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Localization of the gene for rapidly progressive autosomal dominant parkinsonism and dementia with pallido-ponto-nigral degeneration to chromosome 17q21

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Rapidly progressive autosomal dominant parkinsonism and dementia with pallido-ponto-nigral degeneration (PPND) is a neurodegenerative disorder which begins later in life (>30 years of age) and is characterized by rapidly progressive parkinsonism, dystonia, dementia, perservative vocalizations and pyramidal tract dysfunction. The disease is observed in a large American family that includes almost 300 members in nine generations with 34 affected individuals. In this kindred evidence for linkage to chromosome 17q21 was obtained with a maximum lod score of 9.08 for the D17S958 locus. Multilocus analysis positions the disease gene in an ~10 cM region between *D17S250* and *D17S943*. Notably, the disease locus for a clinically distinct familial neurodegenerative disease named 'disinhibition-dementia-parkinsonism-amyotrophy complex' (DDPAC) was recently mapped to the same region of chromosome 17, suggesting that PPND and DDPAC may possibly originate from mutations in the same gene.

INTRODUCTION

We described previously (1) a large family presenting with a strikingly uniform picture of an autosomal dominant disease with parkinsonism that responded poorly to anti-parkinsonian medication. Symptoms included progressive parkinsonism, dementia, ocular motility impairment, pyramidal tract dysfunction, frontal lobe release signs, perservative vocalizations and urinary incontinence. Magnetic resonance imaging demonstrated cerebral atrophy affecting predominantly frontal, parietal and temporal lobes, narrowing of the *substantia nigra pars compacta*, atrophy of the pontine tegmentum (2). Dopaminergic functions were investigated with [^{18}F]-L-6-fluorodopa and positron emission tomography demonstrating significantly decreased nigrostriatal activity (3). Autopsy findings revealed severe neuronal loss with gliosis in the *substantia nigra*, *pontine* and *mesencephalic tegmentum* and *globus pallidus*. Plaques, tangles, Lewy bodies and amyloid deposits have not been seen. Immunohistochemically, abundant neurophil threads, complement activated oligodendrocytes and oligodendroglial microtubular masses were found (4). The disease shows an unique aggressive course with an onset in the fifth decade and an average duration of 8–9 years after which death, usually caused by aspiration pneumonia, occurs. This family appears to represent a distinct neurodegenerative disease with a characteristic combination of genetic, neuropathological and clinical features.

We employed a linkage mapping approach as a first step towards positional cloning of the pallido-ponto-nigral degeneration (PPND) disease gene and a better understanding of the pathophysiological differences between PPND and other forms of hereditary diseases with parkinsonism.

Our findings indicate that the PPND locus maps to chromosome 17q, in the same region where a distinct neurological disease (disinhibition-dementia-amyotrophy complex; DDPAC) gene has been localized (5).

RESULTS

Family studies

The PPND family contains 34 affected individuals over nine generations and has been described (1,6). One of us (Z.K.W.)

