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Two large British kindreds with familial Parkinson's disease: a clinico-pathological and genetic study

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**These authors contributed equally to this work*

Summary

We present the findings of a study of two large unrelated kindreds with autosomal dominant Parkinson's disease. The affected members were assessed clinically and with [^{18}F]6-fluorodopa-PET and were indistinguishable from patients with the sporadic form of Parkinson's disease. In one kindred, an affected member was examined subsequently at autopsy and Lewy bodies were present in a distribution typical of sporadic Parkinson's disease. These kindreds are distinct from other Parkinsonian kindreds with identified genetic loci (*PARK1–4*) and provide further evidence for genetic heterogeneity in familial Parkinson's disease.

Keywords: Parkinson's disease; familial; Lewy body; α-synuclein; parkin

Abbreviations: [^{18}F]dopa = [^{18}F]6-fluorodopa; *PGP9.5* = ubiquitin C-terminal hydrolase isozyme L1 gene

Introduction

Parkinson's disease is a complex disorder of unknown aetiology, believed to involve a combination of genetic and environmental factors (Ben-Shlomo, 1996). The genetic contribution in Parkinson's disease has been debated for over a century, since Gowers noted that 15% of his patients had affected relatives (Gowers, 1893). Frequently, this debate has centred around whether the often noted family history of parkinsonism was a reflection of shared environment rather than genetic factors. Although several pedigrees initially were described with parkinsonian features (Bell and Clark, 1926; Allan, 1937; Spellman, 1962), often there were no supporting pathological data. More recently, an increasing number of well-documented multigenerational parkinsonian kindreds have been reported with evidence of autosomal dominant inheritance with variable penetrance. Only a few kindreds have been reported where the clinico-pathological features are indistinguishable from the sporadic form of the disease, with a late age of onset, good L-dopa response and typical Lewy body neuronal inclusions (Wszolek *et al*., 1995). Others exhibited atypical features, such as young age of onset and rapid disease course (Golbe *et al*., 1996), marked cognitive decline with an atypical distribution of Lewy bodies (Muenter *et al*., 1998) or apathy, hypoventilation and scattered Lewy bodies (Perry *et al*., 1975, 1990). Finally, the chromosome 17-linked syndromes of pallidopontonigral degeneration (Wszolek *et al*., 1992; Clark *et al*., 1998) and frontotemporal dementia (Hutton *et al*., 1998) can show parkinsonism as part of their rather broad phenotype.

The identification of a G209A (Ala53Thr) mutation in exon 4 of the α-synuclein gene on chromosome 4q21–23

in the Contursi kindred (Golbe *et al*., 1996; Polymeropoulos *et al*., 1997) represented the first step in understanding the molecular basis of dominantly inherited Parkinson's disease. Although a second mutation in α-synuclein (Ala30Pro) has been identified in exon 3 of a small German kindred (Kruger *et al*., 1998), mutations within α-synuclein are responsible for only a tiny fraction of familial Parkinson's disease cases (Munoz *et al*., 1997; Bennett and Nicholl, 1998; Farrer *et al*., 1998; Vaughan *et al*., 1998*a*; Zareparsi *et al*., 1998). In addition, other autosomal dominant parkinsonian loci have been described recently: *PARK3* on chromosome 2p13 (Gasser *et al*., 1998) and two separate loci on chromosome 4p [an Ile93Met mutation in the ubiquitin C-terminal hydrolase isozyme L1 gene (*PGP9.5*) on 4p14 in two German siblings (Leroy *et al*., 1998), and a disease-segregating haplotype on chromosome 4p15.1 in a large Iowan kindred (*PARK4*) (Farrer *et al*., 1999) also known as the Spellman–Muenter–Waters kindred (Spellman, 1962; Waters and Miller, 1994; Muenter *et al*., 1998)]. Analysis of these loci by other groups suggests that these are likely to be rare causes of familial and sporadic Parkinson's disease (Harhangi *et al*., 1999; Klein *et al*., 1999; Maraganore *et al*., 1999; Wintermeyer *et al*., 2000).

The other known Parkinson's disease locus, *PARK2*, represents an autosomal recessive form of juvenile parkinsonism linked to mutations/deletions in a ubiquitin ligase gene, *parkin*, on chromosome 6q (Kitada *et al*., 1998; Shimura *et al*., 2000). The PARK2 phenotype differs from that of typical Parkinson's disease in its juvenile onset, mode of inheritance and lack of Lewy body pathology, but mutations in *parkin* appear to be the commonest cause of juvenile Parkinson's disease (Lucking *et al*., 2000). The parkin phenotype seems extremely variable, including dopa-responsive dystonia (Tassin *et al*., 2000), apparently dominant forms of parkinsonism (Klein *et al*., 2000; Farrer *et al*., 2001) and later onset of typical Parkinson's disease in the sixth or seventh decade (Abbas *et al*., 1999; Klein *et al*., 2000).

Thus the study of other large dominantly inherited Parkinsonian kindreds will be important in furthering our understanding of the pathogenesis of Parkinson's disease. Unfortunately, such kindreds are both rare and of limited size, making linkage analysis to identify the gene loci problematic. We describe the clinical and pathological features in two large unreported kindreds who are of sufficient size to identify pre-clinical cases with PET and the genetic defect(s) using linkage analysis and positional cloning.

Material and methods

Patients

The families were recruited as part of an ongoing European study of familial Parkinson's disease (Vaughan *et al*., 1998*a*). The diagnosis of Parkinson's disease was made using: (i) a pathologically proven diagnosis according to the UK Parkinson's Disease Society Brain Bank criteria (Hughes *et al*., 1992) or (ii) a clinical diagnosis of idiopathic Parkinson's disease using a similar study design on familial Parkinson's disease (Maraganore *et al*., 1991) with at least two of the three cardinal signs present: tremor, rigidity and bradykinesia; responsiveness to L-dopa; and unilateral/asymmetric symptoms at onset and no atypical features. One affected member from each kindred was scanned with [^{18}F]6-fluorodopa ([^{18}F]dopa)-PET. In some cases, a retrospective diagnosis of Parkinson's disease was made in deceased family members via a review of medical records, family documentation and videos where at least two of the three cardinal signs (bradykinesia, rigidity or tremor) were present. A diagnosis of possible Parkinson's disease was based on the historical account from other family members, if there was insufficient information to make a reliable diagnosis based on the above criteria.

Genealogical methods

Genealogical data were collected via civil and church records of births, deaths and marriages. Familial lineages had been traced extensively by a distant member of one kindred for reasons unrelated to this study.

