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Genetic assessment of familial and early-onset Parkinson's Disease in a Greek population

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Abstract

Background: Although the first mutation associated with Parkinson's Disease (PD) was identified several years ago in the alpha-synuclein (SNCA) gene in families of Greek and Italian ancestry, a more systematic study of this and other known PD mutations has not been performed in the Greek population.

Methods: We performed a genetic analysis in 111 familial or sporadic with early-onset (≤50 years, EO) PD patients for the presence of the A53T SNCA mutation. In separate subgroups of these patients, we searched for further mutations in the SNCA, LRRK2, Parkin, PINK1 and DJ-1 genes. Additionally, we analysed a subgroup of familial cases for mutations in the glucocerebrosidase (GBA) gene.

Results: In total, we identified 5 patients (4.5% of our whole population) with the A53T SNCA mutation, two with a heterozygote dosage mutation and one with a heterozygote point mutation in the Parkin gene, and 7 patients (10,3% of our familial cohort) with GBA gene mutations.

Conclusions: The A53T mutation in the SNCA gene, although uncommon, does represent a cause of PD in the Greek population, especially of familial EOPD with autosomal dominant inheritance. GBA mutations in the familial cohort tested here were equally common as in a cohort of sporadic cases previously examined from the same centers. For the remainder of the genes, we did not identify genetic defects that could definitively account for the disease. These results suggest that further Mendelian traits that lead to PD in the Greek population remain to be identified.

Introduction

Although Parkinson's Disease (PD) was long considered a non-genetic disease, it is now well appreciated that there are genetic and hereditary factors underlying its pathogenesis. It is estimated that about 15% of PD patients have a family history of the disease. Furthermore, a genetic component is increasingly being recognized in sporadic PD, particularly due to mutations in the genes encoding for LRRK2, GBA and Parkin. [1-3] As genetic loci and causative genes are increasingly being identified, it is important to examine genetically specific populations of PD patients in different countries, in order to establish the relative frequency of such genetic defects, which appears to differ substantially across populations. [4,5] Such studies are important as they may guide tailored genetic testing depending on the ethnic origin, and may also reveal clusters of cases that, if tested negative for known genetic causes of PD, may provide the basis for the discovery of novel PD-related genes.

With this in mind, we have embarked on a systematic study of known Mendelian genetic defects linked to PD in a Greek population.

Patients

Subjects were recruited from four sites in Southern Greece: the two Neurology Departments of the University of Athens in "Attikon" and "Aeginition" hospitals (54 and 9 patients respectively), the Neurology Department of "Henry Dunant" hospital in Athens (18 patients), and the Neurology Department of the local hospital of Syros, an island of 21400 inhabitants in the Cyclades (30 patients). Most of the patients originated from areas in Southern Greece or the Cycladic islands. The study's procedures and purpose was explained to all participants or their next-of-kin who provided signed informed consent. The Institutional Review Boards of all sites approved the study's protocol and relevant forms.

For the purposes of the present work, we have elected to focus on a population enriched in possible genetic load, comprising of PD patients who either have a positive family history of the disease (at least one affected relative up to the second degree), or are sporadic but

manifest early onset, at or below the age of 50 years (EOPD patients). Moreover, Syros is an area with probable increased genetic significance, as it is a geographical constrained area with suspected consanguinity among its inhabitants. About half (14/29) of the familial PD patients included in this study from Syros have a sibling with PD, while the remainder have autosomal dominant or unclear inheritance.

In total, we recruited 111 patients. Of those, 90 were familial (26 with EOPD) and 21 were sporadic EOPD patients. All were analysed for the presence of the A53T mutation in the SNCA gene. None was known to be a relative of any patient known to harbor the mutation. DNA of 81 patients of our total sample (63 familial, 9 familial with EOPD, 18 sporadic with EOPD) (subgroup A) was analysed for further mutations in the SNCA gene and for mutations in the LRRK2, Parkin, PINK1 and DJ-1 genes. Analysis included direct sequencing of the genes, depending on age at onset and mode of inheritance, and Multiplex ligation-dependent probe amplification (MLPA) for the detection of dosage mutations. More precisely, autosomal dominant familial patients (PD in a parent, n=28, subgroup A1) were sequenced for mutations in the SNCA gene, familial patients with autosomal dominant or unclear inheritance, plus sporadic EOPD patients with AAO>45≤50 years (n=48, subgroup A2) were sequenced for mutations in the LRKK2 gene, and autosomal recessive PD patients (PD in a sibling) plus sporadic EOPD patients with AAO≤45 years (n=33, subgroup A3) were sequenced for mutations in the Parkin, PINK1 and DJ-1 genes. All 81 patients of subgroup A were analysed by MLPA for the detection of dosage mutations in Parkin, PINK1, DJ-1 and SNCA genes. We also analysed 68 of the 90 familial patients of our total sample (subgroup B) for 8 mutations in the glucocerebrosidase (GBA) gene; GBA analysis of sporadic EOPD patients of our population has been already included in the article of Moraitou M et al, 2011. [6] The characteristics of the study subjects are summarized in Table 1.

