

Parkin and *LRRK2*/Dardarin Mutations in Early Onset Parkinson's Disease in the Basque Country (Spain)

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Abstract

We have performed a complete screening of the Parkin gene (*PRKN2*) and looked for p.Gly2019Ser (G2019S) and p.Arg1441Gly (R1441G) *LRRK2*/dardarin gene mutations in twenty seven patients with Parkinson's disease (PD) with an age at onset younger than 50 years (EOPD), living in Gipuzkoa (Basque Country, Spain). Thirteen of them (48%) were *PRKN2* mutation carriers. The c.255-256DelA mutation was the most frequent, followed by a deletion involving exons 3 and 4. A deletion involving exons 3 and 12 of the *PRKN2* gene and R1441G *LRRK2* mutation was found together in one PD patient. Four out of fourteen *PRKN2* negative patients carried the p.G2019S mutation. Both *PRKN2* mutation carriers and non-carriers presented frequently with family history (10 *PRKN2* mutation carriers and 8 *PRKN2* non-carriers); in fact, five patients without a known gene mutation had a first degree relative affected, suggesting another monogenic disease. *PRKN2* carriers presented with a younger age at onset (36.7 vs. 41.7) and more benign disease progression. Indeed, those PD patients younger than forty who initially presented with unilateral tremor became shortly bilateral. Relatively, symmetric parkinsonism and slow disease progression carried more frequently *PRKN2* mutations than patients with unilateral akinetic rigid parkinsonism and age at

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onset later than 40 years. As expected in a recessive disease, *PRKN2* patients present more often with affected siblings and unaffected patients. The G2019S *LRRK2* mutation, less prevalent than R1441G in our area, may be also a frequent cause of PD in EOPD (4 patients).

Keywords

Parkin, Early Onset Parkinsonism, Parkinson's Disease, *LRRK2*, Dardarin

1. Introduction

The probability that Parkinson's disease (PD) would be a mendelian disorder is higher in patients with age onset younger than 50. Three different genes, *PRKN2* (OMIM # 602544), *PINK1* (OMIM #608309) and *PARK7* (OMIM #602533), have been identified to cause autosomal recessive young onset PD forms [1]-[3]. *PRKN2* encodes a protein of 465 amino acids named Parkin which functions as an E3-Ubiquitin protein ligase [4] [5]. The clinical symptoms of *PRKN2* patients are variable, but similar to those found in idiopathic PD. Its phenotype is usually characterized by onset before the age of 40 years (may range from 7 to 72 years), good response to levodopa therapy, dyskinesias under low doses of levodopa and slow progression. Additional features such as dystonia and absence of Lewy bodies (LBs) in the substantia nigra [1] [6] [7] are also frequent. Although the absence of LBs in the substantia nigra seems to be frequent in *PRKN2* mutation carriers, three *PRKN2* cases have been reported to show LB pathology [8] [9].

LRRK2/dardarin mutations are now a well established cause of typical PD [10]. In our geographical region, the Basque Country (Spain), the prevalence of the R1441G mutation is relatively high; in fact, a PD familial patient of Basque origin has a probability of 46% to carry this mutation [11]. The prevalence of G2019S in this region is similar to other European countries [11].

Here, we analysed the prevalence of *PRKN2* and both R1441G and G2019S *LRRK2*/dardarin mutations in patients with PD with an age at onset younger than 50 years of a geographical area (Gipuzkoa) of the Basque Country. In addition, we inspected the phenotypic differences between PD patients carrying *PRKN2* mutations and those who were non carriers to establish a range of clinical manifestations that allowed us to make a more accurate molecular analysis in our patients with early onset parkinsonism (EOPD)

2. Subjects and Methods

2.1. Subjects

Twenty seven patients were recruited from the Movement Disorders Unit of the Hospital Universitario Donostia, Neurology Department. This is a tertiary hospital in the province of Gipuzkoa, Basque Country (Spain), which has a population of 700,000 inhabitants. Physical examinations were done by 3 neurologists specialized in movement disorders. PD was diagnosed according to Gelb criteria [12]. A brief clinical questionnaire was completed providing information on gender, current age, and age at onset, as well as PD affection status for all first-degree relatives of the proband.

Family history was considered positive if the presence of PD was reported in at least one first degree relative. The clinical stages of Parkinsonism were assessed according to the classification of Hoehn and Yahr [13] (H & Y) and Unified Parkinson Disease Rating Scale [14] (UPDRS). All participants in this study lived in the Basque Country (Spain), mainly in the province of Gipuzkoa, and gave their written consent before participation. All PD patients whose onset symptoms began before the age of 50 years were included in this study. Four individuals with no family history, absence of movement disorders and an age at onset older than 80 years were also used in the gene dosage analysis as neurologically normal individuals.

