Frequency of the *LRRK2* G2019S mutation in Italian patients affected by Parkinson's disease

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Abstract Mutations in the gene *Leucine-Rich Repeat Kinase 2* (*LRRK2*) have been identified in both dominant and sporadic cases affected by Parkinson's disease (PD). The *LRRK2* G2019S mutation (c.6055G>A) is the most frequent substitution in Caucasians, accounting for approximately 5–6% of familial and 0.5–2.0% of apparently sporadic PD cases. We investigated the frequency of the *LRRK2* G2019S mutation in 98 unrelated Italian PD patients, including 12 probands belonging to families compatible with autosomal dominant inheritance (12%) and 86 sporadic cases (88%). We detected the G2019S mutation in one sporadic female patient (1.2%). These results confirm that the G2019S mutation is a relevant cause of sporadic PD cases in the Italian population and stress the importance of performing this genetic test, which has important implications for genetic counselling.

Keywords Parkinson's disease · PD · *LRRK2* · G2019S · Penetrance · Familial cases · Sporadic cases

Parkinson's disease (PD; MIM 168600) is the second most common neurodegenerative disorder after Alzheimer's disease, affecting more than 1% of individuals aged 55 years (Polymeropoulos et al. 1996). The disease is clinically defined by the association of bradykinesia, resting tremor, muscular rigidity and postural instability (Gelb et al. 1999). Neuropathological features include loss of dopaminergic neurones in the substantia nigra-pars compacta and other brain regions, with formation of ubiquitin-containing inclusions (Lewy bodies) in the surviving neurones (Forno 1996).

The cause of the disease remains unknown in most patients, but a positive family history of PD is found in ~15–25% of cases, and mutations in five genes have been firmly implicated in the aetiology of these rare inherited forms of the disease (Polymeropoulos et al. 1997; Kitada et al. 1998; Bonifati et al. 2003; Valente et al. 2004; Zimprich et al. 2004).

An autosomal dominant form of parkinsonism (PARK8) was first mapped to chromosome 12 in a Japanese family (Funayama et al. 2002). This linkage was successively confirmed in Caucasian families and mutations in the gene *Leucine-Rich Repeat Kinase 2* (*LRRK2*) have been identified in PARK8-linked families (Paisan-Ruiz et al. 2004; Zimprich et al. 2004). The *LRRK2* gene has 51 exons and encodes a protein of 2,527 amino acids. The *LRRK2* protein has unknown function, although there are five predicted functional domains: a leucine-rich repeat, a Roc (Ras guanosine triphosphatase) domain, a COR (C-terminal

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of Roc) domain, a WD40 (beta-transducin repeats) domain and a tyrosine kinase catalytic domain (Paisan-Ruiz et al. 2004; Zimprich et al. 2004).

Until now, several different missense mutations have been identified in the *LRRK2* gene (Zimprich et al. 2004; Aasly et al. 2005; Hernandez et al. 2005; Kachergus et al. 2005; Khan et al. 2005; Mata et al. 2005; Paisan-Ruiz et al. 2005; Zabetian et al. 2005). In particular, the G2019S substitution in exon 41 accounts for approximately 5–6% of familial and 0.5–2% of apparently sporadic PD cases in populations of European origin (Di Fonzo et al. 2005; Farrer et al. 2005; Gilks et al. 2005; Kachergus et al. 2005; Nichols et al. 2005; Gaig et al. 2006). Furthermore, recent studies have indicated an extremely high prevalence of the G2019S mutation in Ashkenazi Jews (13.3% sporadic; 29.7% familial) and North African Arabs (40.8% sporadic; 37.0% familial) affected by PD (Lesage et al. 2006; Ozelius et al. 2006). Kachergus et al. (2005) reported that G2019S penetrance is age-dependent, increasing from 17% at age 50 to 85% at age 70 years. However, very recent studies found that G2019S penetrance could be lower than previously reported (S. Goldwurm, personal communication). In the Italian population, the prevalence of the G2019S mutation is 5.1% among PD familial cases and 0.9% among sporadic patients (Goldwurm et al. 2005). The G2019S mutation alters a critical residue in the kinase domain of the protein, and it has been postulated that the mutation may have an activating effect on LRRK2 kinase activity (Kachergus et al. 2005). A mutation linked to a “gain of function” of the resulting protein is also compatible with the dominant mode of inheritance of the disease observed in family studies.