PET

An affected member from each kindred was scanned using an ECAT EXACT3D (CTI/Siemens 966) 3D-only PET tomograph after intravenous injection of 3.5–4.5 mCi of [^{18}F]dopa. Analysis of data was performed using in-house software written in IDL (Research Systems, Inc, Boulder, Col., USA). Region of interest analysis was performed using a standard template as previously described (Rakshi *et al*., 1996). [^{18}F]Dopa influx constants (K_i/min values) were calculated for right and left caudate and putamen using the multiple time graphical analysis approach with occipital activity as a reference tissue. Both scans were analysed by a single observer (P.P.).

Molecular analysis

Genomic DNA was extracted from peripheral blood using standard techniques. PCR (polymerase chain reaction) was performed by using 75 ng of genomic DNA per reaction as previously described (Vaughan *et al*., 1998*a*), and the PCR products were analysed on an ABI 377 automated sequencer (ABI, San Francisco, Calif., USA) using Genescan 2.1 and Genotyper 2.1 software. Linkage/haplotype analysis of the known loci for parkinsonism was performed. Genotype data from the markers shown in Fig. 5 were managed and recoded for linkage analysis using Cyrillic 2.1.3. LOD scores were generated using the FASTLINK version of the MLINK program (Cottingham *et al*., 1993; Dwarkadas *et al*., 1994)

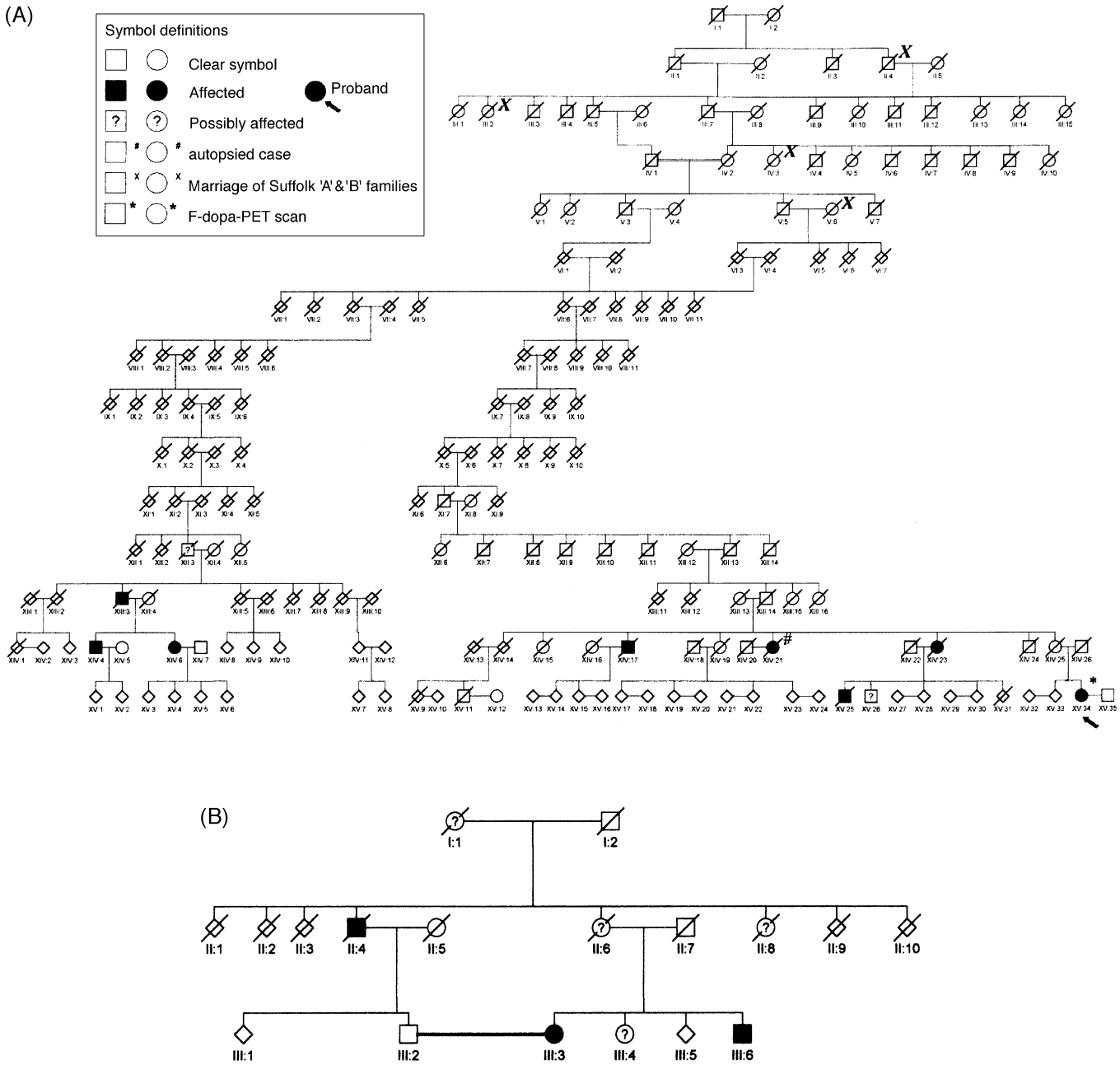


Fig. 1 Pedigree drawing of Suffolk kindreds A (**A**) and B (**B**).

under an 'affecteds-only' analysis. This included only clinically definite Parkinson's disease as indicated in the pedigrees shown in Figs 1 and 4. Affecteds-only analysis was applied due to the wide range of disease onset and because penetrance may be uncertain, as has been observed in other loci predisposing to familial Parkinson's disease (Polymeropoulos *et al*., 1996). 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; de Rijk *et al*., 1997), and marker allele frequencies were set equal. A phenocopy rate for Parkinson's disease in the general population was set at 1.5% (de Rijk *et al*., 1997). Multipoint analysis was performed subsequently using FASTLINK (LINKMAP) affecteds-only analysis (Cottingham *et al*., 1993; Dwarkadas *et al*., 1994). In the multipoint analyses, all markers were included in a single two-point linkage calculation apart from the 2p13 locus, where three overlapping partial linkage analyses were performed. Intermarker distances were taken from the Marshfield map (http://

