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ORIGINAL ARTICLE

Indication for molecular testing by multiplex ligation-dependent probe amplification in parkinsonism

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Abstract

Background and purpose: The monogenic forms of Parkinson's disease represent <10% of familial cases and a still lower frequency of sporadic cases. However, guidelines to orient genetic testing are lacking. The aim was to establish the interest of multiplex ligation-dependent probe amplification (MLPA) as a primary screening test and to propose clinical criteria to guide genetic diagnostic tests for patients with suspected Mendelian Parkinson's disease.

Methods: In all, 567 patients with parkinsonism from 547 unrelated families were recruited and two MLPAs were performed for each. All pathogenic G2019S variants in the *LRRK2* gene were confirmed by Sanger sequencing and the *PRKN* gene was screened for a second mutation in the cases of one heterozygous structural variant in the *PRKN* gene.

Results: The performance of MLPA was 51/567 (9%) for the entire cohort and included 27 (4.8%) *LRRK2* G2019S mutations, 19 (3.4%) *PRKN* mutations and five (0.9%) *SNCA* locus duplications. The variables significantly associated with a positive test in the total cohort were North African ancestry ($p < 0.0001$), female sex ($p = 0.004$) and younger age at onset ($p < 0.0008$).

Conclusions: Retrospective analysis allowed us to refine our indication criteria: (i) North African ancestry, (ii) an age at onset <40 years or (iii) a familial history of parkinsonism with at least one affected first-degree relative. Our study highlights the interest of MLPA

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testing for other parkinsonism cases with a family history, especially for patients with dementia with Lewy bodies or a multiple-system-atrophy-like phenotype.

KEYWORDS

dementia with Lewy bodies, genetic testing, Mendelian transmission, multiple system atrophy, Parkinson's disease

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder and involves multiple environmental and genetic factors. The monogenic forms of PD represent <10% of familial cases and a still lower frequency of sporadic cases [1]. The most frequently involved genes in Mendelian PD are leucine-rich repeat kinase 2 (*LRRK2*) [2, 3], parkin RBR E3 ubiquitin protein ligase or parkin (*PRKN*) [4, 5] and synuclein alpha (*SNCA*) [6]. *LRRK2* pathogenic variants are the most frequent cause of autosomal dominant PD (Parkinson disease 8, OMIM #607060). To date, only nine pathogenic variants have been reported and all consist of missense variants [7, 8]. The recurrent pathogenic variant G2019S is by far the most common, causing 4% of familial PD and 1% of sporadic PD cases worldwide and up to 29% in Ashkenazi Jewish and 37% in North African Berber populations [7]. Pathogenic *SNCA* variants are the second most frequent cause of autosomal dominant forms of PD (*PARK1* and *PARK4*, OMIM #168601 and #605543) and other dominant parkinsonisms, which include dementia with Lewy bodies (DLB) (OMIM #127750). Most pathogenic variants in *SNCA* are genomic duplications and triplications, but five rare missense variants (Ala30Pro, Glu46Lys, Gly51Asp, Ala53Glu and Ala53Thr) have also been reported to be pathogenic [9]. Pathogenic *PRKN* variants are involved in the most frequent autosomal recessive form of PD (*PARK2*, OMIM #600116), accounting for 27.6% of autosomal recessive families [10]. The most frequent pathogenic variants are structural variants with exon deletion or multiplication (43.2%) or missense (22.3%) or frameshift (16.5%) variants [11]. Other less frequently involved genes have been reported, such as *PINK1* (*PARK6*), *DJ1* (*PARK7*), *VPS35* (*PARK17*), *TAF1*, *ATP13A2* (*PARK9*), *DCTN1*, *DNAJC6* (*PARK19*), *FBXO7* (*PARK15*), *PLA2G6* (*PARK14*), *SYNJ1* (*PARK20*), *CHCHD2*, *LRP10*, *TMEM230*, *UQCRC1* and *VPS13C* [12, 13].

There has been a recent increase in the availability of genetic testing, mostly next-generation sequencing (NGS) or targeted analyses using multiplex ligation-dependent probe amplification (MLPA). However, there are few official guidelines for genetic testing in PD. Currently, the only European recommendations for genetic mutation testing in PD patients, but not applied in practice, date from 2009 [14].

Given the large number of structural variants and recurrent pathogenic variants in Mendelian PD, the aim was to establish the cost-effectiveness of MLPA for PD patients as a primary screening test. Secondary objectives were to establish genotype-phenotype correlations and to propose clinical criteria to guide genetic diagnostic tests for patients with Mendelian PD.