The aim of this study was to determine the frequency of the *LRRK2* G2019S mutation in Italian patients with idiopathic PD who have been referred to our Medical Genetics Unit by neurologists from the University Hospital of Siena. We performed the analysis of the *LRRK2* G2019S mutation in 98 unrelated PD patients, originating mostly from the Tuscan region, central Italy. In this group of patients, 12 belong to families compatible with autosomal dominant inheritance (12%) and 86 are sporadic cases (88%). Among familial cases, 6 are early onset (mean age at onset: 41.2 ± 4.4 years) and 6 late onset cases (mean age at onset: 62.2 ± 6.8 years). The sporadic group includes 24 early onset (mean age at onset: 40.7 ± 8 years) and 62 late onset patients (mean age at onset: 62.6 ± 6.7 years).

DNA was extracted using standard methods (Sambrook et al. 1989). Mutation analysis was performed by denaturing high performance liquid chromatography

(DHPLC) using Transgenomic WAVE (Transgenomic, San Jose, CA). For PCR, we used the following primers: LRRK2_41_F: 5'-ATGCTTGACATAGTGGACA-3' and LRRK2_41_R: 5'-AGGTCAGTGGTTATCCATC-3' (amplicon size: 316 bp). The optimal column temperature for fragments analysis (59°C) was calculated using WaveMarker Software (Transgenomic). Direct sequencing of the purified PCR products was carried out in both directions on an ABI310 Automated Sequencer.

We detected the *LRRK2* G2019S mutation in one sporadic case (1.2%, 1/86 sporadic patients). The patient is a 53-year-old female who developed PD symptoms at the age of 47 years. The disease started with bradykinesia, severe dyskinesias and rigidity. At present, psychiatric disturbances are not present and the patient is treated with levodopa. Her parents are still alive, are 78 and 75 years old, and a recent neurological examination did not show any sign of PD. DNA from the parents is not available for genetic testing. The proband has a healthy 16-year-old son.

These results confirm previous studies that demonstrated a frequency of about 1% for the G2019S mutation in sporadic cases with idiopathic PD in the Italian population (Goldwurm et al. 2005). On the other hand, the mutation was not detected in any of the 12 familial PD probands. This result is not unexpected since the *LRRK2* G2019S mutation is reported in about 5% of PD familial cases (Goldwurm et al. 2005).

Concerning disease onset, previous studies reported the G2019S mutation prevalently associated with late onset PD (>50 years) (Kachergus et al. 2005; Infante et al. 2006). In addition, Goldwurm et al. (2005) observed that the average disease onset of females carrying the G2019S mutation is about 10 years earlier than that of males (47.1 years vs 56.5 years). Our female patient developed PD symptoms at the age of 47 years and this is in accordance with previous data. However, a larger number of mutated cases is needed to firmly confirm the age of disease onset associated with the G2019S mutation.

Since genetic predisposition is important in the pathogenesis of PD, and the *LRRK2* G2019S mutation contributes to a significant percentage of cases, screening for this substitution may become a key component of genetic testing for PD in the near future. As already underlined in previous studies, such genetic tests should also be extended to sporadic cases. In fact, the late onset of PD in G2019S mutated patients together with the reduced penetrance of this mutation, can sometimes mask its familial occurrence.

