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Sequencing of the α-synuclein gene in a large series of cases of familial Parkinson's disease fails to reveal any further mutations

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A mutation in exon 4 of the human α-synuclein gene was reported recently in four families with autosomal dominant Parkinson's disease (PD). In order to examine whether mutations in this exon or elsewhere in the gene are common in familial PD, all seven exons of the α-synuclein gene were amplified by PCR from index cases of 30 European and American Caucasian kindreds affected with autosomal dominant PD. Each product was sequenced directly and examined for mutations in the open reading frame. No mutations were found in any of the samples examined. We conclude that the A53T change described in the α-synuclein gene is a rare cause of PD or may even be a rare variant. Mutations in the regulatory or intronic regions of the gene were not excluded by this study.

INTRODUCTION

A recent report described an American-Italian kindred (Contursi kindred) with autosomal dominant inheritance of a levodopa-responsive parkinsonian syndrome. Affected patients exhibited the core triad of tremor, rigidity and bradykinesia characteristic of classical Parkinson's disease (PD) as well as pathological evidence of Lewy bodies. Genomic analysis of this family ultimately led to mapping of a locus to chromosome 4q21–23, designated PD-1 (1). These authors have since identified a G→A transition at position 209 in exon 4 of the α-synuclein gene causing an alanine to threonine substitution at position 53 [Ala53Thr (2)] and proposed this as the causative mutation necessary for the development of the parkinsonian phenotype in the kindred. Support for this hypothesis was given by the finding of the same mutation in affected members of three apparently unrelated Greek families. However, shortly before identification of the α-synuclein gene mutation, polymorphic markers spanning the locus PD-1 were examined in 13 European multigenerational PD families with an autosomal dominant pattern of inheritance, and showed no linkage in 11 out of the 13 families (3). In two small families, positive lod scores were obtained, indicating the possibility of linkage.

Following identification of the Ala53Thr mutation in the α-synuclein gene, we sequenced all seven exons of α-synuclein in 30 index cases of familial PD. Some of these families were found previously to be unlinked to the PD-1 locus (3). Sequencing of the α-synuclein gene would allow us to confirm or refute the linkage data obtained and, more importantly, to estimate the numerical importance of the α-synuclein gene in autosomal dominant PD.

RESULTS AND DISCUSSION

Polymerase chain reaction (PCR) products were generated from genomic DNA from 30 index cases of familial PD using a panel of primer pairs flanking each exon of the α-synuclein gene. Each of these products were of a size consistent with published data (2). These were then sequenced directly in order to screen for the presence of polymorphisms and/or mutations, but no coding base pair differences were found relative to the published sequence in any of the 30 families.

The recent description of a mutation in the α-synuclein gene in four families with autosomal dominant PD may aid in the understanding of the genetic basis of familial PD. The mutation, if it is indeed pathogenic, is a rare cause of PD (4,5). While these authors reported the same amino acid change in the Contursi kindred and in three apparently unrelated Greek kindreds, it remains a possibility that they may share a common origin as the two locations formerly were connected by a trade route. Thus, in the absence of haplotype data, a founder effect cannot be excluded.

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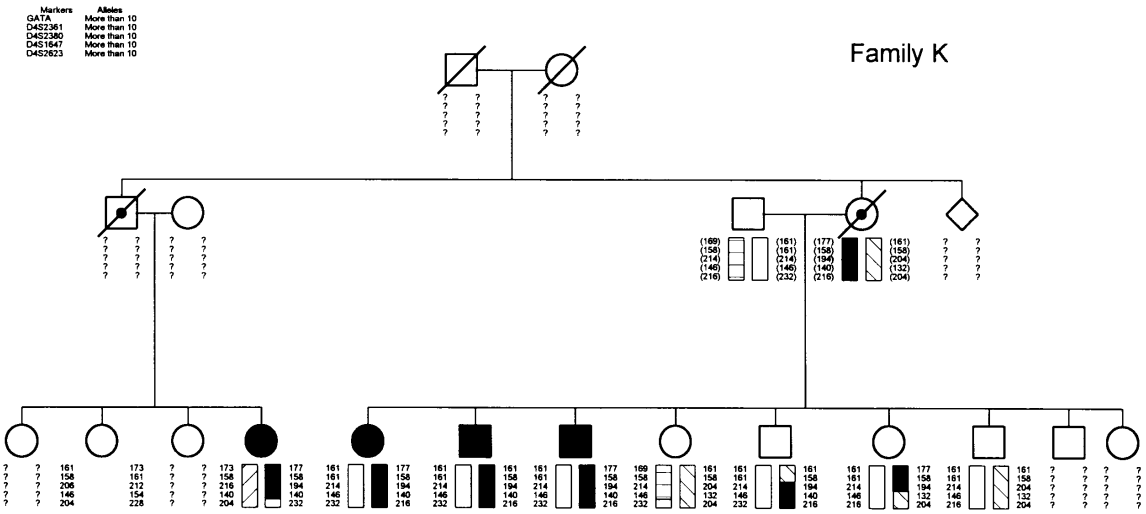

Figure 1. Haplotype data obtained from polymorphic markers spanning the PD-1 locus on chromosome 4q21–q23 in family K.

