Letters to the Editor

Am. J. Hum. Genet. 65:555–558, 1999

Genetic Analysis of Families with Parkinson Disease that Carry the Ala53Thr Mutation in the Gene Encoding α-Synuclein

To the Editor:

Linkage of Parkinson disease (PD; MIM 601508) to a highly penetrant genetic locus on chromosome 4q21-q23 (Polymeropoulos et al. 1996) was soon followed by the detection of a missense mutation, in the α-synuclein gene (SNCA; MIM 163890) segregating with the disease (Chen et al. 1995; Polymeropoulos et al. 1997). The mutation was a 209G→A substitution in exon 4 of the gene, resulting in an Ala53Thr mutation in the α-synuclein protein and this change was predicted to revert the whole structure of the protein into beta-pleated sheets, which, in turn, may be involved in the self-aggregation of proteins. This mutation was first identified in a large Italian kindred and three unrelated Greek families, and later it was reported in two more Greek families (Papadimitriou et al. 1999). However, it was absent from several hundred cases of familial PD investigated by groups in the United States (Chan et al. 1998*a*, 1998*b*; Farrer et al. 1998) as well as in Europe (Munoz et al. 1997; Bennett and Nicholl 1998; Vaughan et al. 1998; Zareparsi et al. 1998), indicating that it is indeed a rare cause of PD. A 88G→C nucleotide substitution in exon 3, resulting in an Ala30Pro mutation was subsequently detected in a German patient with autosomal dominant PD (Kruger et al. 1998), giving further support to the hypothesis that α-synuclein could participate in the pathogenesis of the disease. That impaired degradation of abnormal proteins could play a role in PD—and, possibly, in other degenerative disorders—was also suggested by the detection of a mutation in the ubiquitin C-terminal hydrolase-L1 (UCH-L1) in a German family (Leroy et al. 1998).

α-Synuclein is a presynaptic-nerve terminal protein, identified as a precursor protein for the non-β amyloid component of amyloid plaques in Alzheimer Disease (AD) (Ueda et al. 1993; Ueda et al. 1994; Campion et al. 1995; Jensen et al. 1995). The wild type α-synuclein protein is present in the Lewy bodies of familial and sporadic PD patients (Spillantini et al. 1997; Baba et al. 1998).

We present here a molecular-genetic analysis of PD families, with respect to mutations in the α-synuclein gene. Our study was approved by the Ethics Committee of the Medical School of the University of Patras and involved patients with familial PD and as many of their relatives as possible, as well as sporadic PD patients, all voluntary donors of a blood sample. Donors were informed of the content and purpose of the research project and signed an informed-consent form. Ten ml of blood were collected, in presence of EDTA, from each donor. When possible, a second blood sample was drawn from a patient in each family and was used for lymphocyte transformation to provide permanent access to their DNA.

We studied a total of 19 unrelated families, in each of which there were at least two first- or second-degree relatives affected with PD. The three Greek families reported on elsewhere (Polymeropoulos et al. 1997) were included in the study and are analyzed here in an expanded form. Our study involves the recording of pedigrees of at least three successive generations, the recording of available clinical data, and the molecular analysis of the DNA extracted from the blood of patients and their unaffected relatives. Seven of these families had multiple affected members and showed a pattern consistent with autosomal dominant inheritance. The penetrance appeared high, since among family members of age >47 years who were offspring of an affected individual, approximately half were affected (6/13–3/5). All affected individuals had one affected parent, and both males and females transmitted the trait (Mange and Mange 1994). The first clinical data available for the patients of these seven families are given in table 1.

DNA was extracted from peripheral blood and was used for PCR amplification of α-synuclein exon 4, by use of primers 3 and 13 (Polymeropoulos et al. 1997). Exon 3 was amplified by use of forward primer ACTTTGGAGGGTTTCTCATG and reverse primer TGTTATCCTAACCCATCAC. PCRs were prepared in a volume of 100 μl, and 2.5 units of DNA polymerase (GIBCO-BRL) were used. The *Tsp*45I and *Mva*I digestions of PCR products containing exons 4 and 3, respectively, were performed according to the supplier's

Table 1

Clinical and Molecular Analysis of Patients Carrying the Ala53 Thr Mutation

					Status[a]			
Family	Patient	Sex	Age (years)	Age at Onset (years)	Bradykinesia	Muscular Rigidity	Resting Tremor	Ala53Thr Mutation
PDGR1	II3	M	58	52	+ + +	+ + +	−	+
PDGR2	III1	F	54	50	+ + +	+ + +	−	+
PDGR5	III1	F	80	76	+ + +	+ + +	+	−
PDGR5	III2	M	68	58	+ + +	+ + +	−	+
PDGR5	IV5	F	40	36	+ + +	+ + +	−	+
PDGR8	IV1	M	48	43	+ + +	+ + +	+	+
PDGR8	IV6	M	48	47	+ + +	+ + +	−	+
PDGR11	III1	F	57	49	+ +	+ + +	−	+
PDGR11	III9	F	57	51	+ +	+ + +	−	+
PDGR15	III15	M	49	40	+ +	+ + +	−	+
PDGR18	III4	M	61	58	+ + +	+ + +	−	+

+ = Symptom weakly present; ++ = present; +++ = strongly present; − = absent.

directions. The digested material was electrophoresed in 4% Nusieve agarose gel.

