J Neurol (2002) 249 : 1127–1129
DOI 10.1007/s00415-002-0757-6

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Exclusion of the Nurr1 gene in autosomal recessive Parkinson's disease

Received: 17 August 2001
Received in revised form: 15 January 2002
Accepted: 29 January 2002

Sirs: Studies suggest that the etiology behind Parkinson's disease (PD) might be related to abnormal development of the midbrain dopaminergic neurons. This would be particularly relevant for early onset cases, since maintenance of malfunctioning dopaminergic neurons over a normal life span is difficult to imagine [3, 5, 9, 10]. The nuclear orphan receptor Nurr1 is widely expressed in the CNS [3]. $Nurr1^{-/-}$ mice fail to develop midbrain dopamine neurons whereas other dopaminergic populations seem to be largely unaffected [15]. Overexpression of Nurr1 is, however, insufficient for dopaminergic differentiation, suggesting that Nurr1 is one of several factors governing this developmental process [11, 14]. Given the putative role of Nurr1 in the development and maintenance of dopaminergic neurons, it was of interest to test it for a causal role in autosomal recessive PD (ARPD). Two monogenic forms of PD with autosomal recessive (AR) inheritance have been described. Mutations in parkin (PARK2 locus) account for a significant proportion of ARPD and a new locus, PARK6 on chromosome 1, has been identifed [6, 8] (Rawal et al., submitted) [12]. In this study we tested the Nurr1 locus, by means of linkage and haplotype analyses, in 12 families with a spread genetic background, after excluding linkage to the PARK2 and PARK6 loci.

Twelve families were included according to the following criteria: i) two out of the three cardinal signs of PD (tremor, bradykinesia and rigidity) were present and reduced by at least 30 % with levodopa treatment, ii) apparent autosomal recessive transmission and iii) absence of parkin mutations after screening all 12 coding exons [1, 8] (Rawal et al., submitted) and exclusion of linkage to the PARK6 locus [13]. Patients with Babinski signs, early dementia, ophthalmoplegia or early autonomic failure were excluded. There were 5 families (one consanguineous) with at least two affected siblings, and 7 consanguineous isolated cases. The families had a spread genetic background: France (n = 5), Italy (n = 3), Algeria (n = 2), Brazil (n = 1) and Portugal (n = 1). Five of the families had early-onset PD (onset < 40 years in at least one affected sibling) while the remaining seven families had an age at onset > 40 years. Clinical information and blood for DNA extraction was collected for each patient with written informed consent.

The PARK2 locus was analysed with four intragenic markers (D6S1559, D6S305, D6S411 and D6S1550) located within the parkin gene. For the Nurr1 locus, four markers spanning 0.7 cM on chromosome 2, that flank the Nurr1 gene were used: D2S142/D2S141/D2S2360–0.7-D2S284. Primer sequences and PCR conditions were according to GDB. Amplified products were analysed by denaturing 5 % polyacrylamide gel electrophoresis with an automated sequencer (ABI PRISM 377) and GeneScan 3.1.2 and Genotyper 1.1.1 software (Applied Biosystems). Haplotypes were reconstructed manually. Two point LOD scores were calculated with Fastlink for all markers flanking the Nurr1 gene [4]. Multipoint LOD scores were calculated for the consanguineous cases using the Linkage program [7]. The following parameters were used: autosomal recessive inheritance, gene frequency of 1/100, equal recombination rates in males and females, allele frequencies according to GDB.

Nurr1 was tested using four closely flanking microsatellite markers. Neither haploidentity among affected sibs nor homozygosity in patients with consanguinity was observed for the Nurr1 markers (see figure). The two point LOD scores were negative at $\emptyset = 0.00$ for at least one marker. The multipoint LOD scores were negative over the entire interval containing Nurr1, thereby excluding linkage in all families. There is therefore no evidence that Nurr1 constitutes an important locus for ARPD, or for sporadic cases [2]. However, the implication of Nurr1 in other forms of PD (e. g. autosomal dominant) cannot be ruled out. The excludison of the known loci for ARPD, PARK2 and PARK6, in addition to the candidate gene Nurr1, points to the existence of yet unknown genes that account for a large proportion of families with ARPD.

Acknowledgements We thank Drs. Nacer Abbas and Giovanni Stevanin for their contribution and Dr. Merle Ruberg for critical reading of the manuscript. This study was supported by grants from the Assistance Publique-Hôpitaux de Paris, the Association

Family	Mean age at onset in years (range)	No. of affected	Origin	Consanguinity
FPD 237	33 (26-40)	2	Portugal	No
FPD 232	65 (53-76)	4	France	No
FPD 290	56 (52-60)	3	France	No
FPD 268	64 (52-73)	4	Brazil	No
FPD 134	48 (35-60)	2	Algeria	Yes
SPD 130	47	1	France	Yes
SPD 072	38	1	France	Yes
SPD 162	66	1	Algeria	Yes
SPD 189	45	1	France	Yes
JMP 11 (SPD)	32	1	Italy	Yes
JMP 38 (SPD)	37	1	Italy	Yes
JMP 32 (SPD)	42	1	Italy	Yes

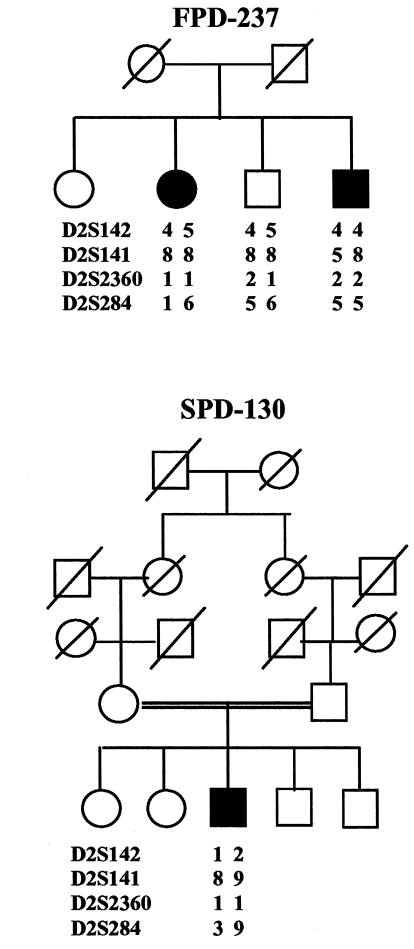

Figure Characteristics of the included families and haplotype reconstruction in 2 representative families. Left: characteristics of the families included. Right: genotype or haplotype reconstruction for microsatellite markers D2S142, D2S141, D1S2360 and D2S284, which flank the Nurr1 are shown for two representative families (FPD 237 and SPD 130). Circles and squares represent females and males, respectively. Black symbols indicate affected individuals. Double horizontal lines represent marriage between related individuals. Alleles are numbered according to GDB.

France-Parkinson, from the Italian Ministry of Health and MURST to GDM, from the European Community Biomed 2 (BMHCT960664), and Aventis-Pharma.

Appendix

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Genome Database, http://www.gdb.org
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