Table 1. Clinical characteristics of 16 of the families

Family	No. of affecteds	No. of affecteds examined	Region of origin	Mean age at onset	Atypical features	Reference
A	12	4	German-Canadian	51 (35–60)	Amyotrophy/dementia	(9,10)
B	8	4	Danish-American	62 (51–82)	Dementia (some)	(9,10)
C	11	4	German-American	60 (55–66)	None	(9,10)
D	18	5	English-American	63 (48–78)	None	(10,11)
G	11	4	German-American	56 (48–74)	Dementia (some)	(12)
IT-027	9	4	Italian	55 (46–67)	None	unpublished
IT-1	6	4	Italian	54 (36–89)	None	(13)
IT-0	3	2	Italian	56 (28–74)	None	(14)
K	4	4	German	56 (45–63)	None	unpublished
L	6	2	English	60 (52–66)	None	unpublished
M	3	3	English	45 (30–55)	None	unpublished
N	16	8	English	53 (42–70)	None	unpublished
O	4	4	English	48 (38–55)	None	unpublished
P	7	3	English	64 (58–70)	None	unpublished
Q	3	3	Welsh	63 (59–65)	None	unpublished
R	8	6	English	64 (42–75)	None	unpublished

All affected members exhibited at least two out of four of the cardinal parkinsonian signs (akinesia, resting tremor, rigidity, postural instability), and improvement on l-Dopa therapy.

To date, no patients with sporadic PD have been found to carry the Ala53Thr α-synuclein mutation (2). In addition, the amino acid at position 53 is not conserved between mammalian species, in contrast with neighbouring residues. For instance, in rats, a threonine at position 53 is the native sequence. To test whether the alanine/threonine substitution at this position in the Contursi kindred was a neutral, perhaps ancestral, variant we also sequenced exons 3–5 of African green monkey α-synuclein cDNA and found that monkey and man have an identical sequence (unpublished data). Therefore, the Contursi Ala53Thr mutation does not reflect the ancestral human gene.

The possibility that the α-synuclein gene is in linkage disequilibrium with the true causative gene defect should also be considered. In support of this, we previously reported a lod score of 1.5 at the PD-1 locus for family K, close to the theoretical maximum for this small family (3), yet in the present study no mutations were identified in the exons of α-synuclein. This does not exclude other candidates in the region as responsible for the parkinsonian syndrome in this kindred. The pedigree shown in Figure 1 gives haplotype data obtained for markers spanning the PD-1 locus in family K.

Histopathological studies of brainstem and cortex from patients with sporadic PD and dementia with Lewy bodies have shown that Lewy bodies are strongly immunoreactive for α-synuclein (6). However, as Lewy bodies are composed of many different proteins, the presence of α-synuclein in these structures does not necessarily implicate it in their formation.

If mutations in the α-synuclein gene rarely cause PD, an understanding of its biochemical function and interactions may suggest logical candidate genes for investigation, as mutations in heterogeneous gene products in a biochemical pathway may ultimately lead to a similar parkinsonian phenotype. However,

until further novel mutations in the α-synuclein gene are found, the assignment of the α-synuclein gene as the PD-1 locus must be regarded as provisional rather than certain.

Table 2. PCR primers for the exonic amplification of α-synuclein

NACP exon	Primers 5′/3′, forward and reverse	Product size (bp)
Exons 1 and 2	GAGAAGGAGGAGGACTAGGAGG CGGCGTTCTCCAGGATTTC	499
Exon 3	GTCTCACACTTTGGAGGGTTTC CACCTACCTACACATACCTCTGACTC	395
Exon 4	GCTAATCAGCAATTTAAGGCTAG GATATGTTCTTAGATGCTCAG	215
Exon 5	CGATGGCTAGTGGAAGTGG CGATGGCTAGTGGAAGTGG	325
Exon 6	CGGAGGCATTGTGGAGTTTAG CCACGTAATGAGCATGTAGAGAGC	373
Exon 7	GACTGGGCACATTGGAACTGAG GCTGTCAGTGCTGATGCGTAATTG	189

MATERIALS AND METHODS

Patients

Thirty index cases from Caucasian families exhibiting apparent autosomal dominant inheritance were examined. In these families, at least two affected individuals in each family were personally examined in order to ensure fulfilment of the diagnostic criteria of idiopathic PD, in accordance with a similar study design (7). Post-mortem information was available from members of two kindreds, and this confirmed the presence of Lewy bodies in a typical distribution. Three members of family K, which previously had given a maximum multipoint lod score of 1.5 at locus PD-1, were included in the study (3). Clinical characteristics of 16 kindreds are shown (Table 1). Index cases of seven American Caucasian families were ascertained by The Mayo Clinic (onset ages of the affecteds sequenced were 69, 75, 53, 72, 67 and 45 years), and seven further families are also referenced for information (5).

Laboratory methods

Primers were designed to human genomic non-Aβ component of Alzheimer's disease amyloid precursor protein, NACP/synuclein (sequences submitted to NCBI database; accession nos U46896–U46901). They were designed to amplify sequences flanking each exon at ~50–100 bp proximal and distal to the coding sequence. The exact exon–intron organisation of the human α-synuclein gene is not yet known. Primers were designed using Gene Runner 3.05, Hastings Software, Inc. Exons 1 and 2 (untranslated) were amplified together as one product. The 5′ intron sequence flanking exon 7 is not known. Thus intron 6–7 was PCR amplified using exon 6F and exon 7R primers and Expand Long Template PCR system (Boeringer Mannheim) (for primers, see Table 2). A 2.8 kb PCR fragment was excised from an agarose gel, TA cloned (Invitrogen) and sequenced. Exon 4 primers used were as published (2). 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 analysed using PolyPhredPhrap (8).

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