Table 1 *Suffolk kindreds A and B—clinical summary*

Case	Sex	Age at onset (years)	Disease duration (years) (and current status)	First symptoms	Bradykinesia	Rigidity	Rest tremor	Postural instabilty	Other features	Response to L-dopa	Source
Suffolk kindred: family A											
XV:34	F	65	7 (alive)	Asymmetric RT	+	+	+	+	O–OF; D	+	PE
XIV:21	F	65	20 (dead)	Asymmetric RT	+	+	+	+	O–OF; D; autopsied	+	PE
XIV:23	F	75	5 (dead)	Asymmetric RT	+	+	+	+		+	PE
XV:25	M	42	11 (dead)	Asymmetric B	+	+	+	+	Painful dystonias; panic attacks	+	PE
XIV:17	M	73	10 (dead)	B	+		+			Not given	V; H
XIV:6	F	57	14 (alive)	Asymmetric RT	+	+	+	–	D	+	PE
XIV:4	M	68	6 (alive)	Asymmetric RT	+	+	+	+	O–OF; D	+	PE
XIII:3	M	91	2 (dead)	RT	+	+				Not given	H
XV:26	M	54	3 (alive)	Asymmetric tremor	–	–	–	–	Asymmetric action tremor; impassive face	Unclear	PE
III:3	F	58	16 (alive)	Asymmetric RT/B	+	+	+	+	O–OF; D	+	PE
III:6	M	73	5 (alive)	Asymmetric B/RT	+	+	–	–	O–OF; D	+	PE
Suffolk kindred: Family B											
I:1	F	76	10 (dead)	Tremor							H
II:4	M	Late 50s	~10 (dead)	Asymmetric B/RT	+		+			Not given	H
II:6	F	85	10 (dead)	Tremor							H
II:8	F	72	~10 (alive)	Tremor	+						H
III:3	F	58	15 (alive)	Asymmetric B/RT	+	+	+	+	Micrographia	+	PE
III:4	F	75	7 (alive)		+	+	–		Probable neuroleptic induced PD, akathisia; dementia		PE
III:6	M	73	5 (alive)	Asymmetric B/RT	+	+	+	+		+	PE

Individuals in bold correspond with 'affecteds' in Fig. 1A and B. RT = rest tremor; O–OF = on–off fluctuations; B = bradykinesia; D = dyskinesias; PE = personal examination; V = review of family video; H = historical report from family members.

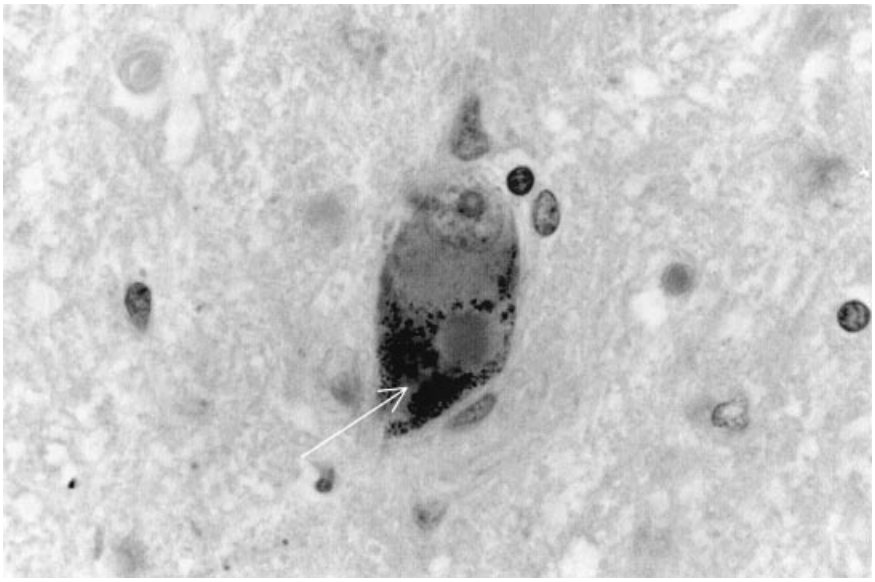


Fig. 2 Suffolk kindred XIV:21: photomicrograph of a pigmented neurone in substantia nigra containing a Lewy body characteristic of idiopathic Parkinson's disease. Haematoxylin–eosin ×350.

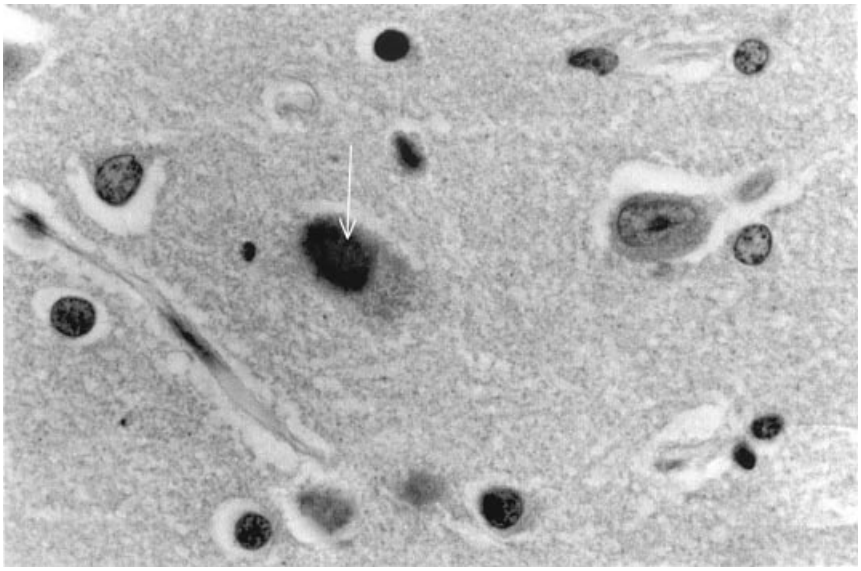


Fig. 3 Suffolk kindred XIV:21: occasional cortical Lewy bodies identified using immunocytochemistry for α-synuclein (1 : 2000; courtesy of Dr D. Hanger). Haematoxylin counterstain ×350.

www.marshmed.org/genetics/) and are given in Kosambi sex-averaged centiMorgans (cM).