Ten microsatellite markers (Gyapay et al. 1994) were used in the haplotype analysis, including two new polymorphisms. The order of the eight previously described genetic markers, from centromere to telomere, is D4S2361-D4S2460-D4S2371-D4S2461-D4S3006-D4S1089-D4S414-D4S2380. The genetic markers were ordered by use of a minimal physical YAC contig. The two additional genetic markers used were (1) TA46, a $(TA)_n$ repeat that was generated from the bacterial artificial chromosome clone 225H6 (Research Genetics) and that contains marker D4S2461 and (2) marker SYN 24,25, which was designed to flank a dinucleotide repeat in the 5′ noncoding region of the α-synuclein gene (GenBank U46895) (table 2).

The Ala53Thr α-synuclein mutation (Polymeropoulos et al. 1997) was detected in 10 patients belonging to the seven autosomal dominant families but was not found in any member of the remaining 12 families (table 1). In patients carrying the mutation the mean age at onset of the disease is 47 ± 11 years, which is considered to be "early onset" PD. Interestingly, one patient from family PDGR5, individual III-1, did not carry the Ala53Thr mutation, although the mutation was detected in two other affected members of that family. This patient had a much later age at onset of the disease, 76 years, and may represent "sporadic" PD. None of 41 sporadic PD patients of local origin, in 13 of whom the age at onset was 35–55 years, carried the mutation. The DNAs from 116 nonaffected members of the seven families were also analyzed. Among them, we found 11 unaffected carriers of the Ala53Thr mutation (not shown), 10 of whom were younger than the mean age at onset. All of these 11 patients had one affected parent carrying the mutation. However, these results are consistent with a high penetrance of the Ala53Thr mutation in the families that we studied, with age at onset >60 years, as was the case in the Contursi family, the original family studied (Polymeropoulos et al. 1997). Finally, we analyzed 100

Table 2

Markers Used for Haplotyping, and the Primers that Generated the Respective Alleles

		PCR Primer (5′→3′)					
Marker	Repeat	Forward	Reverse	PIC	Allele	Size (bp)	Allele Frequency[a]
TA46	$(TA)_{25}$	TGTTTGCTACGACATCTCTC	CTTGAGCCAGAAGGTTGAGG	.76	1	107	.07
					2	109	.3
					3	111	.28
					4	113	.07
					5	115	.13
					6	117	.11
					7	119	.04
SYN24,25	$(TA)_7(CA)_{11}$	AGGATGGATTAGTAGCTATG	CCTATGGAAGACATGAAGAC	.40	1	181	.27
					2	183	.67
					3	185	.04
					4	187	.01

[a]Frequencies based on 56 chromosomes for TA46 and on 92 chromosomes for SYN24,25

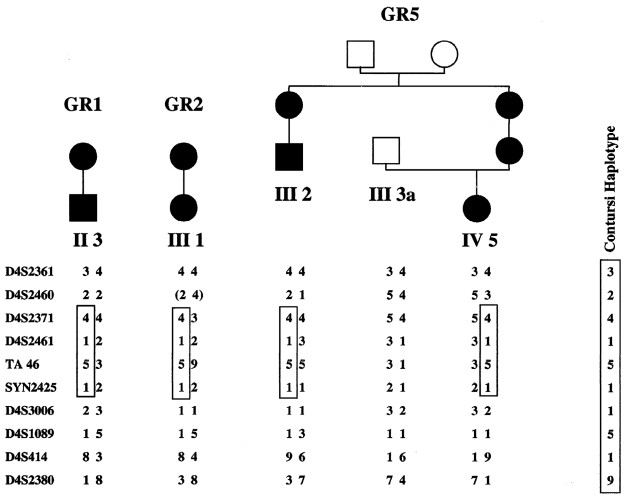

Figure 1 Chromosome 4 haplotype analysis of families carrying the Ala53Thr mutation. The unblackened and blackened symbols represent unaffected and affected individuals, respectively. Roman numerals indicate generations, and arabic numerals indicate individuals. The shared haplotype is boxed.

chromosomes from 50 healthy control individuals, deriving mainly from the Peloponnese and from western Greece, and found none carrying the mutation. Neither any of the patients with familial PD nor any of those with sporadic PD carried the Ala30Pro mutation.