Methods

SNCA-A53T

DNA was extracted from peripheral blood and was used for PCR amplification of SNCA exon 4. For mutation analysis genomic DNA was amplified with primer 3 (5-GCTAATCAGCAATTTAAGGCTAG-3) and primer 13 (5-GATATGTTCTTAGATGCTCAG-3) of the DNA sequence (GenBank ID U46898) under standard PCR conditions. Restriction digestion was done after the PCR with Tsp45 I according to the manufacturer's protocol (New England Biolabs, Beverly, MA). The digested PCR products were separated by electrophoresis on a 3% agarose gel and visualized by ethidium bromide staining. Mutant alleles carrying the G209A substitution were identified through the presence of 128 and 88 bp digestion products. [7]

GBA

Molecular analysis was carried out in the Department of Enzymology and Cellular Function of the Institute of Child Health in Athens. Genomic DNA was extracted from peripheral blood leucocytes using the modified salting out procedure. [8] All patients were tested using PCR and restriction enzyme analysis for 8 mutations in the GBA gene that cover approximately 87% of the identified mutations in patients with Gaucher disease diagnosed in Greece (mutations investigated are depicted in Table 2). [6]

SNCA, LRRK2, Parkin, PINK1, DJ-1

DNA was extracted as described above. All exons of SNCA, Parkin, PINK1 and DJ1, and those exons of the LRRK2 gene known to harbor pathogenic mutations (exons 24, 27, 30, 31, 35, 40 and 41) were analysed by conventional Sanger Sequencing following PCR-amplification of the respective exon and immediately adjacent intronic sequences on an ABI automated sequencer. Primer sequences and PCR conditions are available by request.

MLPA

The Multiplex Ligation-dependent Probe Amplification (P052-C1 kit from MRC Holland) was used according to the manufacturer's specifications. The kit contains specific probes to identify whole exon deletions/duplications in Parkin, PINK1, DJ1 and SNCA.

In a first step, the specific probes are hybridized, followed by ligation and in a third step the ligated probes are amplified and quantitatively analysed on an ABI sequencer. Due to the fact that MLPA is a technique based on relative quantification, two calculating steps have to be performed. First, peak areas are normalized with respect to an internal standard, and then compared to healthy controls. A ratio of peak areas ≤ 0,6 is considered to be indicative for a heterozygous deletion, whereas results ≥ 1,3 usually indicate a heterozygous duplication.

Results

SNCA

The A53T mutation in SNCA gene was identified in 5 out of our 111 patients (4.5%). All were familial autosomal dominant cases with age at onset ranging from 31 to 61 years (31, 35, 45, 46 and 61 years). Within the familial cases, the percentage of the mutation carriers was higher (5/90:5.5%), and even higher among EOPD familial cases (4/26:15.4%).

Sequencing of the SNCA gene in the 28 familial patients of subgroup A1 and MLPA analysis in the patients of subgroup A for the detection of dosage mutations revealed no further mutations, including those identified recently. [9,10]

GBA

The analysis of the 68 familial patients of subgroup B yielded 7 (10.3%) with heterozygous GBA mutations. Only one mutation carrier had EOPD. Patients positive for a GBA mutation tended to have an earlier age at onset than those without a mutation (mean age at onset: 60.9 vs 64.2 years), but this difference was not statistically significant (p=0.2). Mutations identified were L444P: 3 patients, D409H;H255Q (both mutations on the same allele): 2 patients, N370S and R120W: one patient each.

LRRK2, Parkin, PINK1, DJ-1

None of the 7 known pathogenic mutations in the LRKK2 gene (G2019S, N1437H, R1441C, R1441G, R1441H, V1699C, and I2020T) were detected. Three familial patients with late-

onset typical PD were found to carry genetic variants: one patient carried the T1410M variant and two patients carried the R1398H-K1423K variants.

Sequencing of Parkin, PINK1 and DJ-1 genes revealed a possibly pathogenic variant (c.101 del ag) in the Parkin gene and several sequence variants of no pathogenic significance. Analysis by MLPA for dosage mutations in the Parkin, PINK1 and DJ-1 genes revealed a heterozygous Parkin duplication of exons 2+3 and a heterozygous Parkin deletion of exon2. The clinical characteristics of the Parkin mutation carriers of our sample are summarized in Table 3. All genetic variants detected in LRRK2, Parkin, PINK1 and DJ-1 genes are summarized in Table 4.