PATIENTS AND METHODS

Patients

Since 2007, the Laboratory of Neurobiology of Lille University Hospital has been one of the two laboratories performing the molecular diagnosis of inherited PD in France. The patients came from French university hospitals (Lille, Amiens, Bordeaux, Besançon, Caen, Dijon, Lyon, Marseille, Montpellier, Nancy, Nice, Poitiers, Reims, Rennes, Saint-Etienne, Toulouse), French regional hospitals (Béthune, Chambéry, Épinal, Lens, Libourne, Millau, Nîmes, Orléans, Tourcoing, Troyes and Valenciennes) and one Belgian regional hospital (Fleurus). The request for genetic analysis was at the discretion of the clinician. However, the following criteria have been proposed previously for MLPA testing of inherited PD: (i) PD in a proband with an age at onset ≤ 45 years and/or (ii) PD patients with a family history defined by at least two relatives with a parkinsonism, all of them being related in the first degree. All samples were retrospectively tested independently of the proposed criteria.

Between June 2007 and December 2020, peripheral blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes from 567 individuals with PD or atypical parkinsonism referred by neurologists or geneticists for a molecular diagnosis. The medical records of all patients for whom an analysis by MLPA was carried out between June 2007 and December 2020 were retrospectively examined. The data collected consisted of the sex, age at onset, number of first-degree and further relatives affected by parkinsonism, and geographical origin. Moreover, the clinical diagnosis of all patients followed in the Lille University Hospital or regional hospitals related to the Lille expert center for PD during the same period were reviewed. Clinical diagnoses of the patients and their affected relatives were reviewed according to the diagnostic criteria for PD [15], progressive supranuclear palsy [16], corticobasal degeneration [17], multiple system atrophy (MSA) [18] and DLB [19].

Molecular genetics

Multiplex ligation-dependent probe amplification

Genomic DNA was isolated from peripheral blood according to standard procedures. MLPA was performed on all patients using SALSA MLPA Probe mixes P051 and P052 for the molecular diagnosis of

Mendelian PD (MRC Holland, Amsterdam, The Netherlands) according to the manufacturer's instructions. Both kits contain 50 MLPA probes for the detection of structural variants in the *ATP13A2*, *LRRK2*, *PARK7*, *PINK1*, *PRKN* (*PARK2*), *SNCA*, *UCHL1* and *GCH1* genes. They also include two probes specific for (i) the rare pathogenic variant NM_000345.3(*SNCA*):c.88G>C, p.(Ala30Pro) and (ii) the recurrent pathogenic variant NM_198578.3(*LRRK2*):c.6055G>A, p.(Gly2019Ser). Electrophoresis of the MLPA products was performed on an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The MLPA data were analyzed using GeneMapper® 2.5 (Applied Biosystems) and COFFALYSER (MRC Holland) software. Structural variants detected by only a single probe were confirmed both by qPCR SYBR® Green (Applied Biosystems) and Sanger sequencing to detect putative variations located in the probe hybridization site.

Sanger sequencing

All pathogenic G2019S variants in the *LRRK2* gene detected by MLPA were confirmed by Sanger sequencing. In the cases of a heterozygous structural variant in the *PRKN* gene, the 12 coding exons and flanking introns were screened for a second mutation by polymerase chain reaction followed by direct Sanger sequencing on an ABI 3730 XL sequencer. All primers are listed in Table S1.

Statistical analysis

Categorical variables are expressed as number (percentage). Continuous variables are expressed as mean (standard deviation) in cases of a normal distribution or median (interquartile range) otherwise. Normality of the distribution was assessed using histograms and the Shapiro–Wilk test. Comparisons of baseline patient characteristics between the two groups of MLPA were performed using chi-squared tests (or Fisher exact tests when the expected cell frequency was <5) for categorical variables and the Mann–Whitney *U* or Student *t* tests (depending on the normality of the distributions) for continuous variables.

Statistical testing was performed at the two-tailed α level of 0.05. Data were analyzed using SAS software, release 9.4 (SAS Institute, Cary, NC, USA).

Ethical standards

Written informed consent for genetic testing for diagnosis, research and publication of relevant findings was obtained from all the study participants according to the local ethics committee (CNIL registration number 726/2020 and 1418/2022). The study was conducted in accordance with the World Medical Association Declaration of Helsinki.

RESULTS

Description of the cohort

In all, 567 patients were recruited from 547 unrelated families. The sex ratio was 1.28 (319/248). The median age at onset was 47 (40–57) years (range 5–87; $n = 535$). Details of the cohort are provided in Table 1 and in Data S1.

Performance of MPLA testing

Amongst the 567 patients, the MLPA was positive (MLPA+) for the *LRRK2* G2019S pathogenic variant in a heterozygous state for 27 (4.8%) patients from 26 unrelated families. Two structural variants in the *PRKN* gene were observed for eight patients and one structural variant for 12 patients. Sanger sequencing of the entire coding sequence of *PRKN* for these 12 patients showed conventional variants for 11. There were 19 (3.4%) patients from 19 families with two genetic variants in the *PRKN* gene and after segregation analysis these two variants were observed to be in *trans* for eight patients. A duplication of the entire *SNCA* gene for five (0.9%) patients from four families was observed. No other pathogenic variants tested by the MLPA kit were identified.

