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


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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 [18F]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: [18F]dopa = [18F]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., 1992; Clark et al., 1998). 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 (Muentet 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...
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 \(^{18}\text{F}\)fluorodopa (\(^{18}\text{F}\)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 \(^{18}\text{F}\)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). \(^{18}\text{F}\)Dopa influx constants \((K_i/\text{min values})\) 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., 1998a), and the PCR products were analysed on an ABI 377 automated sequencer (ABI, San Francisco, Calif., USA) using Genescan 2.1 and Genotype 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)

**Material and methods**

**Patients**

The families were recruited as part of an ongoing European study of familial Parkinson’s disease (Vaughan et al., 1998a). The diagnosis of Parkinson’s disease was made using: (i) a pathologically proven diagnosis according to the UK
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>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age at onset (years)</th>
<th>Disease duration (years) and current status</th>
<th>First symptoms</th>
<th>Bradykinesia</th>
<th>Rigidity</th>
<th>Rest tremor</th>
<th>Postural instability</th>
<th>Other features</th>
<th>Response to L-dopa</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>III:3</td>
<td>F</td>
<td>58</td>
<td>16 (alive)</td>
<td>Asymmetric RT/B</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O–OF; D</td>
<td>+</td>
</tr>
<tr>
<td>II:6</td>
<td>M</td>
<td>73</td>
<td>5 (alive)</td>
<td>Asymmetric B/RT</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>O–OF; D</td>
<td>+</td>
</tr>
<tr>
<td>Suffolk kindred: Family A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>F</td>
<td>65</td>
<td>7 (alive)</td>
<td>Asymmetric RT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O–OF; D</td>
<td>+</td>
</tr>
<tr>
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<td>F</td>
<td>65</td>
<td>20 (dead)</td>
<td>Asymmetric RT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O–OF; D</td>
<td>+</td>
</tr>
<tr>
<td>XIV:23</td>
<td>F</td>
<td>75</td>
<td>5 (dead)</td>
<td>Asymmetric RT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>PE</td>
</tr>
<tr>
<td>XV:25</td>
<td>M</td>
<td>42</td>
<td>11 (dead)</td>
<td>Asymmetric B</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Painful dystonias; panic attacks</td>
<td>+</td>
</tr>
<tr>
<td>XIV:17</td>
<td>M</td>
<td>73</td>
<td>10 (dead)</td>
<td>B</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Not given</td>
<td>V; H</td>
</tr>
<tr>
<td>XIV:6</td>
<td>F</td>
<td>57</td>
<td>14 (alive)</td>
<td>Asymmetric RT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>D</td>
<td>+</td>
</tr>
<tr>
<td>XIV:4</td>
<td>M</td>
<td>68</td>
<td>6 (alive)</td>
<td>Asymmetric RT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O–OF; D</td>
<td>+</td>
</tr>
<tr>
<td>XIII:3</td>
<td>M</td>
<td>91</td>
<td>2 (dead)</td>
<td>RT</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Asymmetric action tremor; impassive face</td>
<td>Not given</td>
</tr>
<tr>
<td>XV:26</td>
<td>M</td>
<td>54</td>
<td>3 (alive)</td>
<td>Asymmetric tremor</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Not given</td>
<td>H</td>
</tr>
<tr>
<td>III:3</td>
<td>F</td>
<td>58</td>
<td>16 (alive)</td>
<td>Asymmetric RT/B</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O–OF; D</td>
<td>+</td>
</tr>
<tr>
<td>III:6</td>
<td>M</td>
<td>73</td>
<td>5 (alive)</td>
<td>Asymmetric B/RT</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>O–OF; D</td>
<td>+</td>
</tr>
<tr>
<td>Suffolk kindred: Family B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I:1</td>
<td>F</td>
<td>76</td>
<td>10 (dead)</td>
<td>Tremor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
</tr>
<tr>
<td>II:4</td>
<td>M</td>
<td>Late 50s</td>
<td>~10 (dead)</td>
<td>Asymmetric B/RT</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Not given</td>
<td>H</td>
</tr>
<tr>
<td>II:6</td>
<td>M</td>
<td>85</td>
<td>10 (dead)</td>
<td>Tremor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not given</td>
<td>H</td>
</tr>
<tr>
<td>II:8</td>
<td>F</td>
<td>72</td>
<td>~10 (alive)</td>
<td>Tremor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H</td>
</tr>
<tr>
<td>III:3</td>
<td>F</td>
<td>58</td>
<td>15 (alive)</td>
<td>Asymmetric B/RT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Micrographia</td>
<td>+</td>
</tr>
<tr>
<td>III:4</td>
<td>F</td>
<td>75</td>
<td>7 (alive)</td>
<td>Asymmetric B/RT</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Probable neuroleptic induced PD, akathisia; dementia</td>
<td>+</td>
</tr>
<tr>
<td>III:6</td>
<td>M</td>
<td>73</td>
<td>5 (alive)</td>
<td>Asymmetric B/RT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>PE</td>
</tr>
</tbody>
</table>

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.
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.

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.
Table 2  *Lincolnshire kindred—clinical summary*

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

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.
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 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 proband (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 [18F]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 ([18F]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 [18F]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
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 ($\alpha$-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 $\alpha$-synuclein gene was sequenced in an index case in each family and no mutations were found (Vaughan et al., 1998b).

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).

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
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 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 $\alpha$-synucleinopathies (Kruger et al., 2000), even though mutations in $\alpha$-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., 1998a; 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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