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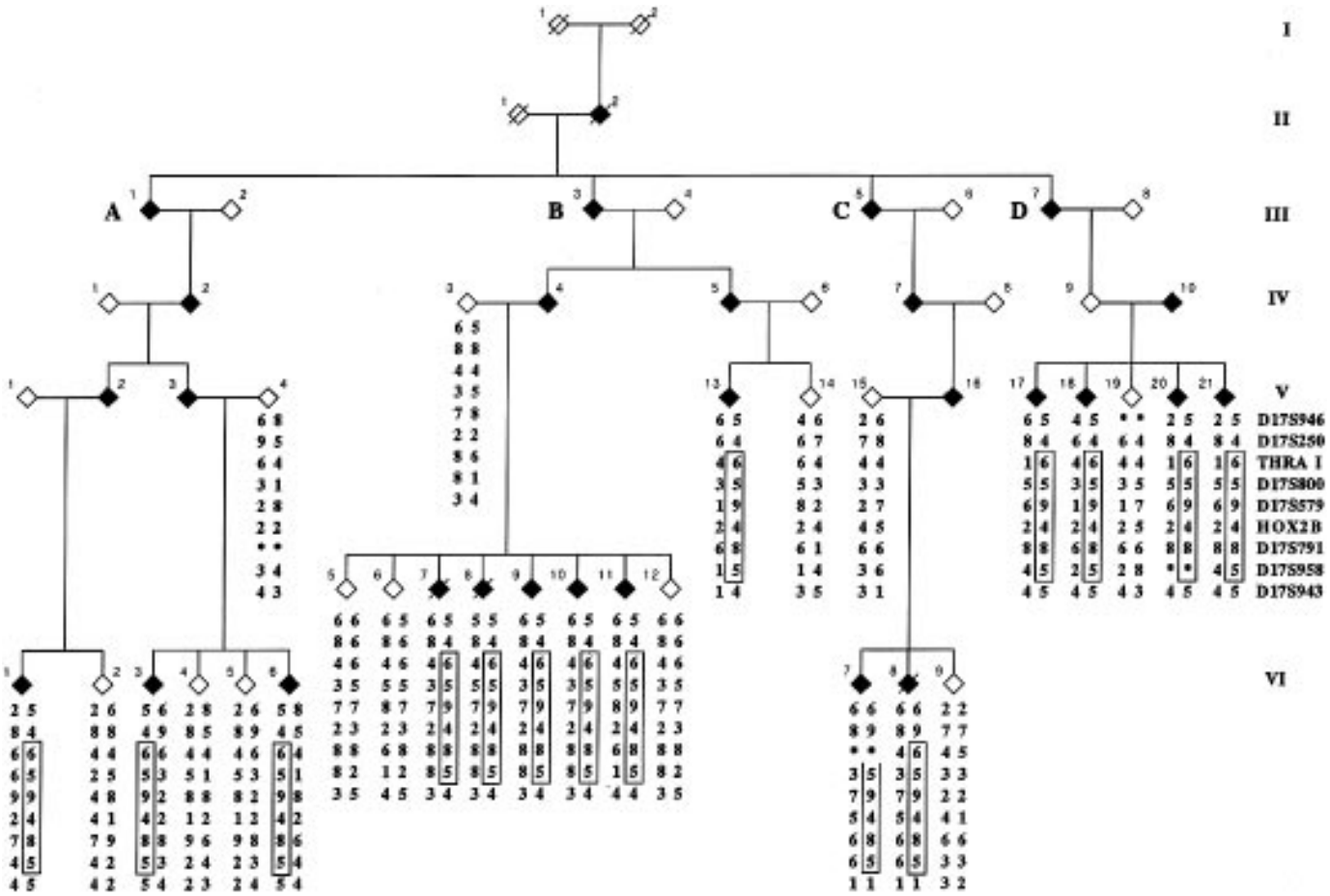

Figure 1. Partial pedigree with haplotype data of the PPND family. Affected individuals are indicated by solid symbols. No male or female symbols are shown and order of birth is changed for confidentiality reasons. The disease associated haplotype is boxed. Branch C shows recombination between D17S250 and THRA1. Recombination occurred between D17S958 and D17S943 in branches B and C.

investigated all the living patients and most of their sibs. An individual was diagnosed as affected based on the presence of parkinsonism, personality change and/or dementia.

The age of onset of the disease varies from 32 to 58 years. We estimate that the disease penetrance in this pedigree is 15% by age 40, 80% by age 45 and >90% after 50.

Blood samples from 182 family members including 15 affected individuals were obtained (Fig. 1). We selected 27 individuals that would be most informative for our linkage study. The average age of phenotypically healthy sibs included in this study was 49 years, so we assumed a penetrance of 90%. The youngest generation was not included since no patients were observed yet.

Linkage analysis

Our initial linkage studies focused on a few candidate regions. Markers located close to the prion-like protein on the short arm of chromosome 20, close to the dopamine D2 receptor on chromosome 11q and markers covering 90% of chromosome 4p were tested, but none of them suggested linkage.

Subsequently, we started a systematic genome search with polymorphic microsatellite markers evenly distributed over the human autosomes with an average distance of ~20 cM. Computer simulation showed that the maximum LOD score for a 10% linked marker (assuming four equally frequent alleles) was >4.