Mendelian PD was diagnosed in 40/567 (7%) patients from 49 unrelated families with one or two “pathogenic” or “likely pathogenic” variants according to the American College of Medical Genetics and Genomics criteria for the classification of genetic variants [20]. Eleven patients were highly suspected to have autosomal recessive Mendelian PD but other samples from family members were not available to demonstrate that the variants were in *trans*. The performance of MLPA was 51/567 (9%) for the entire cohort. Examination of the patients from the Lille expert center for PD ($n = 422$), disregarding the prescriptions that did not fulfill our indication criteria ($n = 181$), showed the performance of MLPA to be 31/241 (12.9%). However, 3/31 (9.7%) patients from the Lille expert center for PD were MLPA+ but did not respect our indication criteria. These results show that our previous indication criteria were too stringent. These three patients all had a pathogenic *LRRK2* G2019S variant in the heterozygous state. One had an age at onset of 63 years and no family history, another had an age at onset of 47 years and only one affected sister, and the age at onset and family history were missing for the third patient. All variants obtained during this study are listed in Table 2.

Patient phenotypes associated with being MLPA+

Amongst the 51 patients who were MLPA+, the clinical diagnosis was available for 34 and encompassed 30 clinically established or probable cases of PD (88.2%). The characteristics of the Mendelian PD patients are summarized in Tables 3 and S2.

TABLE 1 Details of the cohort

Sex ratio	1.28 (319/248)
Criteria for MLPA testing	
Early onset	216
Family history ^a	82
Both	31
None	171
Missing data	67
Median age at onset (years)	47 (40–57) (range 5–87, n = 535)
Median number of affected relatives	1 (0–2) (range 0–11, n = 461)
Sporadic cases	226
Ancestry	
Caucasian	516
North African	51
Patients from Lille University Hospital	422
PD	313/422 (73.9%)
Probable MSA	19/422 (4.5%)
Possible or probable DLB	14/422 (3.3%)
Atypical parkinsonism	5/422 (1.2%)
Possible or probable PSP	3/422 (0.7%)
Possible or probable CBD	3/422 (0.7%)
Not defined degenerative atypical parkinsonism	5/422 (1.2%)
Nondegenerative movement disorders	64/422 (15.2%)

Abbreviations: CBD, corticobasal degeneration; DLB, dementia with Lewy bodies; MLPA, multiplex ligation-dependent probe amplification; MSA, multiple system atrophy; PD, Parkinson's disease; PSP, progressive supranuclear palsy.

^aFamily history defined by at least two relatives suffering from a parkinsonism, all of them being related in the first degree.

Four patients were observed with a parkinsonism corresponding to two MSA-like cases (patients with one *LRRK2* G2019S or one *SNCA* duplication), one probable case of DLB (in a patient with *SNCA* duplication) and one case of atypical parkinsonism with an age at onset of 41 and early cognitive impairment for a patient with chronic psychosis (*LRRK2* G2019S).

The patient with the *SNCA* duplication initially fulfilled the criteria for clinically probable MSA as she presented dysautonomic dysfunction with severe neurogenic orthostatic hypotension, rapid progression of parkinsonism, and an unexplained pyramidal syndrome at her first evaluation at age 64. The follow-up was then marked by dopa-responsive parkinsonism with motor fluctuations and late dementia. She died at 74. The other patient carrying a G2019S *LRRK2* mutation fulfilled the clinically established criteria for MSA, as he developed a severe dopa-resistant parkinsonism at 51 with cerebellar ataxia and severe dysautonomic dysfunction, with neurogenic hypotension and urogenital failure. Genetic testing for spinocerebellar ataxia was negative. Cerebral magnetic resonance imaging showed marked

pontocerebellar atrophy. The disease duration at death was 5 years but no pathological confirmation was obtained. The second patient with *SNCA* duplication fulfilled the criteria for probable DLB, as she presented with parkinsonism with early cognitive impairment that interfered with daily activities, visual hallucinations, rapid eye movement sleep behavior disorder and fluctuating cognition at age 65. All four patients had a family history of parkinsonism with one to four affected relatives and at least one affected first-degree relative.

Definition of indication criteria

First, the variables that were significantly associated with an MLPA+ test in the total cohort and, more specifically, in the various groups of patients, depending on the inheritance pattern of their genetic disorder (autosomal dominant for *LRRK2* and *SNCA* patients, autosomal recessive for *PRKN* patients), were individually assessed. Amongst the entire cohort, the MLPA test was more frequently positive for (i) patients with North African ancestry, with 24/52 (46.2%) positive tests versus 27/515 (5.2%) in the remaining cohort ($p < 0.0001$), (ii) women, with 32/248 (12.9%) positive tests versus 19/316 (6%) for men ($p = 0.004$), and (iii) patients who were younger at onset. The age at onset in the MLPA+ group was 41 (31–51) years (range 10–73, $n = 46$) versus 47 (40–57) years (range 5–87, $n = 489$) ($p < 0.0008$). As expected, the significant variables for the patients with autosomal dominant forms of PD were (i) North African ancestry ($p < 0.0001$), (ii) a family history of parkinsonism with at least one affected first-degree relative ($p = 0.023$), (iii) a higher number of affected first-degree relatives ($p = 0.003$) and (iv) the total number of affected relatives ($p = 0.01$). Unexpectedly, it was observed that (v) sex was also a significant variable, with 21/237 (8.9%) positive tests for women versus 11/311 (3.5%) for men ($p = 0.009$). For the patients with autosomal recessive forms of PD (*PRKN* patients), only a younger age at onset was significantly associated with a more frequent MLPA+ test ($p < 0.0001$). The significant variables associated with an MLPA+ test are summarized in Table 4. There was no difference in the age at onset between men and women amongst the *LRRK2* ($p = 0.99$) or *PRKN* patients ($p = 0.26$).

