria for probable essential tremor, and one met the criteria for definite essential tremor according to Louis *et al*. (13). The offspring of 2023 has probable essential tremor (11). There were no stigmata of Parkinson's disease in individuals stated to have essential tremor.

RESULTS

Power analysis suggested that the pedigree was large enough to detect linkage to Lewy body parkinsonism (Table 1). For the genome search, we used the Cooperative Human Linkage Center/Weber Human Screening Set (version 8) consisting of 365 autosomal markers supplemented with extra markers to give coverage of the genome at a 10 cM resolution. On the first pass of this screen, four markers showed positive scores ($Z > 1.0$, $\theta = 0$); for these markers, we analyzed adjacent markers to test for true linkage. This strategy eventually led us to run 392 markers. From the power calculations, this number of markers was deemed

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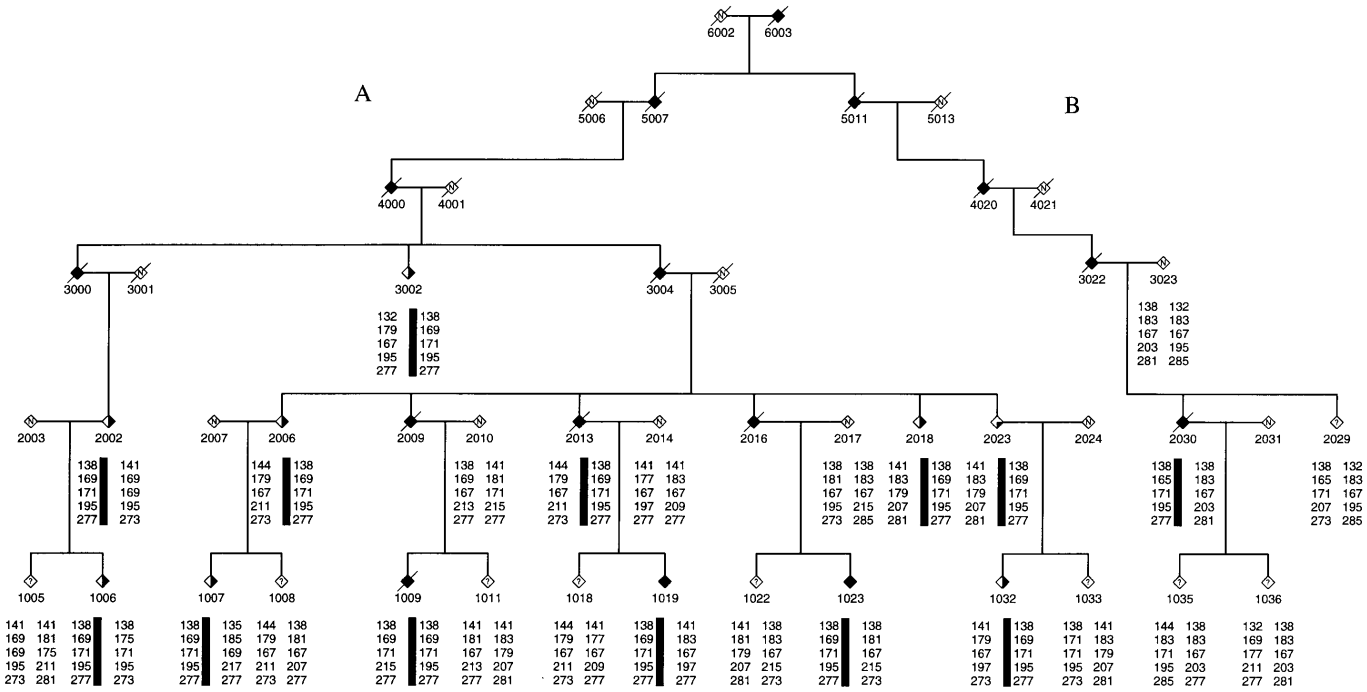

Figure 1. Pedigree diagram. (**A**) Spellman–Muenter branch of the kindred (9,11); (**B**) Waters and Miller branch (10). The gender of individuals and the number of sibs in different branches have been disguised for family confidentiality. Chromosome 4p marker alleles, in base pairs, within the minimum haplotype are shown, and correspond to *D4S2397*–3.0 cM–*D4S391*–0.6 cM–*D4S1609*–0.6 cM–*D4S230*–1.20 cM–*D4S2408*, reading from the top of the haplotype (shaded). For individuals without parental genotypes, or phase information deduced from their spouse and children, composite haplotypes have been inferred (see text). Individual 2023 has no documented disorder, although the only member of the kindred yet to be examined. This individual has a sib, compatible with inheritance of the 4p haplotype, with postural and activation tremor. The full pedigree is available from the authors. N, unaffected; filled diamonds, Parkinson's disease; ?, at risk; quarter-filled diamond, offspring with essential tremor; half-filled diamonds, essential tremor.