The identification of a frequent genetic determinant of PD also has important implications for genetic

counselling. The lifetime risk of developing PD for the son of the patient described here shifts from 2%, reported for first-degree relatives of an affected individual, to about 42.5%, considering the penetrance of the mutation (Marder et al. 1996; Kachergus et al. 2005). Analysis of the G2019S *LRRK2* mutation in large cohorts of PD cases is necessary to better assess penetrance rate prior to offering presymptomatic tests to relatives.

References

- Aasly JO, Toft M, Fernandez-Mata I, Kachergus J, Hulihan M, White LR, Farrer M (2005) Clinical features of *LRRK2*-associated Parkinson's disease in central Norway. *Ann Neurol* 57:762–765
- Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M, van Dongen JW, Vanacore N, van Swieten JC, Brice A, Meco G, van Duijn CM, Oostra BA, Heutink P (2003) Mutations in the *DJ-1* gene associated with autosomal recessive early-onset parkinsonism. *Science* 299:256–259
- Di Fonzo A, Rohe CF, Ferreira J, Chien HF, Vacca L, Stocchi F, Guedes L, Fabrizio E, Manfredi M, Vanacore N, Goldwurm S, Breedveld G, Sampaio C, Meco G, Barbosa E, Oostra BA, Bonifati V (2005) A frequent *LRRK2* gene mutation associated with autosomal dominant Parkinson's disease. *Lancet* 365:412–415
- Farrer M, Stone J, Mata IF, Lincoln S, Kachergus J, Hulihan M, Strain KJ, Maraganore DM (2005) *LRRK2* mutations in Parkinson disease. *Neurology* 65:738–740
- Forno LS (1996) Neuropathology of Parkinson's disease. *J Neuropathol Exp Neurol* 55:259–272
- Funayama M, Hasegawa K, Kowa H, Saito M, Tsuji S, Obata F (2002) A new locus for Parkinson's disease (*PARK8*) maps to chromosome 12p11.2-q13.1. *Ann Neurol* 51:296–301
- Gaig C, Ezquerro M, Marti MJ, Munoz E, Valldeoriola F, Tolosa E (2006) *LRRK2* mutations in Spanish patients with Parkinson disease: frequency, clinical features, and incomplete penetrance. *Arch Neurol* 63:377–382
- Gelb DJ, Oliver E, Gilman S (1999) Diagnostic criteria for Parkinson disease. *Arch Neurol* 56:33–39
- Gilks WP, Abou-Sleiman PM, Gandhi S, Jain S, Singleton A, Lees AJ, Shaw K, Bhatia KP, Bonifati V, Quinn NP, Lynch J, Healy DG, Holton JL, Revesz T, Wood NW (2005) A common *LRRK2* mutation in idiopathic Parkinson's disease. *Lancet* 365:415–416
- Goldwurm S, Di Fonzo A, Simons EJ, Rohe CF, Zini M, Canesi M, Tesi S et al (2005) The G6055A (G2019S) mutation in *LRRK2* is frequent in both early and late onset Parkinson's disease and originates from a common ancestor. *J Med Genet* 42:e65
- Hernandez DG, Paisan-Ruiz C, McInerney-Leo A, Jain S, Meyer-Lindenberg A, Evans EW, Berman KF, Johnson J, Auburger G, Schaffer AA, Lopez GJ, Nussbaum RL, Singleton AB (2005) Clinical and positron emission tomography of Parkinson's disease caused by *LRRK2*. *Ann Neurol* 57:453–456
- Infante J, Rodriguez E, Combarros O, Mateo I, Fontalba A, Pascual J, Oterino A, Polo JM, Leno C, Berciano J (2006) *LRRK2* G2019S is a common mutation in Spanish patients with late-onset Parkinson's disease. *Neurosci Lett* 395:224–226
- Kachergus J, Mata IF, Hulihan M, Taylor JP, Lincoln S, Aasly J, Gibson JM, Ross OA, Lynch T, Wiley J, Payami H, Nutt J, Maraganore DM, Czyzewski K, Styczynska M, Wszolek ZK, Farrer MJ, Toft M (2005) Identification of a novel *LRRK2* mutation linked to autosomal dominant parkinsonism: evidence of a common founder across European populations. *Am J Hum Genet* 76:672–680