Secondly, a retrospective decision-tree based approach was developed and the combination of variables and the most discriminating cut-off for MLPA testing were defined (Data S1, Figure S1). Thirdly, the significant variables to define the most relevant indication criteria were retrospectively applied. When consideration was given to (i) North African ancestry, (ii) an age at onset <40 years, (iii) or a family history of parkinsonism with at least one affected first-degree relative, 48/51 MLPA+ patients within the total cohort and 30/31 MLPA+ patients from the Lille expert center for PD were found. The three missed PD patients using these criteria were one Caucasian woman with two *PRKN* mutations and an age at onset of 56 without a family history and two Caucasian men with an *LRRK2* G2019S mutation. One *LRRK2* patient had no data about the age at onset but a family history, whereas the other had an age at onset of 41 but no family history.

TABLE 2 Variants observed during this study

ID	Gene	Variant 1	Comments variant 1	Variant 1	Variant 2	Comments variant 2	ACMG classification variant 2	Status
PARK102-111-112-135-174-212-259-270-303-318-384-385-396-439-445-449-456-466-491-510-625-668-703-725-744	LRRK2 (NM_198578.3)	c.6055G>A, p.(Gly2019Ser)	Most common LRRK2 mutation in Parkinson's disease	P	n.c.	n.c.	n.c.	Het
PARK103	PRKN (NM_004562.2)	c.(534+1_535-1) _{733+1_734-1} dup	Exons 5-6 duplication	P	c.(534+1_535-1) _{733+1_734-1} dup	Exons 5-6 duplication	P	Hom
PARK149		c.(171+1_413-1)dup	Exon 3 duplication	P	c.823C>T, p.(Arg275Trp)	Previously reported mutation	P	Com Het?
PARK202		c.(412+1_595-1)del	Exon 4 deletion	P	c.823C>T, p.(Arg275Trp)	Previously reported mutation	P	Com Het?
PARK309		c.(7+1_172-1)dup	Exon 2 duplication	P	c.(7+1_172-1)dup	Exon 2 duplication	P	Hom
PARK369		c.(171+1_172-1) _{733+1_734-1} del	Exon 3-6 deletion	P	c.(734+1_872-1)dup	Exon 7 duplication	P	Com Het
PARK387		c.(618+1_735-1)del	Exon 6 deletion	P	c.1283dup, p.(Asn428Lysfs*141)	Previously reported mutation	P	Com Het?
PARK403		c.(171+1_172-1) _{533+1_534-1} del	Exons 3-4 deletion	P	c.823C>T, p.(Arg275Trp)	Previously reported mutation	P	Com Het?
PARK497		c.(534+1_535-1) _{734+1_619-1} del	Exon 5 deletion	P	c.(618+1_735-1) _{734+1_872-1} del	Exons 6-7 deletion	P	Com Het?
PARK498		c.(171+1_413-1)del	Exon 3 deletion	P	c.7+5G>T, p.?	Novel splice variant	US	Com Het?
PARK503		c.(412+1_535-1)dup	Exon 4 triplication	P	c.(534+1_535-1) _{1082+1_1083-1} del	Exons 5-9 deletion	P	Com Het
PARK509		c.(871+1_934-1)del	Exon 8 deletion	P	c.971del, p.(Val324Alafs*111)	Previously reported mutation	P	Com Het?
PARK575		c.(171+1_413-1)del	Exon 3 deletion	P	c.(171+1_172-1) _{533+1_534-1} del	Exons 3-4 deletion	P	Com Het
PARK616		c.(171+1_172-1) _{533+1_534-1} del	Exons 3-4 deletion	P	c.155del, p.(Asn52Metfs*29)	Previously reported mutation	P	Com Het
PARK628		c.(171+1_172-1) _{733+1_734-1} del	Exons 3-6 deletion	P	c.1283dup, p.(Asn428Lysfs*141)	Previously reported mutation	P	Com Het
PARK678		c.(171+1_413-1)dup	Exon 3 duplication	P	c.155del, p.(Asn52Metfs*29)	Previously reported mutation	P	Com Het?
PARK714		c.(618+1_735-1)del	Exon 6 deletion	P	c.(618+1_735-1)del	Exon 6 deletion	P	Hom
PARK742		c.(171+1_413-1) _{733+1_734-1} del	Exons 1-3 deletion	P	c.1299A>C, p.(His433Pro)	Novel missense variant	LP	Com Het
PARK798		c.(7+1_172-1)del	Exon 2 deletion	P	c.(734+1_872-1)del	Exon 7 deletion	P	Com Het?
PARK808		c.(618+1_735-1) _{734+1_872-1} del	Exons 6-7 deletion	P	c.1274T>A, p.(Val425Glu)	Novel missense variant	LP	Com Het