Discussion

This is the first systematic analysis in Greece of a number of major known genetic causes of PD in a population of familial and sporadic EOPD patients. The A53T missense mutation in the SNCA gene was found in the considerable frequency of 4.5% of our total and 5.5%.of our familial PD population. This is the first mutation to have been associated with PD in one Italian and three unrelated Greek families originating from Northern Peloponnese. [7] Later on, a few more Greek families were reported to carry the mutation [11,12], while it has been very rarely detected in patients of non-Greek origin. [4,13] Therefore, SCNA-A53T represents a rare cause of familial PD, basically confined to families originating from Greece and Southern Italy, where a common single founder may exist. All 5 carriers of the mutation had autosomal dominant EOPD, apart from one patient who had a later age of onset (61 years). The frequency of the A53T mutation in our population of familial PD patients is the highest reported so far, indicating that this mutation is indeed a cause of familial PD in Greece and should be excluded, especially in autosomal dominant EOPD.

Mutations in the GBA gene were detected in 10.3% of our familial patients tested, while this percentage was found to be 4% in 101 controls of the same population, by Moraitou M et al, 2011, [6] (difference marginally not significant, p=0.1). In that same article, the percentage of carriers of a GBA mutation in 105 sporadic patients of the same population (10/105:9.5%)

was similar to that in our present cohort of familial patients, suggesting that family history does not enrich for GBA mutations in this Greek PD population. In the present study, GBA mutations are not overrepresented in familial EOPD, in contrast with the findings of two prior Greek studies, mainly in sporadic PD. [6,14] A caveat of our GBA analysis is that we did not perform sequencing analysis of the entire GBA gene. Instead, we searched for the presence of 8 specific mutations, which, on the other hand, cover the great majority of the mutations identified in Greek patients with Gaucher disease.

Mutations in LRKK2 have been identified both in familial and sporadic PD patients with variable frequency in different ethnic populations. [5,15,16] Previous studies have shown that LRRK2 mutations are rare in Greek familial and sporadic patients. [17-20] Consistent with this, none of our familial or EOPD patients carried any known pathogenic mutations. One patient with typical late-onset PD and remote family history (an affected paternal aunt) carried the T1410M variant, which has been reported to be of possible pathogenicity. [21] The other sequence variations identified (R1398H-K1423K) are unlikely to be pathogenic, instead they may actually confer protection in the framework of a common three-variant protective haplotype, G-A-A N551K-R1398H-K1423K, identified in various ethnic populations. [21] Due to cost constraints, our sequencing analysis was limited to the exons known to bear pathogenic mutations; we can therefore not exclude the possibility of mutations in other exons of the gene.

Deletions in the Parkin gene have been described previously in a Greek pedigree with EOPD. [22] In our cohort of familial or sporadic EOPD patients we have identified two heterozygous dosage mutations and a heterozygous point mutation. The duplication of exons 2+3 and the deletion of exon2 are both known pathogenic mutations that cause recessive parkinsonism, while c.101 del ag is of unknown pathogenic significance. [23] There is continuing discussion whether Parkin haploinsufficiency could cause disease. Many studies have reported a high proportion of EOPD patients with a single heterozygous Parkin dosage mutation. [24-26] It

seems possible that one single dosage mutation could suffice for disease presentation, while this is more unlikely for single point mutations. [27] The possible causal role of Parkin heterozygous mutations could be explained by the following hypotheses: a) certain mutations may be dominant, conferring dominant-negative or toxic gain of function, b) other mutations remain to be discovered in other regions of the gene, or c) such mutations act rather as susceptibility factors that need to be combined with additional genetic or environmental insults to confer disease status. [28] In our study, both the patient with a deletion in exon 2 and his mother have a benign phenotype compatible with Parkin-associated parkinsonism (Table 3), suggesting a possible dominant pathogenic effect. The second patient, carrying a dosage mutation (duplication of exons 2+3), has typical late-onset PD (Table 3). This patient originated from Syros, and her mutation, together with 3 GBA mutations, are the only pathogenic mutations found in our Syros PD population. Although inheritance here is compatible with autosomal recessive (PD in a sibling), the phenotype is quite distinct from the usual Parkin-associated parkinsonism. However, it has been reported that patients with single heterozygous mutations could have phenotypes more compatible with typical PD with significantly later and more asymmetrical onset than patients with compound heterozygote or homozygote mutations, [29] so a relationship of this mutation to disease cannot be excluded. Our third Parkin mutation carrier of a heterozygous point mutation has sporadic EOPD with a benign phenotype compatible with Parkin-associated parkinsonism, (Table 3) so it seems possible that her Parkin mutation is relevant for her disease status.