Two-point linkage analysis and multipoint analysis were performed using three linked markers to the *PARK1* locus on chromosome 4q21–q23 as previously described (Polymeropoulos *et al*., 1996). D4S2380 co-localizes with D4S423 which flanks the α-synuclein gene. Exclusion analysis with four polymorphic markers (D6S1550, D6S305, D6S411 and D6S1579) spanning the *PARK2* locus was performed (Matsumine *et al*., 1997). These markers co-localize on the Marshfield map and, therefore, two-point LOD scores for chromosome 6 markers were calculated assuming an autosomal dominant model (data not shown). Multipoint analysis was performed using data from eight polymorphic markers spanning the region from D2S2320 to D2S286 (*PARK3* locus) (Gasser *et al*., 1998) and from four markers linked to *PARK4* (Farrer *et al*., 1999). Multipoint linkage analysis in this area also included examination of D4S405, a marker 12.29 cM distal to the 4p15.1 locus [location of the gene ubiquitin C-terminal hydrolase isoenzyme L1 (*PGP9.5*)]. Where multipoint data were not conclusive across the region (i.e. LOD scores were not less than –2.0, the accepted criteria for exclusion), the relevant gene was sequenced in an index case from that kindred.

Table 2 *Lincolnshire kindred—clinical summary*

Case	Sex	Age at onset (years)	Disease duration (years) and (current status)	First symptoms	Bradykinesia	Rigidity	Rest tremor	Postural instability	Other features	Response to L-dopa	Source
I:1	M	65	10 (dead)	Asymmetric RT	+	+	+			+	H
II:1	F	78	8 (dead)	Asymmetric RT	+	+	+			+	H
II:3	F	40	12 (dead)	Asymmetric RT	+	+	+	+		Not given	H
II:6	M	60	10 (dead)	Asymmetric RT	+	+	+	+		+	H
II:9	M	55	15 (dead)	Asymmetric RT	+	+	+	+		+	H
II:11	F	69	10 (dead)	Asymmetric RT	+	+	+	+		Not given	H
III:1	M	–	80 years old at examination		–	–	–	–	Facial hypomimia and stooped gait only. Not progressed over 2 years	Not given	PE
III:3	F	48	9 (dead)	Asymmetric RT	+	+	+			+	H
III:7	F	–	78 years old at examination	–	–	–	–	+	Slight unsteadiness of gait	Not given	PE
III:8	F	57	19 (alive)	Asymmetric RT	+	+	+		O–OF, D, lower limb dystonia	+	PE
III:11	M	47	8 (dead)	Asymmetric RT	+	+	+		Psychosis	+	H
III:12	M	64	10 (alive)	Asymmetric RT	+	+	+			+	H
III:13	F	48	11 (dead)	Asymmetric RT	+	+	+		Rapid progression	+	H
III:14	F	54	15 (alive)	Asymmetric RT	+	+	+		O–OF, D, lower limb dystonia	+	PE
III:16	F	44	19 (alive)	Asymmetric RT	+	+	+		Severe 'yes–yes' head tremor; O–OF, D, lower limb dystonia	+	PE
III:17	M	58	8 (alive)	Right-sided B and tremor	+	+	+/–		Slight upper limb tremor	+	PE
IV:1	M	44	1	Asymmetric RT	–	+	+	–	Pyramidal signs R leg	+	MR

Individuals in bold correspond with 'affecteds' in Fig. 4. RT = rest tremor; O–OF = on–off fluctuations; B = bradykinesia; D = dyskinesias; PE = personal examination; H = historical report from family members; MR = review of medical records.

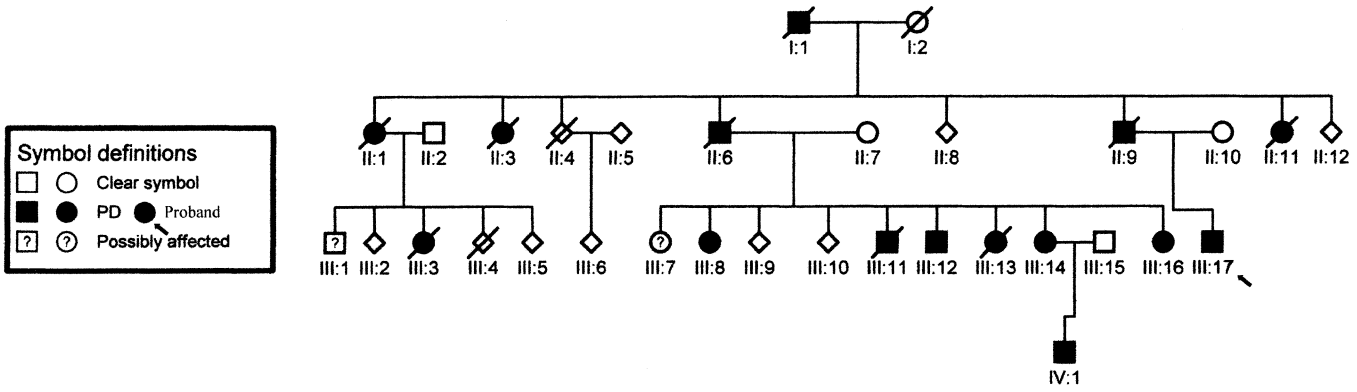


Fig. 4 Pedigree drawing of the Lincolnshire kindred.

Neuropathology

The whole brain was fixed in 10% neutral formalin for 6 weeks prior to cutting. Tissue blocks were taken from the frontal [anterior (Brodmann area 9) and precentral region (Brodmann area 4)], temporal, parietal and occipital cortex, hippocampus, parahippocampus, striatum, thalamus, hypothalamus, subthalamus, substantia innominata, cerebellar vermis and hemisphere, midbrain, pons and medulla. For light microscopy, sections of cerebrum, brainstem and cerebellum were examined using haematoxylin–eosin, luxol fast blue Nissl, Bielschowsky silver impregnation and immunocytochemistry for glial fibrillary acidic protein (Dako; 1 : 400), ubiquitin (1 : 150) and α-synuclein (1 : 2000).

Informed written consent was obtained from all subjects, and the study received approval from the ethical committees of the University Hospital NHS Trust, Birmingham, National Hospital for Neurology and Neurosurgery, London, and the Hammersmith Hospitals Trust Research Ethics Committee. Approval to administer radiolabel ligands was obtained from the Administration of Radioactive Substances Advisory Committee of the UK.

Results

Kindred 1: the Suffolk kindred—family A

The main pedigree is shown in Fig. 1A and has been abbreviated to protect family confidentiality.