(Continues)

TABLE 2 (Continued)

ID	Gene	Variant 1	Comments variant 1	Variant 1	Variant 2	Comments variant 2	ACMG classification variant 2	Status
PARK247	SNCA (NM_000345:3)	c.(?_1471)_(*575_?)dup	SNCA gene duplication	P	n.c.	n.c.	n.c.	Het
PARK279								
PARK329								
PARK366								
PARK529								

Abbreviations: ACMG, American College of Medical Genetics and Genomics; Com Het, compound heterozygous; Com Het?, probable compound heterozygous; Het, heterozygous; Hom, homozygous; LP, likely pathogenic; n.c., not concerned; P, pathogenic; US, uncertain significance.

TABLE 3 Characteristics of the Mendelian PD patients

Clinical diagnosis	34/51
PD	30/34 (88.2%)
Other parkinsonism	4/34 (11.8%)
MSA-like	2/34 (5.9%)
DLB	1/34 (2.9%)
Atypical parkinsonism	1/34 (2.9%)

Abbreviations: DLB, dementia with Lewy bodies; MSA, multiple system atrophy; PD, Parkinson's disease.

DISCUSSION

Good performance of MLPA was obtained in our cohort (9% positivity in the entire cohort). Other studies enriched for early onset and/or familial cases have reported positivity from 1.4% to 3.5% [21, 22], but the methodologies used in these studies differed from ours, making comparisons difficult.

Multiplex ligation-dependent probe amplification cannot detect all the conventional variants, especially in the *PRKN* gene or in other rarely implicated parkinsonism genes. However, MLPA tests the main pathogenic variants in Mendelian PD which are located in *LRRK2*, *PRKN* or *SNCA* genes [12]. The *LRRK2* G2019S pathogenic variant is by far the most frequent known cause of familial and sporadic PD with a worldwide distribution [23], whereas the other eight variants have been reported only in a few families. Pathogenic missenses, including A30P, in the *SNCA* gene are thought to be extremely rare, whereas the frequency of whole *SNCA* gene duplication/triplication in familial PD is ~2% [24]. Structural variants in *PRKN* account for 43.2% of mutations [11], although this frequency is probably underestimated because structural variants were not assessed in all previous studies. Considering a systematic search for a second pathogenic variant in *PRKN* when only one structural variant was found, it can be estimated that *PRKN* variants could be evidenced in more than two-thirds of cases.

Our criteria would not account for late-onset and sporadic PD patients with *LRRK2* G2019S, but considering the variable distribution and penetrance of this variant across populations [25] it is very difficult to estimate the proportion of missed mutations. It should be noted, however, that such patients with no family history and late onset generally have fewer questions about the genetic origin of their disease. The interest of evidencing an *LRRK2* G2019S variant, known for its reduced penetrance, is also very debatable in patients with no family history and no specific treatment.

Next-generation sequencing analyses (panel genes, whole exome or whole genome sequencing) would detect conventional mutations, be more exhaustive and can include deletion/duplication analysis, especially the whole genome sequencing. MLPA has a low cost per patient of ~\$20, which is approximately 15, 25 and 35 times less than a gene panel, an exome or a genome sequencing. NGS requires significant investment (sequencers and computer servers), needs bioinformaticians and its analysis time is much longer. Other studies used different NGS methods in PD [21, 26] or early-onset PD [22,

TABLE 4 Significant variables associated with a positive MLPA test

Total cohort			
Ancestry	North African	Other	$p < 0.0001$
	24/52 (46.2%)	27/515 (5.2%)	
Sex	Women	Men	$p = 0.004$
	32/248 (12.9%)	19/316 (6%)	
Age at onset (years)	MLPA+	MLPA-	$p < 0.0008$
	41 (31-51) (range 10-73, $n = 46$)	47 (40-57) (range 5-87, $n = 489$)	
Autosomal dominant inheritance (<i>LRRK2</i> and <i>SNCA</i>)			
Ancestry	North African	Other	$p < 0.0001$
	23/51 (45.1%)	9/497 (1.8%)	
Sex	Women	Men	$p = 0.009$
	21/237 (8.9%)	11/311 (3.5%)	
>1 affected first-degree relative	MLPA+	MLPA-	$p = 0.023$
	19/228 (8.3%)	7/215 (3.3%)	
Number of affected first-degree relatives	MLPA+	MLPA-	$p = 0.003$
	1 ± 0.8 ($n = 21$)	0.7 ± 0.8 ($n = 417$)	
Number of affected relatives	MLPA+	MLPA-	$p = 0.01$
	1.5 ± 1.3 ($n = 21$)	1.2 ± 1.5 ($n = 417$)	
Autosomal dominant inheritance (<i>PRKN</i>)			
Age at onset (years)	MLPA+	MLPA-	$p < 0.0001$
	28 (26-35) (range 10-56, $n = 19$)	47 (40-57) (range 5-87, $n = 489$)	

Abbreviation: MLPA, multiplex ligation-dependent probe amplification.