sufficient to exclude ($Z < -2.0$) the great majority of the genome and would enable any region with positive lod scores ($Z > 1.0$) to be examined further, and linked to disease ($Z > 3.0$) (Table 1). Two-point lod scores of loci for familial parkinsonism were calculated using both age-associated liability classes and affecteds-only analysis (Table 2). The former takes advantage of all the genetic information in the kindred for phase and haplotype construction, albeit that some individuals, especially those within two standard deviations of the mean onset age for disease, must be considered at risk. Affecteds-only analysis is appropriate when disease penetrance is reduced or uncertain, as has proven the case with other loci predisposing to familial PD (5,7).

In general, lod scores observed mirrored expectations based on *a priori* power analysis (Table 1). Only at a locus on chromosome 4p did adjacent markers show positive lod scores at $\theta = 0$ (Table 2). However, lod scores were sensitive to marker allele frequencies due to the genotypes that must be inferred to connect parts 'A' and 'B' of the family. Assigning equal allele frequencies to 4p markers increased lod score values to the mean score predicted by simulation studies, which suggests low marker informativity in this region. There was no evidence for linkage to either the chromosome 2p or the α-synuclein loci (14), and the α-synuclein gene sequence was normal (15). At the chromosome 4p locus, six markers tested within 8.5 cM showed positive lod scores at $\theta = 0$ (Table 2), with five adjacent markers, spanning 20.3 cM, giving positive scores at $\theta > 0.2$ (data not shown). Thus, an inferred haplotype of 8.5 cM < **h** < 28.8 cM segregated with disease. The likelihood of finding the minimum haplotype (*D4S1609–D4S2408*) in-phase with a disease phenotype is $<1 \times 10^{-6}$ (based on allele frequencies taken from the published data and assuming a disease allele frequency, in the population, of 0.0001). The confidence coefficient calculated from phase known meioses was 99.80% [0.001, 0.497 ($k = 1$, $n = 14$)] (16). Subjects 1020 and 1034 (data not shown) are at risk, carry the 4p haplotype, but await clinical examination.

Haplotypes were assigned to older members of the kindred without phase information based on the minimum number of recombination events and composite haplotypes that can be inferred. To our surprise, other individuals sharing the chromosome 4p haplotype included all of those within the pedigree who had a previously documented history of postural tremor (individuals 2002, 2006, 3002 and 2018). However, postural tremor is not an early manifestation of early-onset parkinsonism in this family, which typically presents with asymmetrical limb 'heaviness' and rigidity (11). One individual who shared the haplotype did not have documented postural tremor, although this individual (2023) has an offspring with the haplotype who has postural and activation tremor (11). There are no family members with tremor, without the haplotype.

Table 1. Probabilities of generating lod scores for linked and unlinked markers, and summary of scores observed

Z	Expected[a] Linked (θ = 0)	Linked (θ = 0.05)	Unlinked (θ = 0.01)	Observed[b] Marker scores (θ = 0)
> –2.0	–	–	0.46	352
> –1.0	–	–	0.20	7
0	1.0	1.0	0.08	17
>0.5	0.90	0.71	0.04	10
>1.0	0.73	0.52	0.02	6
>2.0	0.32	0.18	0.01	–
>3.0	0.03	0.02	–	–

[a]'Age-associated penetrance' model gives an Elod of 1.54 at (θ = 0) (E_{max} = 3.34). Probabilities of *Z* for linked (θ = 0 and 0.05) and unlinked (θ = 0.01) markers are shown (28).
[b]Observations are based on 392 genotyped markers. Age-associated and affecteds-only predictions were comparable (data not shown).