A total of eight affected members (four males and four females) were identified, including one subject (XIV:21) who was examined neuropathologically. Six cases (XV:25, XV:34, XIV:4, XIV:6, XIV:21 and XIV:23) were examined personally by the authors (D.J.N., J.R.V. and S.L.H.), along with 22 other unaffected family members. Two cases were designated as affected based on interviews with multiple first-degree relatives. One case (XV:26) was examined (by D.J.N.) and it was unclear whether there were early signs of Parkinson's disease or not. Assuming that a single gene locus was responsible for the parkinsonism in this family, the mode of transmission was consistent with autosomal dominant inheritance with reduced penetrance. Based on deceased family members, who had lived to the age of 75, XIII-1 to XIII-9 and XIV-14 to XIV-XIV:25 (as these were the only individuals for which complete medical information was known and who had lived long enough to fully acquire their Parkinson's disease risk), some 36% (4/11) of individuals were affected.

Clinical features

A summary of the clinical phenotype and response to L-dopa is shown in Table 1 whilst a more detailed description of the proband and autopsied case follows. Clinical status was known for only the three most recent generations (XIV, XV and XVI), but none of generation XVI have fully realized their Parkinson's disease risk.

The mean age of onset for family A was 64 years (42–75; SD ± 11.2). At least two individuals were obligate heterozygotes with no evidence of Parkinson's disease based on interviews with their relatives: XIII:14 died aged 78 years of 'bronchitis' and his daughter (XIV:25) died aged 82 years of 'probable heart disease'. Other disorders that were noted in members of this kindred include XV:11, who died of amyotrophic lateral sclerosis aged 53 years, and XV:26 who had an asymmetrical postural and action tremor for the last 3 years. Dementia has not been a feature in any of the examined affected members.

XV:34: the index case

This case developed an asymmetrical rest tremor and bradykinesia of her right arm at the age of 65 years. She was put on L-dopa aged 66 years with good effect. Parkinsonism has slowly progressed and at last assessment was Hoehn and Yahr stage 2.5 with some postural instability. A video clip of XV:34 is shown at Internet address http://medweb.bham.ac.uk/http/depts/clin_neuro/papers/brain/nicholl-etal.mov and shows her aged 70 years demonstrating all the relevant clinical features of Parkinson's disease: asymmetrical rest tremor, facial impassivity, bradykinesia, reduced arm swing and postural instability. Her [^{18}F]dopa K_i

values were: left caudate = 0.0068/min, right caudate = 0.0071/min, left putamen = 0.0047/min, right putamen = 0.0052/min ([^{18}F]dopa K_i values for 12 normal volunteers matched for age: right and left caudate = 0.0145/min, right and left putamen = 0.0150/min). The observed pattern of striatal [^{18}F]dopa reduction is characteristic of idiopathic disease, uptake in the putamen being affected more than in the caudate (Brooks *et al*., 1990).

XIV:21: the autopsied case

This case initially presented with a rest tremor in her left arm, first noted whilst holding a pair of binoculars aged 65 years. Her symptoms gradually progressed, with subsequent development of bradykinesia and a hesitant gait. She was put on L-dopa and remained on it for at least a further 16 years. There was a good response with L-dopa (Sinemet) and pergolide throughout. She subsequently developed marked motor fluctuations and dyskinesias. These symptoms slowly progressed over the following 20 years and she was bed-bound for the last 18 months of her life. She had been treated intermittently for depression, but there was no evidence of dementia. She died aged 85 years of septicaemia.

Pathology. Macroscopically, there was mild atrophy involving the posterior frontal region with slight dilatation of the lateral ventricle. Pigment was markedly depleted in the substantia nigra and locus ceruleus. Under light microscopy, substantia nigra and locus ceruleus pigmented neurones were moderately depleted, with several surviving nerve cells containing Lewy bodies (Fig. 2). Above the brainstem, Lewy bodies were also identified in nucleus basalis of Meynert and amygdaloid nuclear complex. The caudate nucleus showed slight increased gliosis; there were no significant abnormalities of thalamus, subthalamus, putamen, pallidum or claustrum.

In cerebral cortex, occasional Lewy bodies were found in anterior cingulate gyrus, parahippocampus, and frontal and temporal neocortex. Lewy body scores according to consensus guidelines (McKeith *et al*, 1996) were area 1 = 0, area 2 (anterior cingulate gyrus) = 13, area 3 = 1, area 4 (parahippocampus) = 3 and area 5 = 0. Lewy neurites were few in number in the CA2/3 region.

All Lewy body pathology was immunoreactive with anti-ubiquitin and anti-α-synuclein (Fig. 3) and was visualized more easily using these techniques. There were additional age-related cortical changes of mature senile plaques which were moderate in number in frontal cortex; neurofibrillary tangles were inconspicuous except in hippocampus and parahippocampus. The overall appearances were characteristic of idiopathic Parkinson's disease.

Genealogy. The kindred had ~10 000 members in some 250 branches, but only the two branches of this pedigree where Parkinson's disease was known to be a feature and with a definite common ancestor are shown in Fig. 1A. The founding couple were both born in a village in Suffolk in the 15th century (I:1 born 1450; I:2 born 1480). The kindred shared an unusual family name, with only 1 in 6000 UK families sharing this name (British Telecom, 1998). The origins of all living individuals with this family name can be traced back to this village in Suffolk.

During the recruitment for this study, a number of other Parkinson's disease kindreds were identified where family members originated from Suffolk. An example of one such kindred, Suffolk kindred B, is shown in Fig. 1B and originates from the same village as Suffolk kindred A. There was strong circumstantial evidence to suggest a genealogical link between Suffolk kindreds A and B: (i) the graves of the two families were intermingled in the same village graveyard and (ii) there were at least four marriages which had taken place between members of family A and persons bearing the same surname as family B in the 15th and 16th century (Fig. 1A: II:4, III:2, IV:3 and V:6). The origins of Suffolk kindred B were traced back to the late 18th century, but no firm genealogical link between the two Suffolk kindreds could be made.