- Khan NL, Jain S, Lynch JM, Pavese N, Abou-Sleiman P, Holton JL, Healy DG et al (2005) Mutations in the gene *LRRK2* encoding dardarin (*PARK8*) cause familial Parkinson's disease: clinical, pathological, olfactory and functional imaging and genetic data. *Brain* 128:2786–2796
- Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392:605–608
- Lesage S, Durr A, Tazir M, Lohmann E, Leutenegger AL, Janin S, Pollak P, Brice A (2006) *LRRK2* G2019S as a cause of Parkinson's disease in North African Arabs. *N Engl J Med* 354:422–423
- Marder K, Tang MX, Mejia H, Alfaro B, Cote L, Louis E, Groves J, Mayeux R (1996) Risk of Parkinson's disease among first-degree relatives: a community-based study. *Neurology* 47:155–160
- Mata IF, Kachergus JM, Taylor JP, Lincoln S, Aasly J, Lynch T, Hulihan MM, Cobb SA, Wu RM, Lu CS, Lahoz C, Wszolek ZK, Farrer MJ (2005) *Lrrk2* pathogenic substitutions in Parkinson's disease. *Neurogenetics* 6:171–177
- Nichols WC, Pankratz N, Hernandez D, Paisan-Ruiz C, Jain S, Halter CA, Michaels VE, Reed T, Rudolph A, Shults CW, Singleton A, Foroud T (2005) Genetic screening for a single common *LRRK2* mutation in familial Parkinson's disease. *Lancet* 365:410–412
- Ozelius LJ, Senthil G, Saunders-Pullman R, Ohmann E, Deligdisch A, Tagliati M, Hunt AL, Klein C, Henick B, Hailpern SM, Lipton RB, Soto-Valencia J, Risch N, Bressman SB (2006) *LRRK2* G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N Engl J Med* 354:424–425
- Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, Lopez de Munain A, Aparicio S, Gil AM, Khan N, Johnson J, Martinez JR, Nicholl D, Carrera IM, Pena AS, de Silva R, Lees A, Marti-Masso JF, Perez-Tur J, Wood NW, Singleton AB (2004) Cloning of the gene containing mutations that cause *PARK8*-linked Parkinson's disease. *Neuron* 44:595–600
- Paisan-Ruiz C, Lang AE, Kawarai T, Sato C, Salehi-Rad S, Fisman GK, Al-Khairallah T, St George-Hyslop P, Singleton A, Rogaeva E (2005) *LRRK2* gene in Parkinson disease: mutation analysis and case control association study. *Neurology* 65:696–700
- Polymeropoulos MH, Higgins JJ, Golbe LI, Johnson WG, Ide SE, Di Iorio G, Sanges G, Stenroos ES, Pho LT, Schaffer AA, Lazzarini AM, Nussbaum RL, Duvoisin RC (1996) Mapping of a 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, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276:2045–2047
- Sambrook J, Fritsch E, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbour Laboratory Press, NY

- Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, Gonzalez-Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G, Wood NW (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 304:1158–1160
- Zabetian CP, Samii A, Mosley AD, Roberts JW, Leis BC, Yearout D, Raskind WH, Griffith A (2005) A clinic-based study of the LRRK2 gene in Parkinson disease yields new mutations. *Neurology* 65:741–744
- Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, Kachergus J, Hulihan M, Uitti RJ, Calne DB, Stoessl AJ, Pfeiffer RF, Patenge N, Carbajal IC, Vieregge P, Asmus F, Muller-Myhsok B, Dickson DW, Meitinger T, Strom TM, Wszolek ZK, Gasser T (2004) Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* 44:601–607