Table 2. Calculated 4p lod scores

Marker	cM	Recombination fraction 0	0.05	0.1	0.2	0.3	0.4
Using age-associated liability classes							
D4S1551	40.70	–8.95	0.00	0.22	0.27	0.17	0.06
D4S2397	45.20	1.38	1.20	1.03	0.71	0.43	0.19
D4S391	46.00	–3.05	0.14	0.31	0.35	0.27	0.15
D4S1609	46.60	1.43	1.24	1.06	0.74	0.45	0.21
D4S230	47.20	1.09	0.98	0.88	0.67	0.46	0.24
D4S1618	48.40	0.47	0.42	0.38	0.28	0.19	0.10
D4S2279	48.40	0.46	0.41	0.38	0.28	0.19	0.10
D4S2408	48.40	0.99	0.87	0.76	0.56	0.39	0.20
D4S3350	53.70	–13.83	–1.84	–0.93	–0.14	0.14	0.16
Using early-onset affecteds-only analysis							
D4S1551	40.70	–1.96	0.02	0.19	0.23	0.15	0.06
D4S2397	45.20	1.42	1.24	1.06	0.73	0.45	0.20
D4S391	46.00	–1.65	0.54	0.64	0.57	0.38	0.27
D4S1609	46.60	1.67	1.45	1.24	0.85	0.52	0.37
D4S230	47.20	1.55	1.36	1.17	0.83	0.53	0.26
D4S1618	48.40	0.66	0.58	0.50	0.35	0.22	0.10
D4S2279	48.40	0.63	0.55	0.47	0.34	0.21	0.10
D4S2408	48.40	1.20	1.04	0.90	0.65	0.42	0.21
D4S3350	53.70	–4.90	–0.28	0.13	0.37	0.34	0.21

In the Iowa kindred, only 4p markers give a haplotype which segregates with disease (Fig. 1). Distances between markers is given in Kosambi, sex-averaged centiMorgans based on the Marshfield genetic map (1998).

The haplotype of 3004 may be inferred from descendants to be homozygous at most 4p marker loci. Haplotypes for 2018 and 2023 accurately reflect these individuals' genotypes and are compatible with the fewest number of recombination events; the most parsimonious composite haplotypes have been assigned. However, 2018 and 2023 may not have inherited the disease chromosome from 3004. For 2002, 2006 and 3002, the disease chromosome has been inherited, as several marker genotypes within the segregating 4p region are also homozygous.

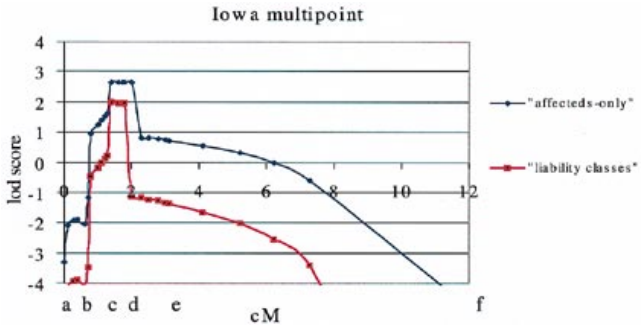

Figure 2. Multipoint analysis of the Iowa kindred using both affecteds-only and age-associated liability classes. Multipoint analysis used the VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recoding and fuzzy inheritance (34); intermarker distances were taken from the Marshfield map. Marker positions, labeled a–f, are indicated on the *x*-axis. The map used was (a) *D4S1091*–0.8 cM– (b) *D4S391*–0.6 cM– (c) *D4S1609*–0.8 cM– (d) *D4S230*–1.2 cM– (e) *D4S1618*–8.9 cM– (f) *D4S3350*, centromeric to telomeric, obtained from the Marshfield map. CEPH allele frequencies were obtained from the public databases.

Reduced penetrance has ramifications for multipoint analysis (Fig. 2). Performance using an early-onset affecteds-only method inflates two-point lod scores to give a maximum multipoint lod score of $Z = 2.64$, $\theta = 0.006$ between *D4S1609* and *D4S230*; multipoint analysis using age-associated liability classes was correspondingly lower, though greater than two-point scores.

DISCUSSION

Overall, these data strongly suggest that there is a locus for autosomal dominant, familial PD on chromosome 4p. This is the third locus identified for this prevalent disorder. While a lod score of >3.0 (1/1000 odds for linkage) was not achieved, the fact that all the affected individuals share the chromosome 4 haplotype but not any other throughout the genome strongly suggests that we are observing true identity by descent at this locus and at no other. Scores observed parallel those expected from *a priori* power analysis and are close to the mean predicted for this pedigree.