Kindred 1: the Suffolk kindred—family B

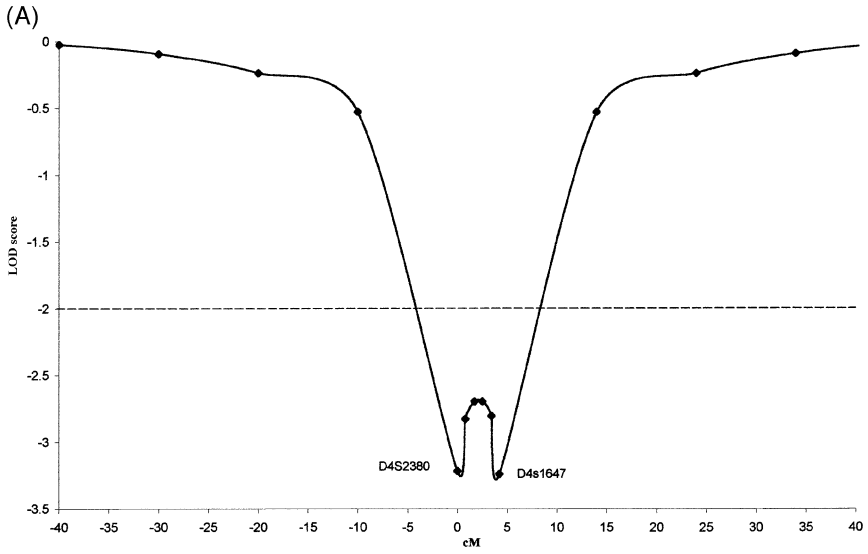
Family B had four affected members (Fig. 1B): III:3 and III:6 had L-dopa-responsive typical Parkinson's disease (examined by D.J.N. and J.R.V.); II:4 and II:8 had Parkinson's disease based on family report. In addition, there were other members of family B who appeared to have either essential tremor (I:1, II:6; based on family report) or highly atypical parkinsonism (III:4; examined by D.J.N.) rather than Parkinson's disease. No post-mortem data were available on this kindred.

Kindred 2: the Lincolnshire kindred

The pedigree for this kindred is shown in Fig. 4. The clinical description of this kindred has not been reported previously, although the linkage data on this family were reported upon briefly (Family UK-A; Gasser *et al*., 1997). Fifteen affected members were identified (seven male; eight female). All six living affected members and nine unaffected members were examined personally (by J.R.V., N.L.K., D.J.N. and G.G.L.). Two further members were examined (by J.R.V. and G.G.L.) in which the presence of Parkinson's disease could not be confirmed unequivocally.

Clinical description

Asymmetrical rest tremor was the most common initial presentation, with a good L-dopa response invariably with subsequent development of motor fluctuations and dyskinesias (Table 2). Clinical course was similar to that of sporadic Parkinson's disease but with an earlier age of onset. Clinical status is known for four of the most recent generations. Segregation ratios are based on generation III as these were the only individuals for whom complete medical information is known and who have lived long enough to acquire their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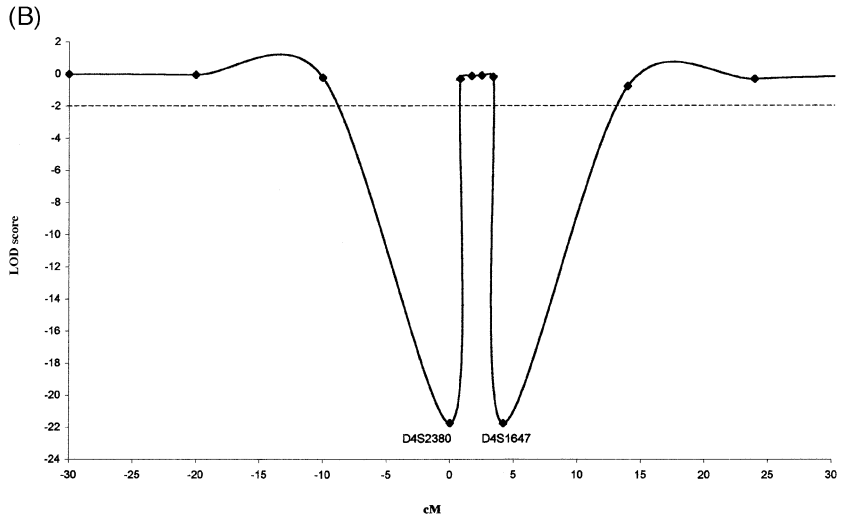
LOD score
cM
D4S2380
D4s1647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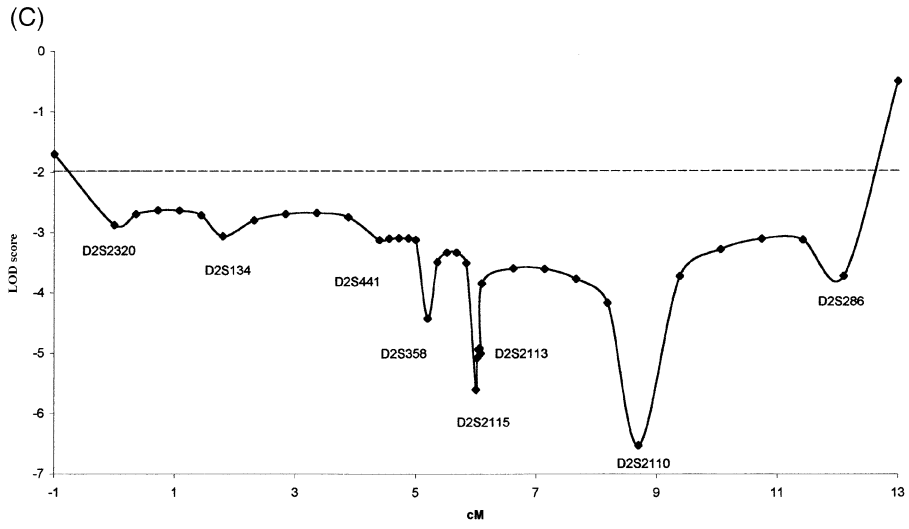
LOD score
cM
D4S2380
D4S1647


(C)
LOD score
cM
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D2S134
D2S441
D2S358
D2S2113
D2S2115
D2S2110
D2S286

