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Original Synthetic Report

New contribution on the *LRRK2* G2019S mutation associated to Parkinson's disease: age estimation of a common founder event of old age in Moroccan Berbers

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Abstract – ***Background:*** The *LRRK2* G2019S mutation is an important genetic determinant of Parkinson's disease (PD) across the world that occurs at an elevated frequency in North Africa. ***Aim:*** To estimate the date of the G2019S mutation in Berbers. ***Material and Methods:*** We determined the *LRRK2* haplotypes in twenty-two G2019S carriers, mostly North Africans, and in one hundred twenty-four Arab, Moroccan Berber and Sephardi Jew controls, using seven microsatellite and two SNP genetic markers. ***Results:*** A single haplotype was detected, with some variations, in all mutation carriers. Using a maximum–likelihood method, we estimate that Moroccan Berbers with G2019S share a common ancestor who lived ~128 (95% CI 107-180) generations ago. ***Conclusion:*** Our conclusion is that the G2019S mutation of the *LRRK2* gene originates 3,840 (95% CI 3,210-5,400) years ago in parkinsonian Moroccan Berbers patients.

Key words: Parkinson's disease (PD), Leucine-rich repeat kinase 2 *(LRRK2)* gene, G2019S mutation, Haplotype, Founding mutation.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, with a prevalence of 1-2 % in those aged sixty years old and older (De Rijk et al., 1997). Analysis of multigenerational pedigrees in which PD segregates in a Mendelian fashion has yielded five "causal" genes for the disease; *SNCA*, *DJ-1*, *LRRK2*, *PAKK2* and *PINK1* (Prankratz and Foroud, 2007). Of these five genes, mutations in *LRRK2* (Leucine-Rich Repeat Kinase 2) are the most frequent in both familial and sporadic PD; the G2019S mutation in the *LRRK2* gene has been detected in familial and sporadic PD patients of various origins, particularly Europeans, North Africans and Jews (Gaser, 2007).

Despite of a wide spectrum of ethnicities, most carriers of the G2019S mutation share a single rare background haplotype (Lesage et al., 2006; Ozelius et al., 2006), suggestive of a common founder. Lesage et al. (2005) used a likelihood-based haplotype approach in the study of six families of North African and European origin carrying G2019S, and estimated that these individuals shared a common founder ~725 years ago. Zabetian et al. (2006a) studied twenty-two families with G2019S and observed two distinct haplotypes: haplotype 1, present in nineteen families of Ashkenazi Jewish and European ancestry, and haplotype 2 occurring in three European-American families; using the same method, they estimated that the families with the more frequent haplotype shared a common ancestor about 2,250 years ago. Finally Warren et al. (2008) identified a single haplotype among thirty-eight Tunisian families and two families from USA, and estimated that they shared a common founder who lived 2,600 years ago.

This wide range of age estimates in these three studies prompted us to address this issue using G2019S carriers of cohorts of Arabs, Berbers from Morocco and Sephardi Jews (Change et al., 2008).

Subjects and Methods

We studied a total of twenty-two unrelated individuals with G2019S: two PD French patients (Funalot et al., 2008), one of them being of Berberian origin, and twenty asymptomatic subjects heterozygous for the G2019S mutation.

Among the latest (Change et al., 2008), one is from France (Marseilles), another is from Spain (Sevilla), eight are from North Africa (one form Libya, two from Tunisia, three from Algeria and two from Morocco), five are Moroccan Berbers, and five others are Sephardi Jews (four from Morocco and one from Djerba - Tunisia). A total of one hundred eighty-four (forty-nine non-Berbers Moroccans, seventy-eight Moroccan Berbers, and fifty-seven Sephardic Moroccan Jews (Lucotte and Mercier, 2003; Gérard et al., 2006), healthy and mutation-negatives, were used as controls.

This study was approved by our local ethic comity. Genomic DNA was isolated from peripheral blood of patients and subjects using standard protocols; a signed informed consent was obtained by the concerned subjects. Detection of *LRRK2* 6055G > A (G2019S) mutation was performed as described previously (Funalot et al., 2006). Limited haplotype data on the two PD patients (one of them being of Berberian origin) and on the twenty-two subjects bearing the G2019S mutation (no individual homozygous) have been published elsewhere (Change et al., 2008); because of the instability of D12S2515, this marker was not taken in consideration in the present study (Zabetian et al., 2006a). The following seven microsatellites and one single nucleotide polymorphism (SNP) were now analysed, based on previous work (Kachergus et al., 2005; Zabetian et al., 2006a): D12S2194, D12S2514, rs28903073, D12S2516, D12S2518, D12S2519, D12S2520 and D12S1048, covering an interval of about 400 kb that spans the entire length of *LRRK2* (Table 1).

SNP genotyping was performed by sequencing with the Big-Dye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, Ca, USA). Microsatellites were amplified by PCR using fluorescently labelled forward primers. Genotypes were determined using an ABI PRISM 3130 Genetic Analyser and GeneMapper 4.0 software (Applied Biosystems). Several Centre d'Etude du Polymorphisme Humain (CEPH) samples were used as a reference for microsatellite allele size determination.