Conventionally, the disease progression was categorized as very benign (VB) if after 10 years there the motor UPDRS score was less than 2/68, benign (B) if the symptoms progressed to an any incapacity (motor UPDRS difference 3 - 6 points), regular(R) if the incapacity was moderate (UPDRS increases 7 - 15 points) and malign (M) if the incapacity developed to be severe (more than 15 points in motor UPDRS).

This work was approved by the ethic committee of Hospital Universitario Donostia.

2.2. Molecular Analysis

DNA was extracted from peripheral blood by a standard phenol chloroform methods and two different approaches were performed to analyze the *PRKN2* gene. A single-strand conformational polymorphism (SSCP) analysis to detect missense mutations and small insertions and deletions [15] was performed after a PCR analysis of the 12 coding exons of the *PRKN2* gene. A direct sequencing analysis to characterize those samples that showed abnormalities in the previous SSCP analyses was also performed using an ABI310 Genetic analyzer (Applied Biosystems, Foster city, CA). Additionally, a semiquantitative multiplex polymerase chain reaction to detect large deletions and duplications was carried out as previously described by Lucking and Brice [16]. This was performed for exons 2 through 12 in five different groups: group 1 consisted of exons 2, 3, 9; group 2 consisted of exons 6, 8, 10; group 3 consisted of exons 7, 11; group 4 consisted of exons 4, 5; group 5 consisted of only exon 12. An external control, a 328 bp sequence of the transthyretin gene (TTR) on chromosome 18, was included in all groups and amplified at same time. All PCR products were resolved on an ABI310 Genetic Analyzer with an internal standard molecular weight (GeneScan 350-ROX; Applied Biosystems, Foster city, CA) and analysed using GeneScan v3.6 and Genotyper v3.7 software (Applied Biosystems). A ratio value was obtained comparing all samples to an external standard; the resulting ratios were also compared with ratios obtained from neurologically normal individuals that were run in parallel. All samples were amplified and ran in triplicate. The ratio values were interpreted as follows:

≤0.6 values as an indicative of a heterozygous exon deletion;

0.8 - 1.2 values as an indicative of a wild type doses;

1.8 - 2.3 values as an indicative of a homozygous exon duplication or heterozygous exon triplication (all primers sequences are available upon requested).

All subjects were also screened for both *LRRK2* c.4321C > G (Ex31, p.Arg1441Gly) and c.6055G > A (Ex41, p.Gly2019Ser) mutations. Single nucleotide polymorphism (SNP) genotyping was performed using TaqMan chemistry on an ABI7300 following manufacturer's protocol (Applied Biosystems, Foster City, CA).

3. Results

Thirteen out of twenty seven patients with *EOPD studied* carried *PRKN2* mutations (further details are described in **Table 1**). In addition, the R1441G *LRRK2* mutation was identified in a PD patient carrying as well a heterozygous deletion involving exons 3 and 12 of the *PRKN2* gene. The G2019S mutation was present in four out of fourteen patients who did not show any pathogenic *PRKN2* variant (further details are described in **Table 2**).

Table 1. Patients with *PRKN2* mutations.

Patients	Sex	Family history	Age at onset (years)	Symptoms	Duration symptoms (years)	L-dopa response	Progression	Dysk	Fluct	H & Y	<i>PRKN2</i> mutations	<i>LRRK2</i> mutations
1	M	Mt	44	T-LL	9	G	+	-	-	1	Delx3-x12	p.Arg1441Gly
2	M	Un	49	T-Un	7	G	+	-	-	2	p.Arg402Cys/Multiple exon rearrangements	-
3	M	B	43	A, G	13	G	++	-	+	3	Delx3-x4/Delx3-x4	-
4	F	B	39	T-Bi	12	G	+	-	-	2	Delx3-x4/Delx3-x4	-
5	F	B	26	A	35	G	+++	+	+	4	c.202_203delAG/Delx4	-
6	F	B	41	T-Uni	18	G	-	+	+	0	c.202_203delAG/Delx4	-
7	M	B	23	T-Uni	27	G	++	+	+	3	c.255-256delA/c.255-256delA	-
8	F	B	27	T-Bi	28	G	+	+	+	2.5	c.255-256delA/c.255-256delA	-
9	M	-	28	T-UL	7	G	-	-	-	1	c.255-256delA/Delx4-x5	-
10	F	B	38	T-Bi	29	G	-	+	+	1	c.255-256delA/c.255-256delA	-
11	M	B	43	T-Bi	29	G	-	+	+	2.5	c.255-256delA/c.255-256delA	-
12	F	-	34	T-Bi	27	NT	-	-	-	2	c.255-256delA/c.255-256delA	-
13	M	-	43	T-UL	27	G	-	+	-	2	Delx5/Delx5	-