In the Contursi kindred, polymorphic markers established a haplotype shared by all the affected individuals of the kindred, within a region of ~6 cM harboring the α-synuclein gene. To assess the possibility that a founder chromosome is shared by the southern Italian kindred and the seven Greek families that carry the Ala53Thr mutation, we used 10 polymorphic markers to genotype members of three Greek families carrying the mutation (fig. 1). The Greek patients with PD share the portion of chromosome 4 shared by the Contursi kindred, delineated by marker D4S2460 at the centromeric end and by marker D4S3006 at the telomeric end.

On the basis of the information currently available, it appears that these patients with the Ala53Thr α-synuclein mutation have an average age at onset that is at or below the average age at onset in sporadic PD. Clinically, they have prominent bradykinesia and muscular rigidity but rarely have tremor. A more detailed study is currently underway to determine the specific clinical phenotype that may be associated with the α-synuclein Ala53Thr mutation.

All seven Greek families with PD originate from three villages of the northern Peloponnese in Greece, two of which are only 17 km apart and are villages of origin for six of the families. The village of origin of the seventh family was 120 km distant . The Contursi kindred comes from southern Italy, a region geographically and historically linked to Greece. Our data suggest that affected members in these families may all be descendents of a single founder. A study of "early-onset" familial PD in the greater Balkan area would help to establish the contribution of α-synuclein mutations to the PD phenotype.

Acknowledgments

We wish to thank Drs. C. Bissas, N. Georgopoulos, P. Ghikas, C. Kremmydas, P. Leonardos, S. Papapetropoulos, and, last but not least, A. Protonotariou, for their great help in sample collection. This work was supported by European Framework Program EPET II grant 236/234/603 to G.M.M.

Aglaia Athanassiadou,[1] Gerassimos Voutsinas,[1] Lambrini Psiouri,[1] Elisabeth Leroy,[3] Mihael H. Polymeropoulos,[4] Achilleas Ilias,[5] George M. Maniatis,[1] and Thodoros Papapetropoulos[2]

Departments of [1]Biology and [2]Neurology, Medical Faculty, University of Patras, Patras, Greece; [3]Laboratory of Genetic Disease Research, National Human Genome Research Institute, National Institutes of Health, Bethesda; [4]Novartis Pharmaceuticals Corporation, Pharmacogenetics, Gaithersburg, MD; and [5]Eginition Hospital, University of Athens, Athens

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim/ (for SNCA and type 1 familial PD)

References

Baba M, Nakajo S, Tu PH, Tomita T, Nakaya K, Lee VM, Trojanowski JQ, et al (1998) Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. Am J Pathol 152:879–884

Bennett P, Nicholl DJ (1998) Absence of the G209A mutation in the alpha-synuclein gene in British families with Parkinson's disease. Neurology 50:1183

Campion D, Martin C, Heilig R, Charbonnier F, Moreau V, Flaman JM, Petit JL, et al (1995) The NACP/synuclein gene: chromosomal assignment and screening for alterations in Alzheimer disease. Genomics 26:254–257

Chan P, Jiang X, Forno LS, Di Monte DA, Tanner CM, Langston JW (1998*a*) Absence of mutations in the coding region of the alpha-synuclein gene in pathologically proven Parkinson's disease. Neurology 50:1136–1137

Chan P, Tanner CM, Jiang X, Langston JW (1998*b*) Failure to find the alpha-synuclein gene missense mutation (G209A) in 100 patients with younger onset Parkinson's disease. Neurology 50:513–514

Chen X, de Silva HA, Pettenati MJ, Rao PN, St George-Hyslop P, Roses AD, Xia Y, et al (1995) The human NACP/alpha-synuclein gene: chromosome assignment to 4q21.3-q22 and TaqI RFLP analysis. Genomics 26:425–427

Farrer M, Wavrant-De Vrieze F, Crook R, Boles L, Perez-Tur J, Hardy J, Johnson WG, et al (1998) Low frequency of alpha-synuclein mutations in familial Parkinson's disease. Ann Neurol 43:394–397

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc P, et al (1994) The 1993–94 Genethon human genetic linkage map. Nat Genet 7:246–339

Jensen PH, Sorrensen ES, Petersen TE, Gliemann J, Rasmussen LK (1995) Residues in the synuclein consensus motif of the alpha-synuclein fragment, NAC, participate in transglutaminase-catalysed cross-linking to Alzheimer-disease amyloid beta A4 peptide. Biochem J 310:91–94

Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, Przuntek H, et al (1998) Ala30Pro mutation in the gene encoding α-synuclein in Parkinson's Disease. Nat Genet 18: 106–108

Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, Harta G, et al (1998) The ubiquitin pathway in neurodegenerative disorders. Nature 395:451–452

Mange EJ, Mange AP (1994) Basic Human Genetics. Sinauer Associates Inc., Sunderland, MA

Munoz E, Oliva R, Obach V, Marti MJ, Pastor P, Ballesta F, Tolosa E (1997) Identification of Spanish familial Parkinson's disease and screening for the Ala53Thr mutation of the alpha-synuclein gene in early onset patients. Neurosci Lett 235:57–60

Papadimitriou A, Veletza V, Hadjigeorgiou GM, Patrikiou A, Hirano M, Anastopoulos I (1999) Mutated alpha-synuclein gene in two Greek kindreds with familial PD: incomplete penetrance? Neurology 52:651–654

Polymeropoulos MH, Higgins JJ, Golbe LI, Johnson WG, Ide SE, Di Iorio G, Sanges G, et al (1996) Mapping of s gene for Parkinson's Disease to Chromosome 4q21-q23. Science 274:1197–1199

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, et al (1997) Mutation in the α-synuclein gene identified in families with Parkinson's Disease. Science 276:2045–2047

Spillantini MG, Schmidt ML, Lee VM-Y, Trojanowski JQ, Jakes R, Goedert M (1997) α-synuclein in Lewy Bodies. Nature 388:839-840

Ueda K, Fukushima H, Masliah E, Xia Y, Iwai A, Yoshimoto M, Otero DA, et al (1993) Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. Proc Natl Acad Sci USA 90:11282–11286

Ueda K, Saitoh T, Mori H (1994) Tissue-dependent alternative splicing of mRNA for NACP, the precursor of non-A beta component of Alzheimer's disease amyloid. Biochem Biophys Res Commun 205:1366–1372

Vaughan JR, Farrer MJ, Wszolek ZK, Gasser T, Durr A, Agid Y, Bonifati V, et al (1998) Sequencing of the alpha-synuclein gene in a large series of cases of familial Parkinson's disease fails to reveal any further mutations. Hum Mol Genet 7: 751–753

Zareparsi S, Kay J, Camicioli R, Kramer P, Nutt J, Bird T, Litt M, et al (1998) Analysis of the α-synuclein G209A mutation in familial Parkinson's disease. Lancet 351:37–38

Address for correspondence and reprints: Dr. Aglaia Athanassiadou, Laboratory of Biology, Medical Faculty, University of Patras, 261 10 Patras, Greece; E-mail: Athanass@med.upatras.gr

0002-9297/99/6502-0032$02.00

Am. J. Hum. Genet. 65:558–562, 1999

Mutation of the XNP/ATR-X Gene in a Family with Severe Mental Retardation, Spastic Paraplegia and Skewed Pattern of X Inactivation: Demonstration that the Mutation is Involved in the Inactivation Bias

To the Editor:

A family in which severe mental retardation (MR) is segregating with spastic paraplegia (SP) has recently been reported (Martinez et al. 1998). The extended pedigree of this family is presented in figure 1. Obligate-carrier females have a totally skewed pattern of X inactivation, detected by amplification of the $(CAG)_n$ microsatellite repeat in the androgen-receptor gene and previous digestion of genomic DNA with the methylation-sensitive restriction endonuclease *Hpa*II, as described elsewhere (Martinez et al. 1998). Genetic analysis in the family has revealed linkage of the morbid locus to the proximal long arm of the X chromosome, with a maximum LOD score in Xq13.3. Three genes involved in X-linked MR (XLMR) have already been reported in this genomic region. One, encoding oligophrenin-1, is involved in nonsyndromic XLMR (Billuart et al. 1998) and thus does not seem to be a good candidate. The other two are involved in two syndromic XLMR conditions: Menkes syndrome (MIM 309400) and alpha-thalassemia with mental retardation (ATR-X [MIM 301040]) syndrome. The latter is an XLMR condition that associates severe MR, mild alpha-thalassemia, typical facial dysmorphy and a skewed pattern of X inactivation in carrier females (Gibbons et al. 1995*a*). The clinical characteristics of the reported MR+SP family are close to this description, and thus the presence of an allelic mutation at the ATR-X locus could be hypothesized. In addition to the already reported clinical features in the MR+SP family, hematologic analysis revealed that 3% of the patients' erythrocytes showed HbH inclusions after cresyl-brilliant staining, which reinforced the possibility that a mutation in the XNP/ATR-X gene is present in this family. However, ATR-X syndrome has always been reported to be associated with neonatal hypotonia, which can be severe (Gibbons et al. 1995*a*). In the case of the family that we studied, affected