27]. All pathogenic variants (excluding *GBA* variants that are risk factors for PD) described in Illés et al. [21] and Kovanda et al. [26] could be observed using MLPA \pm Sanger sequencing to look for a second mutation. Tan et al. [20] reported 15 pathogenic variants in 1725 PD patients and MLPA would have missed two *LRRK2* patients and five *PRKN* patients. Bentley et al. [25] reported 13 pathogenic variants in 2206 PD patients but only one *PRKN* patient would have been missed using MLPA. Considering the low cost, simplicity and performance of MLPA compared to NGS, it is proposed that it is used as a first-line screening test.

The review of clinical diagnoses of patients from Lille University Hospital limited missing data. However, our study still suffered from missing data and further replication in other cohorts or during a prospective study is warranted. A limitation of our study was the composition of our cohort, corresponding to all patients referred to our laboratory by neurologists or geneticists for a molecular diagnosis of PD. There was a selection bias, as mostly patients with characteristics suggestive of Mendelian PD were referred to our laboratory for molecular testing. As a consequence, our cohort is enriched for early-onset PD and patients with a family history of PD and is not representative of the entire PD population. Our results cannot be applied for the whole PD population. However, all PD patients should not be tested, as Mendelian PD concerns only <10%

of all PD patients [1]. In addition, genetic screening is generally time consuming and can be expensive. Previous studies on large cohorts used various methods (including MLPA) to grossly assess the same mutations and genes in all PD patients with little selection bias [21, 26, 28]. They identified 0.6%–1.4% pathogenic variants for PD. Our retrospective study had the advantage of more faithfully reproducing the conditions of real life in neurology departments and directly addressing the prescriber's questions: when to think and test for a genetic cause in patients with PD or parkinsonism?

Retrospective analysis of the MLPA results allowed us to refine our indication criteria: (i) North African ancestry or (ii) an age at onset <40 years or (iii) a family history of parkinsonism with at least one affected first-degree relative. The prevalence of the *LRRK2* G2019S mutation (up to 41% of familial PD in the North African Berber population [29]) justifies the use of MLPA for PD patients of North African ancestry, but other populations should also be considered. For example, Orr-Urtreger et al. reported a rate of PD of \approx 15% amongst Ashkenazi Jews [30]. French laws prohibit collecting data on ethnicity or religious origin. No information on religion could therefore be retrieved, which could have been particularly interesting, considering the high prevalence of the G2019S mutation in Ashkenazi Jews, estimated to be 18.3%–28% [31]. That is why the prescription of MLPA for PD patients from Israel and/or of

Ashkenazi Jewish origin should also be considered. The early onset of PD in our population (≤ 39 years old) is similar to the findings of the Movement Disorder Society Genetic mutation database that led to recommending testing for *PRKN* mutations for patients with PD onset before the age of 40 years [11]. The association of the sex ratio for positive MLPA tests is more puzzling. It was observed that women had a higher probability of being positive. This may reflect that women have a higher risk of developing Mendelian PD. Such a gender effect has previously been observed for PD patients with *LRRK2* mutations, especially those carrying the G2019S mutation [32]. However, it cannot be excluded that men may be less willing to consult a neurologist and/or a medical geneticist, or that men develop a disease with a more marked environmental origin, such as pesticide exposure [33].

Our study highlights the interest of MLPA testing for other parkinsonisms with a family history, especially for patients with DLB or an MSA-like phenotype. *SNCA* duplication has been previously shown to be associated with a rare Mendelian cause of DLB (OMIM #127750) [34]. However, the involvement of pathogenic *SNCA* variants in MSA is still debated [35]. Similarly, only one rare variant of uncertain significance in the *LRRK2* gene has been previously shown to be associated with clinically diagnosed and pathologically proven MSA [36]. However, it is commonly accepted that *LRRK2* G2019S and *SNCA* mutations are not Mendelian causes of MSA [37, 38]. Our patient with the G2019S *LRRK2* mutation fulfilled the diagnostic criteria of clinically established MSA but no neuropathological examination was carried out. The other patient with the MSA-like phenotype carrying the *SNCA* duplication only fulfilled the criteria of clinically probable MSA at first evaluation, but the slow evolution of her disease excludes a posteriori the diagnosis of MSA. However, it is argued that at the time of their first consultation the existence of a family history of parkinsonisms in a patient with a diagnosis compatible with MSA should direct the prescriber towards a genetic form and MLPA testing.

