FREQUENCY AND PHENOTYPES OF *LRRK2* MUTATIONS IN PARKINSON'S DISEASE PATIENTS FROM NORTHERN ITALY

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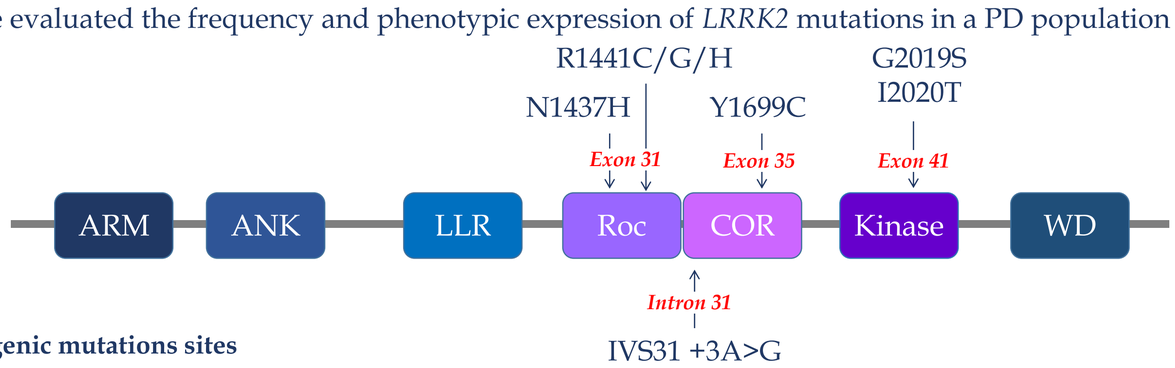
INTRODUCTION

Mutations in leucine-rich repeat kinase 2 (*LRRK2*) gene have been found in patients with both sporadic and familial Parkinson's disease (PD) with a frequency of 0,4 – 2,7 % and 1,7 - 13 % respectively. To date, only seven *LRRK2* mutations have been proved by linkage studies to be pathogenic: p.N1437H, p.R1441C/G/H, p.Y1699C, p.G2019S and p.I2020T.

In this study we evaluated the frequency and phenotypic expression of *LRRK2* mutations in a PD population from Northern Italy.



LRRK2 protein structure – Dardarin and pathogenic mutations sites (*Rosenbusch K.E. et al., 2016*).

MATERIALS AND METHODS

We performed a screening of exons 31, 35 e 41 of *LRRK2* in 276 patients with PD and 200 healthy controls, recruited from the Movement Disorder Centre of Bellaria Hospital, Bologna.
All patients met established diagnostic criteria (*Gelb et al., 1999*) for probable PD. 108 patients presented a positive family history (within the third-degree of kinship) for PD, parkinsonism or tremor. The age at onset of PD ranged from 18 to 82 years (53,9 ± 11,7).

5 patients (1 homozygote) (1,8 %) p.G2019S

		Our population	PD with p.G2019S mutation (Alcalay et al. 2013, Schulte et al. 2011, Yahalom et al. 2012, Marras et al. 2016, Healy et al. 2008)	Idiopathic PD (Alcalay et al. 2013, Schulte et al. 2011, Yahalom et al. 2012, Marras et al. 2016)
Mean age at onset		61,4 years	60 years	62 years
Positive family history for PD		60% (3/5)	39,1%	-
Symptoms at onset		Tremor 60% (3/5) Bradykinesia 20% (1/5)	Tremor 63% Bradykinesia 27%	Tremor 52% Bradykinesia 36%
Non-motor symptoms	Constipation	40% (2/5)	30,3%	39,4%
	Urinary dysfunction	20% (1/5)	41,5%	45,5%
	RBD	20% (1/5)	21,2%	42,4%
	Depression	20% (1/5)	57,6%	36,4%
Levo-dopa response		80% (4/5)	88%	83%
Dyskinesias		40% (2/5) 6 yao*	66% 5,4 yao*	53,2% 4,4 yao*
Dystonia		40% (2/5)	42%	25%

*years after onset

PD POPULATION		
Gender	M	176
	F	100
Positive family History	108 (39,1 %)	
Mean age at onset	53,9 ± 11,7 years	
Early onset (< 50 years)	98 (35,4 %)	