Sex: M: male, F: female; Family history: Mt: mother, Un: uncle, B: brother; Symptoms: T: tremor, LL: low limb, Uni: unilateral, A: akinesia, Bi: bilateral, UL: upper limb, G: gait disturbance; L-dopa response: G: good, R: regular, P: poor, NT: not tolerated; Progression: VB: very benign (-), B: benign (+), R: regular (++) , M: malignant (+++); DysK: dyskinesias; Fluct: fluctuations.

Table 2. Patients without *PRKN2* mutations.

Patients	Sex	Family history	Age at onset (years)	Symptoms	Duration symptoms (years)	L-dopa response	Progression	Dysk	Fluct	H & Y	<i>LRRK2</i> mutations
1	M	-	48	R	3	G	++	-	-	2.5	p.Gly2019Ser
2	F	-	39	T-UL	19	G	+	+	+	3	-
3	M	Mt-GF	47	T-Uni	20	G	++	+	+	3	p.Gly2019Ser
4	M	Mt	24	A	3	Und	Und	-	-	1.5	-
5	M	-	46	T-UL	6	Und	Und	-	-	1	-
6	M	-	38	T-UL	26	G	++	+	+	4	-
7	M	Mt-Un	49	T-UL	10	G	+	+	-	2	-
8	F	-	48	A,R	17	R	+++	+	+	4	-
9	M	F-GF	48	T-bi	20	R	+	-	-	2	-
10	F	F	43	T-Uni	18	R	++	+	+	4	-
11	M	F-B	49	A	3	Und	Und	-	-	1.5	p.Gly2019Ser
12	F	Mt	48	A	11	P	+	-	-	2	-
13	M	F	37	Dy	15	P	++	+	+	4	p.Gly2019Ser
14	M	-	21	A	4	Und	Und	-	-	1.5	-

Sex: M: man, F: woman; Family history: Mt: mother, Un: uncle, B: brother, F: Father, GF: grandfather; Symptoms: T: tremor, LL: low limb, Uni: Unilateral, A: akinesia, Bi: bilaterall, UL: upper limb; G: gait disturbance; R: rigidity, Dy: dystonia; L-dopa response: G: good, R: regular, P: poor, NT: not tolerated, Und: undetermined; Progression: VB: very benign (-), B: benign (+), R: regular (++), M: malign (+++), Und: undetermined; DysK: dyskinesias; Fluct: fluctuations.

3.1. *PRKN2* Mutations

Homozygous c.255-256delA mutations were found in four individuals from two different families (patients 7, 8, 10 and 11), and in an apparently sporadic case (patient 12). This was the most frequent genetic abnormality identified. It was also present along with a heterozygous deletion implying exons 4 and 5 in another apparently sporadic case (patient 9). Two affected individuals from the same family (patients 5 and 6) were found to carry three heterozygous *PRKN2* mutations: an exon 4 deletion (Delx4), a small deletion involving only two bases (c.202_203delAG) and the missense p.Gly94Ser variant. This indicates that one of the parents was also a compound heterozygous *PRKN2* mutation carrier, however neither the father nor mother was known to be affected when they died, both 80 years old. A homozygous exon deletion involving exons 3 and 4 was found in two manifesting members of a PD family (patients 3 and 4). A p.Arg402Cys missense variant along with a large deletion involving exons 3, 4, 7, 8, 9, 10 and 12 were present in an possible recessive inheritance pattern (patient 2), suggesting that this case may belong to a PD family in which there may be more *PRKN2* mutation carriers. Analysis of this large deletion in additional members was not possible to carry out, being unable to determine if these deletions occur in *cis* or in *trans* to one another. The last mutation reported here is a exon 5 homozygous deletion that was identified in a sporadic PD patient (patient 13).

In addition we found 4 different exon deletions involving exons, 3, 4 and 5: *Delx4*, *Delx3-x4*, *Delx4-x5* and *Delx5*.