Over 70 microsatellite markers were typed before linkage was detected with markers mapping to the long arm of chromosome 17. Two point maximum likelihood calculations between D17S579 and the disease phenotype resulted in a highly significant lod score of 8.36 at $\theta = 0$.

Table 1 summarizes the two point maximum likelihood data between chromosome 17q markers [D17S798 (7 cM) D17S946 (0.1 cM) D17S250 (1.8 cM) THRAI (3.8 cM) D17S800 (1 cM) D17S579 (1 cM) HOX2B (1 cM) D17S791 (3 cM) D17S958 (1 cM) D17S943 (3 cM) D17S941 (1 cM) D17S788] and the PPND locus. Order of loci and recombination frequencies were taken from the Genome Database (Johns Hopkins University).

Haplotypes defined by loci listed in Table 1 are shown in Figure 1.

Multipoint analysis using adjacent pairs of marker loci resulted in a multilocus lod score of 10.8 (Fig. 2). Haplotype analysis places the PPND locus between the loci D17S250 and D17S943.

Table 1. Two point lod scores between chromosome 17q markers and the disease locus

Locus	0.00	0.01	0.05	0.10	0.20	0.30	0.40	θ_{max}	Z_{max}
D17S798	–3.80	–1.18	0.15	0.60	0.76	0.56	0.25	0.180	0.77
D17S946	–8.67	0.23	1.28	1.44	1.16	0.68	0.23	0.097	1.44
D17S250	0.69	4.83	5.09	4.78	3.74	2.46	1.09	0.039	5.11
THRAI	5.30	5.20	4.82	4.33	3.26	2.11	0.95	0.000	5.30
D17S800	2.07	2.04	1.90	1.70	1.23	0.74	0.30	0.000	2.07
D17S579	8.47	8.32	7.72	6.93	5.26	3.46	1.61	0.000	8.47
HOX2B	6.60	6.49	6.04	5.43	4.09	2.64	1.17	0.000	6.60
D17S791	5.94	5.84	5.40	4.84	3.63	2.33	1.05	0.000	5.94
D17S958	9.08	8.93	8.33	7.54	5.83	3.94	1.93	0.000	9.08
D17S943	–7.50	–1.22	1.21	1.89	1.92	1.34	0.56	0.147	2.02
D17S941	–20.40	–3.56	–1.54	–0.77	–0.19	–0.01	0.03	0.402	0.03
D17S788	–10.70	–1.87	–0.60	–0.17	0.07	0.06	0.01	0.200	0.07

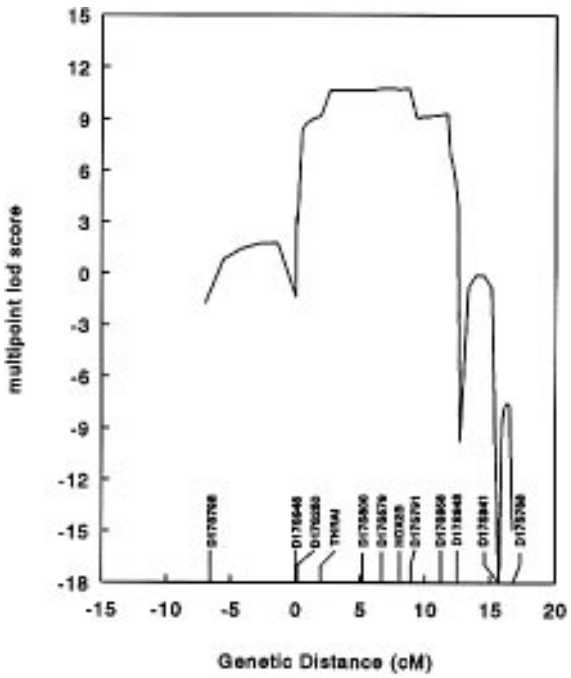

Figure 2. Multipoint Lod score map of the PPND locus versus chromosome 17q markers, using the LINKMAP program. D17S946 was arbitrarily set at 0.