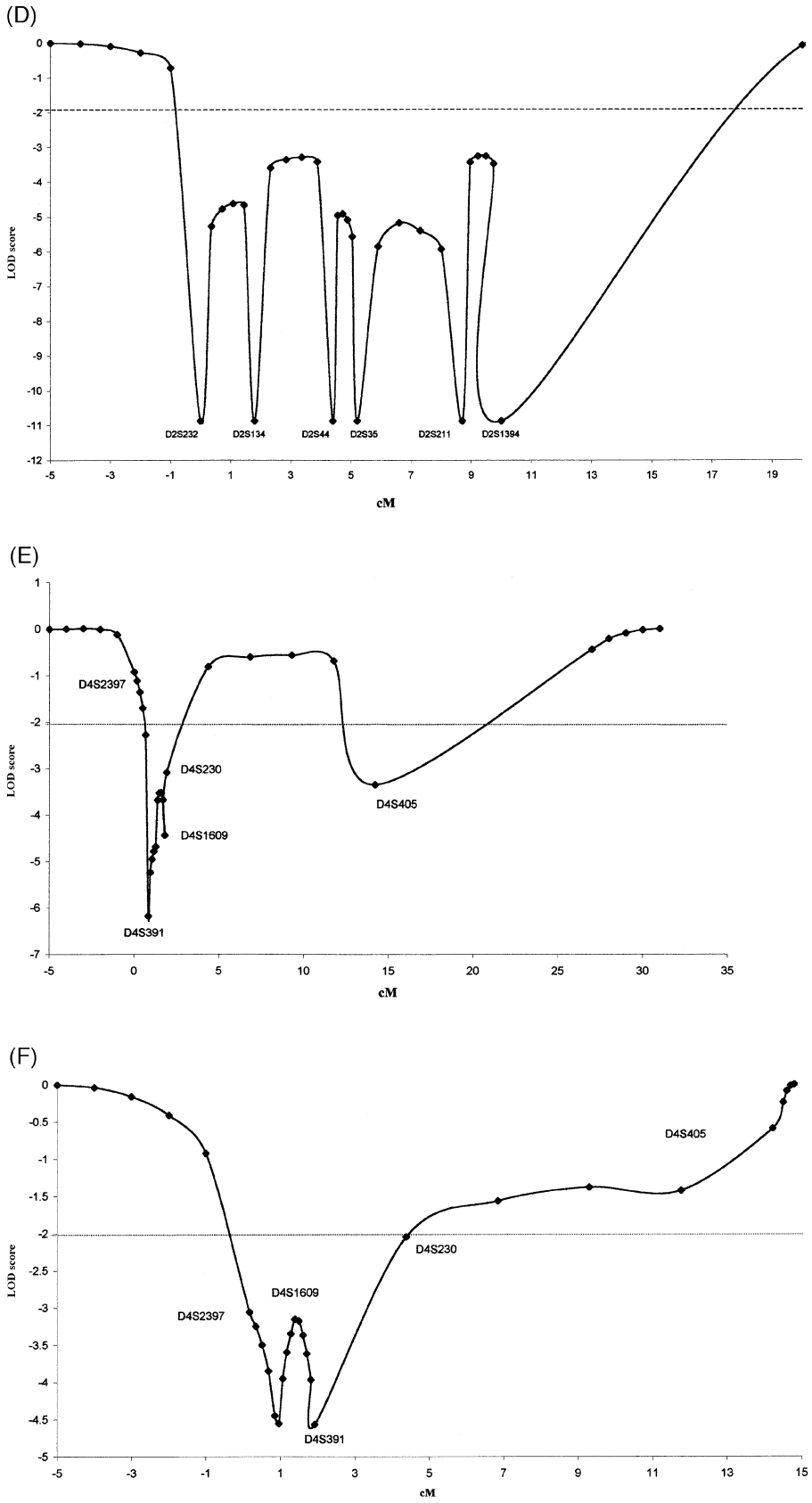

Fig. 5 Multipoint analyses of the *PARK1* (**A** and **B**), *PARK3* (**C** and **D**) and *PARK4* (**E** and **F**) loci in Suffolk kindred A (**A**, **C** and **E**) and the Lincolnshire kindred (**B**, **D** and **F**).

Parkinson's disease risk fully. The mean age of onset for the kindred is 57 years old (44–72; SD ± 13.2).

The mode of inheritance was consistent with autosomal dominant inheritance with reduced penetrance. There were no obligate heterozygotes. No post-mortem data were available on this kindred.

III:17: the index case

This case developed an asymmetrical rest and slight upper limb tremor with bradykinesia of the right arm at the age of 58 years. At presentation, he also had a flexed posture and gait disturbance. He started on L-dopa aged 59 years with good effect. His parkinsonism has slowly progressed and at last assessment was Hoehn and Yahr stage 2.0. His [^{18}F]dopa K_i values were: left caudate = 0.0072/min, right caudate = 0.0074/min, left putamen = 0.0054/min, right putamen = 0.0057/min/min. In this subject, the pattern of striatal [^{18}F]dopa reduction was also characteristic of idiopathic disease.

Linkage analysis of candidate regions

PARK1 locus (α-synuclein) (Fig. 5A and B)

Two-point analysis excluded linkage to the two polymorphic markers most closely linked to *PARK1* (D4S2380 and D4S1647) in these two families, although the multipoint exclusion data were not conclusive across the region (Fig. 5B). For this reason, the entire coding region of the α-synuclein gene was sequenced in an index case in each family and no mutations were found (Vaughan *et al*., 1998*b*).

PARK2 locus (parkin; 6q 25.2–27) (data not shown)

Most parkin families described to date show a recessive model of inheritance. Two-point LOD scores for chromosome 6 markers were calculated assuming an autosomal dominant model to illustrate that neither haplotypes nor consanguineous genotypes could be shared by individuals with the disease in these kindreds.

PARK3 locus (2p13) (Fig. 5C and D)

Linkage of each kindred to locus 2p13 was excluded using two-point and multipoint analysis. Multipoint analysis results are as shown in Fig. 5C and D.This corresponds to D2S2320–1.8 cM–D2S134–2.6 cM–D2S441–0.8 cM–D2S358–0.8 cM–D2S2115–0 cM–D2S2113–2.6 cM–D2S2110–1.3 cM–D2S1394–2.1 cM–D2S286. The data also include markers linked to the segregating haplotype identified for 2p13 (Gasser *et al*., 1998).

PARK4 locus (4p14–15) (Fig. 5E and F)

Multipoint and two-point analyses excluded linkage to *PARK4*. The marker map in this region corresponds to D4S2397–3.0 cM–D4S391–0.6 cM–D4S1609–0.6 cM–D4S230–12.29 cM–D4S405. The coding region of the *PGP9.5* gene was sequenced in an index case from the Lincolnshire kindred as the multipoint linkage data were not conclusive across the region, but no mutations were found.

Discussion

In both the Suffolk and Lincolnshire kindreds, the initial clinical presentation with an asymmetrical rest tremor, followed by the subsequent development of the other features of L-dopa-responsive parkinsonism, was indistinguishable from the sporadic form of Parkinson's disease (Hughes *et al*., 1992), with a comparable age of onset. Likewise, in the Suffolk kindred, the neuropathological appearances with pigment depletion in substantia nigra and adjacent structures, with typical Lewy bodies in surviving neurones, were identical to those found in sporadic Parkinson's disease. This differs from the majority of the published Parkinsonian kindreds which, apart from notable exceptions (Wszolek *et al*., 1995; Gwinn-Hardy *et al*., 2000), often have had atypical features.