3.2. Clinical Phenotype

Patients with *PRKN2* mutations (see Table 1)

Six women and seven men carried *PRKN2* mutations and their age at onset ranged from 23 to 49 years (mean age at onset: 36.76 years). Ten had a family history of PD. The patient with digenic mutations (*PRKN2/LRRK2*) had a family history compatible with an autosomal dominant pattern of inheritance, having the mother suffered from PD. One patient had an uncle affected with PD and the remaining eight had affected siblings who carried

the same mutation following a recessive inheritance pattern.

Tremor was the onset symptom in eleven patients, of which six presented with bilateral hand tremor. The tremor was unilateral in the remaining seven but, in less than 2 years, it progressed to be bilateral. Clumsiness and gait disturbances were the onset symptoms in just two patients.

Duration of disease was at least 7 years at the time of examination (from 7 to 35 years), allowing us to accurately determine their phenotypic characteristics. The response to levodopa therapy was good in all patients, except for one that did not tolerate it well when she first tried. During the 17 years of the disease progression, she never was under levodopa therapy.

The disease progression was extremely benign in some of them who after many years of evolution could have a regular life. Furthermore, it is worth noting that two siblings carrying the same *PRKN2* mutations (202-203ΔAG/Delx4) presented with a completely different disease progression. Patient with severe evolution consumed higher doses of levodopa than those prescribed, while his sister takes always small levodopa doses.

Patients without *PRKN2* mutations (see [Table 2](#))

Among the ten men and four women who were *PRKN2* mutation non-carriers, four carried the G2019S mutation in the *LRRK2* gene. The onset of symptoms ranged from 21 to 49 years with a mean age at onset of 41.7 years, although in eight patients symptoms began after age 45. Three out of four patients carrying G2019S mutation were diagnosed in their fifties. Eight patients described an autosomal dominant pattern of inheritance, 5 patients were recessive and only one who carried the G2019S mutation was sporadic.

Seven patients presented with tremor, being unilateral in six of them and bilateral only in one. An akinetic rigid syndrome was present as the initial symptom in one patient.

The progression of disease has been slow in four patients, regular in five and very bad in one who showed many complications from the disease onset, accumulating significant disability. Overall, the disease progression was significantly more benign in *PRKN2* mutation carriers than non-carriers ($X, p = 0.04$).

4. Discussion

In the Province of Gipuzkoa (The Basque Country, Spain), where the prevalence and incidence of PD is similar to other European countries [17], the prevalence of the p.Arg1441Gly/*LRRK2* mutation is relatively high [11]. In this study we have also found a high prevalence of *PRKN2* mutations. Therefore, one may speculate that other mutations may be more prevalent in different geographic areas. *In this work we present prevalence of PRKN2 and LRRK2 mutations in a cohort of EOPD in a region with a population which is possibly unique and different from others.*

An extensive review of *PRKN2* mutations identified the most common mutations as deletions of exon 4 (7.38%), deletions of exon 3 (6.06%), deletions of exons 3 and 4 (6.06%), a point mutation in exon 7 (c.924C > T, 10.02%) and a single base pair deletion in exon 2 (c.255-256delA, 4.48%) [18]. Four of these five common mutations occurred in our sample, confirming that the hot spots for small mutations may be concentrated in exon 2 and for break points in introns 2 through 4. In our sample, the most common mutation found was c.255-256delA. The frequency of homozygous carriers for c.255-256delA has been estimated at 1/40.000 in the Spanish population [19], supporting the hypothesis of a possible Spanish founder for this mutation.

The presence of family history is relatively high in our sample analyzed (66.67%); indeed, 44.45% showed an autosomal recessive pattern of inheritance with affected siblings carrying the same genetic defect. All autosomal recessive patients carried *PRKN2* mutations, compiling a total of 29.6% of the cases here described. This proportion is similar to those reported in other studies [20]-[22]. In addition, the patient who carries heterozygous *PRKN2* deletions involving exons 3 and 12, and the R1441G mutation of *LRRK2*, had a family history compatible with autosomal dominant inheritance (two unaffected brothers and an affected parent). An additional *PRKN2* mutation carrier had also an affected uncle.

With respect to the cases without *PRKN2* mutations, 6 familial individuals presented with strong genetic background compatible with an autosomal dominant pattern of inheritance.