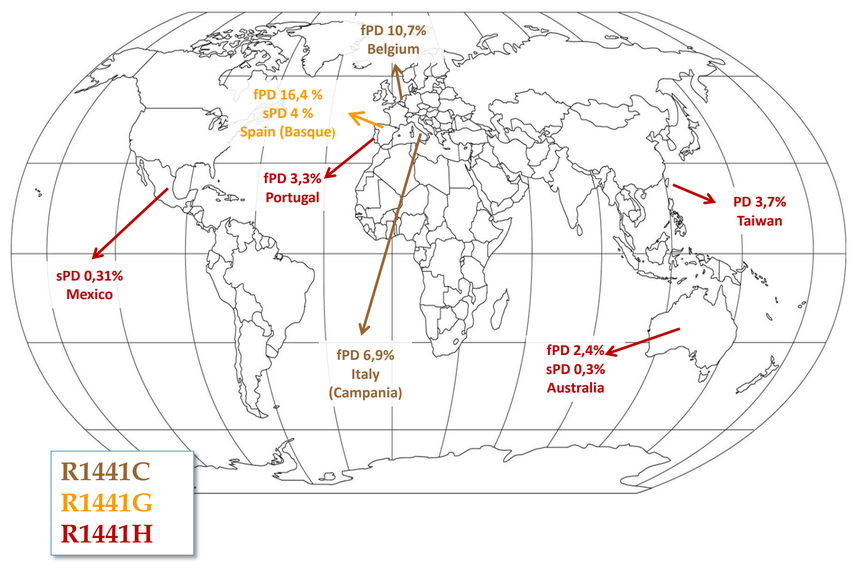
3 patients (1,08 %) p.R1441H

	p.R1441H (Our population)	p.R1441H ([1]Lesage et al. 2009, [2]Ferreira et al. 2007, [3]Lin et al. 2008, [4]Guedes et al. 2013)
Mean age at onset	48,3 years	54,6 years (France) 51,4 years (Taiwan)
Positive family history	100%	50-100%
Symptoms at onset	Bradykinesia and rigidity 66% (2/3) Tremor 33% (1/3)	Tremor, bradykinesia and rigidity 50% (2/4) [2] Bradykinesia and rigidity 100% (2/2)[1]
Non-motor symptoms	«Leg jerking during sleep» 33% (1/3) Cognitive impairment 33% (1/3) Depression 33% (1/3) Nocturia and erectile dysfunction 33% (1/3)	Hallucinations and vivid dreams 50% (1/2) [2] Anxiety and depression 33% (1/3) [3] Anxiety disorder 100% (2/2) [4]
Levo-dopa response	Good 100% Dyskinesias (after 4 years from onset) Dyskinesias and off (after 7 years from onset)	Good

	p.R1441C (Nuytemans et al. 2008 Floris G. et al. 2008)	p.R1441G (Simòn-Sanchez et al. 2006)
Mean onset age	56,5 years	64,6 years
Positive family history	100%	41,2%
Symptoms at onset	Tremor, bradykinesia, rigidity 83% (5/6)	Tremor 53,8% (9/17)
Non-motor symptoms	Depression 100% (2/2)	
Levo-dopa response	Good	Good

2 patients (0,72 %) IVS31 + 3 A>G

	Our population		Zabetian et al. 2005	Shoyaee et al. 2009			Anfossi et al. 2013	Pavlova et al. 2014	Jankovi et al. 2015	
Nationality	Italian	Italian	European	Persian	Persian	Persian	Italian	?	Serbian	Serbian
Onset age	39	36	47	51	41	54	76	62	59	62
Positive family history	Father (Rest tremor) Aunt (MND)	-	-	Uncle (PD)	+	+	Father (PD) Mother (Dementia)	-	-	-
Symptoms at onset	RBD	Bradykinesia	PD	Rest tremor, rigidity, bradykinesia	Healthy	Healthy	Cognitive impairment	Bradykinesia and rigidity	Unknown	Rest tremor and rigidity
Symptoms and Diagnosis	1 yao: sexual impotence, constipation. 4 yao: bradykinesia and rigidity, orthostatic hypotension, urinary dysfunction. 7 yao: camptocormia, postural instability, dysphagia, "en bloc" turning. 10 yao: stridor ***MSA type P, probable***	At onset: monotone speech, hypomimia, micrographia. 1 year after onset: bradykinesia adiadochokinesia						Urinary incontinence, static and gait ataxia, dysarthria, mild cognitive impairment, abasia ***MSA type C, probable***		
Rest tremor	-	-		+			+			
Postural tremor	+	-					+			
Bradykinesia	+	+		+			+			
Rigidity	+	-		+			+			
L-dopa response	Good	In follow up					Good			
Cognitive impairment	Mild	-					+			



Worldwide prevalence of the p.R1441C/G/H

DISCUSSION

p.G2019S is the most frequent mutation in the Caucasian population with a prevalence of 6% in familial and 0,6 - 3% in sporadic PD cases. We found a frequency of 2,8% in familial PD and 1,2% in the sporadic form with a mean onset age of 61,4 years. The phenotype is similar to that one of Idiopathic PD but with less frequent non-motor symptoms, apart from depression (*Jie-Qiong L. et al., 2014*). No significant differences were found between homozygotes and heterozygotes.
p.R1441H is a rare mutation with a high penetrance. We found a frequency of 2,8 % in familial PD and 0% in the sporadic form with a mean onset age of 48,3 years. The phenotype resembles that one of Young-onset PD and is characterized by an early onset age, less frequent non-motor symptoms, a good response to levodopa and early dyskinesias. This mutation should be investigated in EOPD.
IVS31+3A>G has been reported in few other studies. However, the causal link to PD for this genetic variant remains unproven. We found a frequency of 0,72% in our population.
One of our patients presented with RBD associated with autonomic dysfunction, whereas the other one had bradykinesia and asymmetric rigidity, with a mean onset age of 37,5 years. The phenotype in one of our patients and in one previously reported is MSA.

CONCLUSIONS

In conclusion, our study, while confirming that p.G2019S is the most frequent *LRRK2* mutation, describes the IVS31+3A>G variation and reports the high frequency of the p.R1441H mutation in our population. Moreover it underlines the different phenotypes linked to each mutation. Further study are necessary to prove the pathogenicity of the IVS31+3A>G variation, however the association of the mutation with an MSA phenotype should be better investigated.

REFERENCES
1) *De Rosa A et al., Genetic screening for the LRRK2 R1441C and G2019S mutations in Parkinsonian patients from Campania. J Parkinsons Dis. (2014);4(1):123-8*
2) *Kalinderi K et al., The genetic background of Parkinson'sdisease: current progress and future prospects. Acta Neurol Scand. (2016) Feb 12.*
3) *Zabetian CP et al., A clinic-based study of the LRRK2 gene in Parkinson disease yields new mutations. Neurology. (2005);65(5):741-4*