In spite of the clinical similarities and the physical proximity between Suffolk and Lincolnshire (Fig. 6), we are unaware of any genealogical links between the two kindreds and suspect that, due to the reduced degree of penetrance and the slightly later age of onset in the Suffolk kindred, the genetic basis of the parkinsonism in the two kindreds may differ.

Aggregation of Parkinson's disease in current generations along with historic evidence of Parkinson's disease in deceased family members in both kindreds suggested a

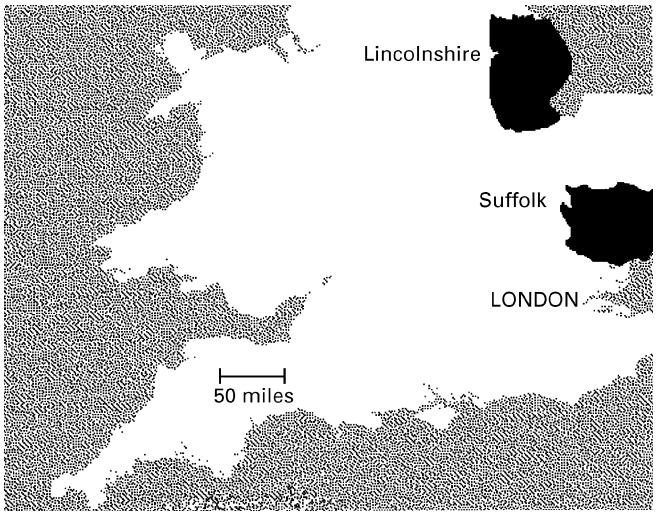

Fig. 6 Map of the UK showing the counties of Suffolk and Lincolnshire.

genetic trait consistent with autosomal dominant inheritance of a major gene with reduced penetrance. The pedigrees were evaluated conservatively for their power to detect linkage using the SLINK program (Ott, 1989; Weeks *et al*., 1990). This revealed that for a linked marker, the Suffolk kindred A may generate a maximum two-point LOD score of 1.24 at $\theta = 0$. In the Lincolnshire kindred, a linked marker may generate a maximum two-point LOD score of only 0.65 at $\theta = 0$ but, if the two pedigrees were linked to the same genetic locus, this generated a maximum combined two-point LOD score of 1.88 at $\theta = 0$. Theoretical modelling assuming that the Suffolk A and the Lincolnshire kindreds do share the same genetic locus revealed that families would have a 40% probability of observing a LOD score of >1.0, $\theta = 0$. However, only at $\theta = 0$ are LOD scores likely to reach –2.0, the accepted criterion for exclusion. In the Suffolk kindred, although there was strong circumstantial evidence that kindreds A and B are related (Fig. 1A), and several other phenotypically similar Parkinson's disease kindreds originate from this region (some of whom have been described previously; Maraganore *et al*., 1991), no direct genealogical link was made between these various families.

Other important factors to consider include the size of the Suffolk kindred. Assuming a prevalence of Parkinson's disease of 2% in those aged over 65 (de Rijk *et al*., 1997), there are likely to be individuals who have had sporadic Parkinson's disease and thus represent phenocopies. One potential example of this was a member of Suffolk kindred A who was a distant relative of the individuals shown in Fig. 1. He had neuropathologically confirmed typical Lewy body Parkinson's disease (S. Daniel, personal communication). This individual had no family history of Parkinson's disease, yet shared the same family name and was traced back to an ancestor who lived in 1400 in a parish adjacent to the founding Suffolk village.

No common environmental factor was identified which could explain the occurrence of Parkinson's disease in either of these kindreds. Although both Suffolk and Lincolnshire are predominantly rural counties with a large proportion of the population involved in agriculture, the affected members have lived in both urban and rural areas throughout the UK with no consistent environmental exposure that could have accounted for their parkinsonism. Likewise, no conjugal Parkinson's disease cases were identified, which would be more suggestive of an environmental cluster, rather than a genetic effect.

Since there was no medical information regarding the earlier generations of the Suffolk kindred, we were unable to exclude the possibility of more than one Parkinson's disease locus in this family. It was thought that this was unlikely given: (i) the similar phenotypes and age of onset of the two branches of the Suffolk kindred and (ii) that the the genealogical data suggested that other families from this region could be related to Suffolk kindred A. Apart from Suffolk kindred B, four other Parkinson's disease families from this region have been identified, including some who have been described previously (Maraganore *et al*., 1991).

Nonetheless, it was impossible to exclude the possibility that the parkinsonism in the Suffolk kindred was due to more than one gene since no medical information is available prior to generation XIII. This has presented problems in other kindreds where a single gene locus has been assumed (e.g. in a large Amish kindred, bipolar depression appeared to be inherited as a complex trait even though all the affected members could be traced back to a single founder in around 1750, and segregation analysis suggested dominant inheritance; Ginns *et al*., 1996; Risch and Botstein, 1996).

The fact that no linkage has been shown to the three major loci or mutations detected in the coding region of the two genes described in autosomal dominant Parkinson's disease to date in either the Suffolk or Lincolnshire kindreds (Polymeropoulos *et al*., 1996, 1997; Gasser *et al*., 1998; Leroy *et al*., 1998; Farrer *et al*., 1999) indicates that at least one (if not two) further loci are yet to be described and that genome screening of both kindreds may demonstrate linkage to a novel autosomal dominant Parkinson's disease locus. Both the mode of inheritance and haplotype analysis excluded linkage to *PARK2*, although there have been recent reports of apparently dominant Parkinson's disease kindreds with parkin mutations (Klein *et al*., 2000; Farrer *et al*., 2001).

The identification of genetic forms of Parkinson's disease in the last 4 years has transformed our understanding of the pathogenesis of parkinsonism and other α-synucleinopathies (Kruger *et al*., 2000), even though mutations in α-synuclein are a rare cause of familial Parkinson's disease (Munoz *et al*., 1997; Bennett and Nicholl, 1998; Farrer *et al*., 1998; Vaughan *et al*., 1998*a*; Zareparsi *et al*., 1998). Parkinsonian kindreds of the size of the Contursi kindred are exceedingly rare (Golbe *et al*., 1996), and the clinico-pathological description of other Parkinson's disease kindreds, distinct from any of the known loci, is an important first step in the identification of the genetic defects involved.

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