The phenotypic characteristics found in the cases reporting *PRKN2* mutations resemble to those described in most of the genetic studies to date reported [20]-[23]. The *PRKN2*-associated phenotype is well defined and broadly similar; the main clinical manifestations are the young age at onset, and the benign disease progression. The majority of the cases began with tremor which can be unilateral and/or progress to be bilateral and occasionally accompanied with postural tremor. Additionally, some of the cases presenting with akinesia or gait disturbances have a more severe disease progression than those initially presenting with tremor, being similar to

that found in *PRKN2* mutation non-carriers who showed dystonia or akinesia as initial symptom. None of our patients have dystonia, diurnal fluctuations, exercise induced dystonia, psychosis or peripheral neuropathy described elsewhere [23].

In this study the evolution in patients with *PRKN2* mutations is more benign. In 5 patients, we considered the evolution very benign, presenting only mild parkinsonian signs in exploration under small doses of antiparkinsonian medication, many years after the onset of symptoms. Phenotype-genotype dissociations were observed in 2 sisters presenting with very different clinical evolution, suggesting that other genetic or environmental factors and behaviours may possibly justify these differences. The PD with double mutation (*PRKN2/LRRK2*) had a similar age at onset (44 years) to the other *PRKN2* mutation carriers; suggesting that to carry pathological mutations in both genes does not have an effect on the disease features.

An important issue is to reflect on the value of the molecular study of the *PRKN2* gene in EOPD. In our series, although the age of onset of symptoms did not differ statistically between both groups (36.7 in *PRKN2* carriers versus 41.7 in the *PRKN2* non carriers), probably because of the sample size, PD patients that present with the disease after age 45 are less likely to have a *PRKN2* mutation. Autosomal recessive patients with an age at onset lower than 45 years and showing a typical idiopathic PD are highly susceptible to carry *PRKN2* mutations.

Given the lack of screening for *PARK7* and *PINK-1* mutations and *PRKN2* promoter variations in our negative *PRKN2* mutation cases, we cannot exclude that in these cases PD may be caused by alterations in one of these genes. Other mutations in *LRRK2* are uncommon and did not be studied. In our study we observed that *PRKN2* mutations are more frequent in early onset familial cases than in early-onset sporadic cases (frequency of 0.26).

We recognize in this work the limitation that suppose of having looked for only the p.Arg1441Gly and p.Gly2019Ser mutations in the *LRRK2* gene, and mutations in *PRKN2* gene. It is true that the study would have been more consistent if the number of patients studied had been greater, and if we had included other pathogenic mutations in the *LRRK2* gene and mutations in other genes involved in recessive forms of PD as *PINK1* and *PARK7*.

In our opinion, with the limitations of this small sample, in our region knowing whether someone may or may not carry *PRKN2* mutations have some advantages, such as warning about the possibility to be a carrier and helping in an accurate genetic counseling, and somehow in the prognosis of the disease; indeed, the therapeutic strategy might be different.

5. Conclusions

Forty eight percent of the 27 PD patients with age of onset before 50 were *PRKN2* mutation carriers. The c.255-256DelA mutation was the most frequent, followed by a deletion involving exons 3 and 4. A deletion involving 3 and 12 of the *PRKN2* gene and R1441G *LRRK2* mutation was found together in one PD patient.

Four out of fourteen *PRKN2* negative patients carried the pG2019S mutation of the *LRRK2* gene.

Both *PRKN2* mutation carriers and non-carriers presented frequently with family history (10 *PRKN2* mutation carriers and 8 *PRKN2* non-carriers); in fact, five patients without a known gene mutation had a first degree relative affected, suggesting another monogenic disease.

PRKN2 carriers presented with a younger age at onset (36.7 vs. 41.7) and more benign disease progression. Indeed, those PD patients younger than forty who initially presented with unilateral tremor became shortly bilateral. Relatively, symmetric parkinsonism and slow disease progression carried more frequently *PRKN2* mutations than patients with unilateral akinetic rigid parkinsonism and age at onset later than 40 years. As expected in a recessive disease, *PRKN2* patients present more often with affected siblings and unaffected parents.

The G2019S *LRRK2* mutation, less prevalent than R1441G in our area, may be also a frequent cause of PD in EOPD (4 patients).

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Authors' Contributions

José F Martí Massó was responsible for study coordination, data collection, and manuscript preparation. Javier Ruiz-Martínez, Alberto Bergareche and Adolfo López de Munain assisted with data collection and manuscript preparation. Coro Paisan-Ruiz, Ana Gorostidi, Ainhoa Alzualde, and Jordi Perez-Tur were responsible for molecular studies and manuscript preparation.

All authors reviewed the manuscript.

Approve

This work was approved by the ethic committee of Hospital Donostia.

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