However, these results surprised us for two related reasons. We had expected the disease penetrance based on segregation ratios to be greater (14), and the data imply that there may be a common underlying pathogenesis between some forms of PD and some forms of postural tremor. Within this kindred, these very different phenotypes appear to be alternative expressions of the same genetic defect; the postural tremor may represent a forme fruste of the parkinsonism syndrome. In support of this hypothesis, we subsequently genotyped the descendants of 2002, 2006 and 2023, and the 4p haplotype segregation supports the hypothesis that this region segregates with both early-onset parkinsonism and essential tremor (Fig. 1). A possible relationship between PD and postural tremor has long been debated (16–18). A constant observation, even in those studies that suggest a lack of association (17), is that there is an increase in the prevalence of essential tremor among first degree relatives of probands with PD. Thus, Cleeves and colleagues make the suggestion that 'there may be an association between essential tremor and a distinct subgroup of PD' (17). Our data strongly support this suggestion. In addition, there are two loci so far reported for familial essential tremor: one on chromosome 2p22–25 (19) and one on 3q13 (20). These loci are not associated with parkinsonism. Thus, we suggest that there is an etiological relationship between a

subgroup of cases of PD and a subgroup of cases of tremor. In support of this suggestion, both non-penetrance and postural tremor in disease haplotype carriers have precedents in the recent literature on autosomal dominant Lewy body parkinsonism. In the Contursi kindred, penetrance of the genetic defect was estimated to be 85% (6). One individual from the Contursi kindred was an obligate gene carrier at 94 years with no symptoms despite a mean age of onset in this family of 46 ± 13 years. There are also individuals in the Contursi kindred who are more than two standard deviations older than the mean onset age in that kindred who appear to have postural tremor rather than classic PD (5,6,21,22). In the families showing linkage to markers on chromosome 2p13, there are many elderly individuals who carry the disease haplotype who are unaffected by parkinsonism. Indeed, within these chromosome 2-linked pedigrees, the estimated penetrance was only 40% (10/25 individuals bearing the haplotype had disease) (7). In these pedigrees (as in the kindred we report here), ascertainment is biased and there is a strong selection for pedigrees multiply affected by PD, so the true penetrance is likely to be much lower. Lewy body parkinsonism is a common, chronic disorder, with a diagnosis encompassing considerable clinical, pathological and now genetic heterogeneity. The disease may appear as an autosomal dominant trait, but the large number of pre-clinical cases and phenocopies may confound parametric genetic analysis. Non-penetrance and variable expressivity of disease haplotypes in this and the other kindreds with parkinsonism probably reflect the sum of both environmental and genetic interactions with genetic mutations of major effect.

Several genes mapping to 4p14–16.3 may be of interest with respect to the PD phenotype, including the G-protein-coupled dopamine D5 receptor (DRD5) (23), which plays a role in dopamine neurotransmission; cholecystokinin type A receptor (CCK-AR) (24) that contributes to the regulation of dopamine-mediated behavior; and ubiquitin C-terminal hydrolase isozyme L1 (PGP 9.5) (25,26), a major component of Lewy bodies involved the proteolytic processing of polymeric ubiquitin. While this paper was under review, LeRoy *et al*. (27) reported a mutation in PGP 9.5 that occurred in two sibs with PD. With this background, we sequenced PGP 9.5 in cDNA from four individuals 1009, 1019, 1023 and 2013. We identified a coding and expressed polymorphism (S18Y) in individuals 2013 and 1019. We have found subsequently that this polymorphism occurs on ~20% of Caucasian chromosomes and that this gene is just outside our obligate candidate region. Although candidates in the region are being investigated, in all probability positional cloning strategies will have to be employed to find the gene after more families with a similar linked locus are identified.

In conclusion, our data suggest both the occurrence of a new locus for PD on the short arm of chromosome 4 and that postural tremor and parkinsonism can be etiologically related. We considered it inappropriate retrospectively to re-score early-onset parkinsonism and postural tremor as manifestations of the same genetic defect although this would give two-point lod scores >3.0 ($\theta = 0$). We suggest that identification of the pathogenic locus in this kindred will provide clues to the molecular processes underlying both disorders.

MATERIALS AND METHODS

Blood was obtained under informed consent with an IRB approved protocol. DNA was prepared by standard procedures. A full clinical, pathological and biochemical description of the families has been published (9–11). The mean age at onset of the disease was 34.1 ± 8.8 years, ranging from 20 to 48 years old. Twenty-nine individuals were collected and genotyped for the genome search (Fig. 1; for family confidentiality, not all individuals are shown). Descendants of 2002, 2006 and 2023 subsequently were haplotyped on chromosome 4p.