In conclusion, good performance of MLPA in Mendelian PD with our indication criteria is reported. It is proposed that patients with a negative MLPA result who fulfill the above-mentioned criteria are also good candidates for complementary analysis by NGS.

AUTHOR CONTRIBUTIONS

E.M: conceptualization, data curation, investigation, supervision, and writing—review and editing. M.S. and N.Ro: data curation and writing—review and editing. C.M., D.D., G.B., A.D., G.R., N.C., A.K., C.S., M.T. and P.K.: investigation and writing—review and editing. N.Ra and M.G: formal analysis and writing—review and editing. B.S: writing—review and editing. L.D: investigation and writing—review and editing. V.H: conceptualization, data curation, investigation, supervision, and writing—original draft preparation.

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CONFLICT OF INTEREST STATEMENT

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary material.

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REFERENCES

1. Trinh J, Farrer M. Advances in the genetics of Parkinson disease. *Nat Rev Neurol*. 2013;9(8):445-454. doi:10.1038/nrneuro.2013.132
2. Funayama M, Hasegawa K, Kowa H, Saito M, Tsuji S, Obata F. A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1. *Ann Neurol*. 2002;51(3):296-301. doi:10.1002/ana.10113
3. Zimprich A, Biskup S, Leitner P, et al. Mutations in *LRRK2* cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron*. 2004;44(4):601-607. doi:10.1016/j.neuron.2004.11.005
4. Matsumine H, Saito M, Shimoda-Matsubayashi S, et al. Localization of a gene for an autosomal recessive form of juvenile parkinsonism to chromosome 6q25.2-27. *Am J Hum Genet*. 1997;60(3):588-596.
5. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*. 1998;392(6676):605-608. doi:10.1038/33416
6. Polymeropoulos MH, Higgins JJ, Golbe LI, et al. Mapping of a gene for Parkinson's disease to chromosome 4q21-q23. *Science*. 1996;274(5290):1197-1199. doi:10.1126/science.274.5290.1197
7. Tolosa E, Vila M, Klein C, Rascol O. *LRRK2* in Parkinson disease: challenges of clinical trials. *Nat Rev Neurol*. 2020;16(2):97-107. doi:10.1038/s41582-019-0301-2
8. Coku I, Mutez E, Eddarkaoui S, et al. Functional analyses of two novel *LRRK2* pathogenic variants in familial Parkinson's disease. *Mov Disord*. 2022;37(8):1761-1767. doi:10.1002/mds.29124
9. Rosborough K, Patel N, Kalia LV. α -Synuclein and parkinsonism: updates and future perspectives. *Curr Neurol Neurosci Rep*. 2017;17(4):31. doi:10.1007/s11910-017-0737-y