DISCUSSION

In this study we report the mapping of the gene for PPND to chromosome 17q. Analysis of recombination events further localizes the disease gene to a region of ~10 cM between D17S250 and D17S943. All patients carry the same 'affected' haplotype for markers within this region (Fig. 1). DDPAC, a neurological disease described in the Mo family as clearly distinct from PPND (7), was recently mapped to an overlapping region on chromosome 17 (5). DDPAC is autosomal dominant with progressive dementia and parkinsonism and is characterized by behavioral and personality changes such as alcoholism, withdrawal and disinhibition. DDPAC patients show postural instability, rigidity and bradykinesia. Neuropathological examination reveals neuronal loss and gliosis in the substantia nigra and amygdala but no Lewy bodies, neurofibrillary tangles or amyloid plaques are observed. Contrary to PPND, DDPAC is found to be a phenotypically heterogeneous disease (7).

Based on the map position of the genes for the two diseases we speculate that mutations in the same gene could cause parkinsonism, dementia and personality changes observed in both conditions. Cloning of the disease genes for DDPAC and PPND will allow us to test this hypothesis. The similar map location also indicates that it may be worthwhile to test other familial neurological disorders with symptoms in common with PPND and DDPAC, for linkage to the chromosome 17q markers.

Candidate genes for neurological diseases in the 17q region are the tau (τ) - protein (8) and $p75^{ngfr}$ (9). Their possible involvement in DDPAC has been discussed recently (5).

We traced the PPND mutation back to a female 'founder' who was born in 1854 and died at the age of 32 years. Her husband died at age 74 years. Her parents died at the ages of 71 and 82 years and her grandparents reached the age of 71, 77, 83 and 90 years. The oldest affected PPND patient died at age 65 years (mean age of death calculated from available data on 22 affected individuals was 52 years). Therefore we assume that she transmitted a *de novo* PPND mutation. Comparison of the disease haplotype in the PPND family, all descending from the 'founder female', with the disease haplotype in the family with DDPAC strongly suggest that a different mutational event is involved in both disorders. Genealogical investigations performed so far have not revealed any connection between these two families.

Cloning the PPND gene and its functional characterization will help us to understand the underlying etiology of parkinsonism and processes involved in neuronal cell loss. The localization of both the PPND and DDPAC locus may also be a further step in the classification of neurological disorders on a genetic basis.

MATERIALS AND METHODS

Family studies

The PPND family, as previously described (1), was clinically evaluated by Z.K.W. At the time of evaluation peripheral blood

samples were obtained for establishment of EBV-transformed cell lines and DNA extraction.

DNA analysis

Genomic DNA was isolated by standard proteinase K digestion, phenol/chloroform extraction and isopropanol precipitation. Analysis of simple sequence CA-repeat markers was performed using published nucleotide primer sequences (10). Microsatellite markers were obtained from the Dutch Microsatellite Marker Collection (established by The Netherlands Organisation of Scientific Research). Markers were selected for spanning the whole genome with ~20 cM spacing. PCR reactions were carried out in 15 µl and 1.7 µM fluorescein-12-dUTP (Boehringer Mannheim) was added. Concentrations dATP, dCTP, dGTP were 200 µM and dTTP was 50 µM. PCR products were separated on 6% polyacrylamide/7 M urea (Biozym) using the A.L.F. sequencer (Pharmacia LKB Biotechnology). The Fragment Manager software version 1.1 (Pharmacia LKB Biotechnology) was used to analyse the data (11). The sizer 50–500 (Pharmacia Biotech) and internal PCR markers were used to calculate exact allele length.

Linkage analysis

The MLINK and the LINKMAP routine of the LINKAGE package (version 5.1) were used to calculate pairwise lod scores and perform multilocus analysis. PPND was deemed to have autosomal dominant inheritance with a disease frequency of 0.000001 and a penetrance of 90%. No new mutations were allowed and no different recombination rates between male and female were used. Allele frequencies and (sex averaged) recombination frequencies between loci were taken from the Genome Database (Johns Hopkins University, G00-270-726, G00-355-556) and the recent literature (12–14). However, one must consider a relatively high female/male recombination frequency ratio in this chromosomal subregion. A ratio of 2 has been used between D17S588 and D17S74 based on data reported by Devilee *et al.* (15).

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