The pedigree was evaluated for its power to detect linkage using the SIMLINK program version 4.12 (28). This analysis suggested that the pedigree, as presently collected, would generate a maximum lod score of 3.34, but predicted an average of 1.53 (an estimate of the true value) if linked markers show no recombination with the disease (Table 1 and Fig. 1). At a recombination fraction of 0.05, approximating to 5 cM, an average two-point lod score of 1.12 was estimated. In a 10 cM genome search, the disease locus will always be within ~5 cM of flanking markers, hence, regions in which markers were observed with positive lod scores >1.0 were analyzed further. The probability that fully informative data would yield a maximum lod score whose value is >3.0 was 1/35 ($\theta = 0$) and 1/67 ($\theta = 0.05$). The estimated mean lod score for an unlinked marker (mean exclusion value) was –2.30 ($\theta = 0.01$) with a range from –6.96 (minimum) to 1.33 (maximum). The estimated probability of generating lod scores greater than –2.0 (1/100 odds for exclusion) at $\theta = 0.01$ (i.e. for an unlinked marker adjacent to the disease locus) was 0.46. Table 1 highlights the probabilities of generating lod scores for linked and unlinked markers. Only early-onset parkinsonism segregating within the pedigree and for whom DNA samples were available for genotyping was modeled (Fig. 1). Late-onset PD was not present in this branch of the Iowa kindred although present in three elderly individuals in a more distant branch descended from 5007. Early-onset parkinsonism was treated as a dichotomous, autosomal dominant trait with the disease allele frequency set at 0.0001 (given the population prevalence of familial parkinsonism). The trait was examined using both affecteds-only and age-associated penetrance models [cumulative normal penetrance function from 0 to 0.95, set about the mean age of onset, 34.1 ± 8.8 (SD) years]. Individuals with postural tremor or with ages within or below two standard deviations of the mean age of onset for disease were considered 'unaffected, but at risk'. Results using either analytical approach were comparable for this pedigree. Marker frequencies were set at 0.40, 0.30, 0.20 and 0.10, and 392 replicates were performed. The probability of a maximum lod score >3.0 gives the probability that the pedigree is sufficient to demonstrate linkage (Table 1). Genotype data were collected at an average spacing of 10 cM, thus the linked gene should be within ~5 cM ($\theta \approx 0.05$) of any marker. Probabilities for Z at $\theta = 0.05$ for a linked marker are shown.

Polymorphic markers were selected from the human genome screening set (version 8), developed by J.L. Weber/CHLC, distributed by Research Genetics. This consists of 365 autosomal markers with an average heterozygosity of 76% and an average spacing of 10 cM. A further 27 markers were used to fill inter-marker gaps >14 cM and to check marker loci generating lod scores of $Z > 1.0$ at $\theta = 0$. Primers were labeled with fluorescent dyes and the PCR products were analyzed on an ABI 377 automated sequencer, using Genescan 2.1 and Genotyper 2.1 software. Two-point linkage analysis was performed using both age-associated liability classes and affecteds-only analysis, with MLINK (29–33). BINOM 1.74 was used to calculate the confidence coefficient (29–33). Age-associated liability classes

define the cumulative probability of becoming affected having inherited the disease haplotype. Six classes up to 95% penetrance were assigned based on the age at disease onset of affected individuals within the pedigree; 0–15 years = 0%; 16–25 years = 11%; 26–35 years = 36%; 36–45 years = 67%; 46–55 years = 89% and >55 years = 95%. Only individuals with early-onset disease were considered affected; pedigree members below or within two standard deviations of the mean age of onset were considered 'unaffected, but at risk'. As the disease segregates in an autosomal dominant fashion, penetrance for homo- and heterozygotes was set equal. The disease allele frequency was set at 0.0001; marker allele frequencies were taken from Marshfield/CEPH. Multipoint analysis was performed using affected-only analysis, and VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recoding and fuzzy inheritance (34); intermarker distances were taken from the Marshfield map.

Ubiquitin C-terminal hydrolase isozyme L1 [NCBI accession nos X17377 (genomic exons 1–2) and X04741 (mRNA, exons 2–9)] was sequenced from cDNA using nested primers designed with Gene Runner 3.05, Hastings software (available on request). PCR conditions were denaturation at 94°C (3 min), followed by 35 cycles of 94°C (20 s), 55°C (30 s), 72°C (45 s), with a final extension at 72°C (10 min). PCR products were purified using QIAquick columns prior to sequencing using dRhodamine terminators on an ABI377. Sequence chromatograms were analyzed using PolyPhredPhrap (35).

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