No pathogenic mutations, but only sequence variations of no pathogenic significance, were detected in PINK1 and DJ-1 genes, suggesting that these genes do not play a significant role in familial or EOPD in the Greek population. Nevertheless, our sample was small and comprised mostly of familial cases with onset >50 years. As other studies assessing the

frequency of PINK1 and DJ-1 mutations in Greek patients are lacking, definitive conclusions cannot be drawn before larger studies focusing on EOPD cases in Greece are performed.

Conclusions

According to the findings of our study, the A53T mutation in the SNCA gene represents a cause of familial autosomal dominant EOPD in the Greek population, and needs to be excluded in such patients. Mutations in GBA are present in a considerable proportion of familial PD cases, but there is no enrichment relative to sporadic cases. Mutations in LRKK2 are rare in the Greek population. In the Parkin gene we have only identified heterozygote genetic defects, but, based on the patients' phenotype, such mutations may be pathogenic. More studies assessing the frequency of PINK1 and DJ-1 mutations, as of more recent genetic defects associated with PD, need to be undertaken. The largest proportion of familial and EOPD cases in Greece do not have an ascertainable genetic cause, and may represent an opportunity for the discovery of novel genetic underpinnings of the disease. Genetic isolated populations in mainland or the Greek islands, such as Syros, could prove valuable in this regard.

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Disclosure of conflicts of interest

No author involved in this study disclosed any conflict of interest.

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Table 1. Characteristics of study subjects

	Total	Subgroup A[c]	Subgroup A1[d]	Subgroup A2[e]	Subgroup A3[f]	Subgroup B[g]
Patients	111	81	28	48	33	68
Males	62	42	10	23	19	33
Females	49	39	18	25	14	35
Age (years) (mean ± SD) (range)	64.6±14.7 (33-92)	67.4±13.2 (39-92)	72.6±8.4 (52-82)	69.9±10.1 (50-85)	63.7±16.2 (39-92)	70.9±11.9 (39-92)
AAO[a] (years) (mean ± SD) (range)	57±14.2 (31-82)	59.7±13.3 (34-82)	65.5±9.7 (48-80)	62.9±10.7 (46-82)	55.1±15.5 (34-82)	64±12.2 (34-82)
Familial	90	63	28	39	24	68
Familial EOPD[b]	26	9	3	3	7	11
Sporadic EOPD[b]	21	18	0	9	9	0

[a] Age at onset
[b] Early-onset PD
[c] Subgroup A: patients analysed by MLPA for the detection of dosage mutations in Parkin, PINK1, DJ-1 and SNCA genes
[d] Subgroup A1: patients sequenced for SNCA mutations
[e] Subgroup A2: patients sequenced for LRKK2 mutations
[f] Subgroup A3: patients sequenced for Parkin, PINK1 and DJ-1 mutations
[g] Sybgroup B: patients analysed for GBA mutations

Table 2: Nomenclature of *GBA* mutations studied

cDNA	Protein (traditional in GD)	Protein (as recommended by HGVS)
c.1226A>G	N370S	p.Asn409Ser
c.1342G>C	D409H	p.Asp448His
c.1448T>C	L444P	p.Leu483Pro
c.1505G>A (IVS10-1G>A)		p.Arg502GlnfsX2
c.882T>G	H255Q	p.His294Gln
c.475C>T	R120W	p.Arg159Trp
c.440A>G	Y108C	p.Tyr147Cys
c.762-2A>G (IVS6-2A>G)	Not determined	Not determined

Modified from Moraitou M et al, 2011 [6]

Table3. Clinical characteristics of patients with a Parkin mutation

Patients	Mutation	Gender	AAO[a]	FH[b]	Presentation	Dopa responsiveness
Patient 1	ex2del	M	41	mother	tremor	Very good
Patient 2	ex2-3dup	F	82	sibling	Tremor, late dementia	Very good
Patient 3	c.101del ag	F	37	negative	Essential tremor, parkinsonism after 6 years	Very good

[a]Age at onset
[b]Family History

Table 4. Variants detected in LRRK2, Parkin, PINK1 and DJ-1 genes

LRRK2	Parkin	PINK1	DJ-1
T1410M[a]	V380L	L63L	R98Q
R1398H;K1423K	D394N	A340T	c -99 T>C
	S167N	N521T	
	Q78P	IVS2-7 A>G	
	IVS3-20 T>C	IVS5-5 G>A	
	E16E	c *37 A>T	
	pos. 62 T>C		
	c. 101 del ag[a]		
	ex2del[b]		
	ex2-3dup[b]		

[a] possibly pathogenic
[b] pathogenic

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