10. Lesage S, Lunati A, Houot M, et al. Characterization of recessive Parkinson disease in a large multicenter study. *Ann Neurol*. 2020;88(4):843-850. doi:10.1002/ana.25787
11. Kasten M, Hartmann C, Hampf J, et al. Genotype-phenotype relations for the Parkinson's disease genes *Parkin*, *PINK1*, *DJ1*: MDSGene systematic review. *Mov Disord*. 2018;33(5):730-741. doi:10.1002/mds.27352
12. Jia F, Fellner A, Kumar KR. Monogenic Parkinson's disease: genotype, phenotype, pathophysiology, and genetic testing. *Gen*. 2022;13(3):471. doi:10.3390/genes13030471
13. Lunati A, Lesage S, Brice A. The genetic landscape of Parkinson's disease. *Rev Neurol*. 2018;174(9):628-643. doi:10.1016/j.neurol.2018.08.004
14. Harbo HF, Finsterer J, Baets J, et al. EFNS guidelines on the molecular diagnosis of neurogenetic disorders: general issues, Huntington's disease, Parkinson's disease and dystonias: neurogenetic guidelines on HD, PD and dystonia. *Eur J Neurol*. 2009;16(7):777-785. doi:10.1111/j.1468-1331.2009.02646.x
15. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease: MDS-PD Clinical Diagnostic Criteria. *Mov Disord*. 2015;30(12):1591-1601. doi:10.1002/mds.26424
16. Höglinger GU, Respondek G, Stamelou M, et al. Clinical diagnosis of progressive supranuclear palsy: the Movement Disorder Society criteria: MDS clinical diagnostic criteria for PSP. *Mov Disord*. 2017;32(6):853-864. doi:10.1002/mds.26987
17. Armstrong MJ, Litvan I, Lang AE, et al. Criteria for the diagnosis of corticobasal degeneration. *Neurology*. 2013;80(5):496-503. doi:10.1212/WNL.0b013e31827f0fd1
18. Wenning GK, Stankovic I, Vignatelli L, et al. The Movement Disorder Society criteria for the diagnosis of multiple system atrophy. *Mov Disord*. 2022;37(6):1131-1148. doi:10.1002/mds.29005
19. McKeith IG, Boeve BF, Dickson DW, et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB consortium. *Neurology*. 2017;89(1):88-100. doi:10.1212/WNL.0000000000004058
20. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the association for molecular pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30
21. Tan MMX, Malek N, Lawton MA, et al. Genetic analysis of Mendelian mutations in a large UK population-based Parkinson's disease study. *Brain*. 2019;142(9):2828-2844. doi:10.1093/brain/awz191
22. Illés A, Csabán D, Grosz Z, et al. The role of genetic testing in the clinical practice and research of early-onset parkinsonian disorders in a Hungarian cohort: increasing challenge in genetic counselling, improving chances in stratification for clinical trials. *Front Genet*. 2019;10:1061. doi:10.3389/fgene.2019.01061
23. Correia Guedes L, Ferreira JJ, Rosa MM, Coelho M, Bonifati V, Sampaio C. Worldwide frequency of G2019S LRRK2 mutation in Parkinson's disease: a systematic review. *Parkinsonism Relat Disord*. 2010;16(4):237-242. doi:10.1016/j.parkreldis.2009.11.004
24. Lesage S, Houot M, Mangone G, et al. Genetic and phenotypic basis of autosomal dominant Parkinson's disease in a large multi-center cohort. *Front Neurol*. 2020;11:682. doi:10.3389/fneur.2020.00682
25. Hentati F, Trinh J, Thompson C, Nosova E, Farrer MJ, Aasly JO. LRRK2 parkinsonism in Tunisia and Norway: a comparative analysis of disease penetrance. *Neurology*. 2014;83(6):568-569. doi:10.1212/WNL.0000000000000675
26. Bentley SR, Bortnick S, Guella I, et al. Pipeline to gene discovery—analysing familial parkinsonism in the Queensland Parkinson's project. *Parkinsonism Relat Disord*. 2018;49:34-41. doi:10.1016/j.parkreldis.2017.12.033
27. Kovanda A, Rački V, Bergant G, et al. A multicenter study of genetic testing for Parkinson's disease in the clinical setting. *Npj Parkinsons Dis*. 2022;8(1):149. doi:10.1038/s41531-022-00408-6
28. Puschmann A, Jiménez-Ferrer I, Lundblad-Andersson E, et al. Low prevalence of known pathogenic mutations in dominant PD genes: a Swedish multicenter study. *Parkinsonism Relat Disord*. 2019;66:158-165. doi:10.1016/j.parkreldis.2019.07.032
29. Lesage S, Dürr A, Tazir M, et al. LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. *N Engl J Med*. 2006;354(4):422-423. doi:10.1056/NEJMc055540
30. Orr-Urtreger A, Shifrin C, Rozovski U, et al. The LRRK2 G2019S mutation in Ashkenazi Jews with Parkinson disease: is there a gender effect? *Neurology*. 2007;69(16):1595-1602. doi:10.1212/01.wnl.0000277637.33328.d8
31. Ozelius LJ, Senthil G, Saunders-Pullman R, et al. LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N Engl J Med*. 2006;354(4):424-425. doi:10.1056/NEJMc055509
32. Chen W, Yan X, Lv H, Liu Y, He Z, Luo X. Gender differences in prevalence of LRRK2-associated Parkinson disease: a meta-analysis of observational studies. *Neurosci Lett*. 2020;715:134609. doi:10.1016/j.neulet.2019.134609
33. Poortvliet PC, Gluch A, Silburn PA, Mellick GD. The Queensland Parkinson's project: an overview of 20 years of mortality from Parkinson's disease. *JMD*. 2021;14(1):34-41. doi:10.14802/jmd.20034
34. Zarranz JJ, Alegre J, Gómez-Esteban JC, et al. The new mutation, E46K, of α -synuclein causes Parkinson and Lewy body dementia: new α -synuclein gene mutation. *Ann Neurol*. 2004;55(2):164-173. doi:10.1002/ana.10795
35. Kiely AP, Asi YT, Kara E, et al. α -Synucleinopathy associated with G51D SNCA mutation: a link between Parkinson's disease and multiple system atrophy? *Acta Neuropathol*. 2013;125(5):753-769. doi:10.1007/s00401-013-1096-7
36. Lee K, Nguyen KD, Sun C, et al. LRRK2 p.Ile1371Val mutation in a case with neuropathologically confirmed multi-system atrophy. *JPD*. 2018;8(1):93-100. doi:10.3233/JPD-171237
37. Ozelius LJ, Foroud T, May S, et al. G2019S mutation in the leucine-rich repeat kinase 2 gene is not associated with multiple system atrophy. *Mov Disord*. 2007;22(4):546-549. doi:10.1002/mds.21343
38. Morris HR, Vaughan JR, Datta SR, et al. Multiple system atrophy/progressive supranuclear palsy: α -synuclein, synphilin, tau, and APOE. *Neurology*. 2000;55(12):1918-1920. doi:10.1212/WNL.55.12.1918

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