Because marker phase cannot be resolved by pedigree data, we used PHASE software v2.1.1 (Stephens and Scheet, 2005); that can introduces uncertainly about age estimates. We estimated the age of the most recent common ancestor between two populations for individuals who carried G2019S using the program Estiage (Génin et al., 2004); this maximum-likelihood algorithm uses information on the recombinant fractions between the mutation and each marker and the position of the first marker in each direction that is no longer shared, to calculate the number of generations (with 95% CI) elasped since the most recent common ancestor introduced the mutation into the population. Genetic map positions for each marker were derived from the linkage-mapping server MAP-O-MAT, physical positions being taken from the National Center for Biotechnology Information (NCBI) human genome assembly Build 36 (Kong and Matise, 2005).

Table 1. Comparison of G2019S-containing haplotypes across the LRRK2 region in two patients (1 and 2) and two subjects (3 and 4) from Western Europe. Alleles concordant to haplotype 1 are indicated in bold.

				Patient/Subject numbers:			
LRRK2	**Position**	**Marker**	**Shared alleles**	**French 1**	**French (Berber) 2**	**French (Marseilles) 3**	**Spanish (Sevilla) 4**
	38738007	D12S2194	257	261/265	249/261	**257**/261	249/261
	38873924	D12S2514	291	**291**	**291**/285	**291**	**291**
	38939777	rs28903073	A	**A**/G	**A**	**A**/G	**A**/G
	38989339	D12S2516	254	**254**/252	**254**	**254**/252	**254**/252
LRRK2	39020469	G2019S	A	**A**/G	**A**/G	**A**/G	**A**/G
	39034922	D12S2518	154	**154**	**154**	**154**	**154**
	39116885	D12S2519	132	**132**	134/140	**132**/152	**132**/140
	39120098	D12S2520	260	254/257	251/257	**260**/257	**260**/257
	39312730	D12S1048	214	**214**	**214**/211	**214**	**214**/211

Results

Three of the four G2019S carriers of European origin shared a core haplotype of 243 kb; from D12S2514 to D12S2519 (Table 1), data indicating that these carriers in the population studied likely shared a common ancestor. Incomplete but significant allele sharing was observed far beyond the core region, extending from marker D12S2194 to D12S21592. Patient number 2, of Berberian origin, bears a shorter haplotype (at one end only).

Figure 1 summarizes data about haplotypes in the eighteen G2019S carriers subjects from North Africa. These haplotypes were not observed among 368 chromosomes from non-carriers. Table 2 indicates allele frequencies in the three control populations for the seven microsatellite markers used.

The proportions of shorter haplotypes among carriers in the three populations are: 50% (4/8) in Arabs, 60% (3/5) in Sephardi Jews and 100% (5/5) in Moroccan Berbers; this indicates that the G2019S mutation appeared earlier in Berbers. Using the shortest plausible haplotypes shared among carriers yielded an age estimate of 128 (95% CI 107-180) generations.

Figure 1. Comparisons of G2019S-containing haplotypes in Arabs (*n* = 8), Berbers (*n* =5) and Sephardi Jews (*n* = 5) subjects from North Africa. Core alleles concordant to haplotype 1 are indicated in bold, and haplotype lengths are shaded.

	(North African Arabs) patient/subject numbers:							
	Libyan 5	Tunisian 6	Tunisian 7	Algerian 8	Algerian 9	Algerian 10	Moroccan 11	Moroccan 12
D12S2194	**257**/249	253/261	261	**257**/261	253/261	**257**	**257**/253	253/261
D12S2514	**291**	**291**	**291**/297	**291**	**291**	**291**	**291**	**291**
rs28903073	**A**/G	**A**	**A**/G	**A**	**A**	**A**/G	**A**	**A**
D12S2516	**254**	**254**	**254**	**254**	**254**	**254**	**254**/253	**254**
D12S2518	**154**	**154**	**154**	**154**	**154**	**154**	**154**	**154**
D12S2519	**132**/138	**132**	**132**	**132**/138	**132**	**132**	**132**	**132**
D12S2520	**260**	**260**/254	**260**	**260**	**260**/257	**260**	**260**	**260**
D12S1048	**214**	**214**	**214**	**214/**211	**214/**217	**214**	**214/**226	**214/**217

	(Moroccan Berbers) patient/subject numbers:				
	Moroccan 13	Moroccan 14	Moroccan 15	Moroccan 16	Moroccan 17
D12S2194	265	265	255/261	253/261	**257**/253
D12S2514	**291**/285	**291**	**291**/285	**291**/297	**291**/294
rs28903073	**A**	**A**	**A**	**A**/G	**A**
D12S2516	**254**	**254**/252	**254**	**254**	**254**/253
D12S2518	**154**/158	**154**	**154**	**154**/168	**154**
D12S2519	**132**	**132**	134/140	**132**	134/140
D12S2520	254/257	251/257	**260**/251	**257**	**260**
D12S1048	**214**/217	**214**/223	**214**/226	**214**/220	**214**/226

	(Sephardi Jews) patient/subject numbers:				
	Moroccan 18	Moroccan 19	Moroccan 20	Moroccan 21	Tunisian (Djerba) 22
D12S2194	**257**/249	**257**/261	253/261	**257**/253	261/263
D12S2514	**291**/297	**291**/294	**291**	**291**	**291**
rs28903073	**A**	**A**	**A**/G	**A**	**A**
D12S2516	**254**	**254**/252	**254**/252	**254**	**254**/252
D12S2518	**154**	**154**/168	**154**	**154**	**154**
D12S2519	**132**/138	**132**	**132**/134	**132**	134/140
D12S2520	**260**/254	257	**260**/257	**260**	**260**/257
D12S1048	**214**	**214**/223	**214**	**214**/217	**214**/211

Table 2. Allele frequencies for seven microsatellite markers in the three control populations (for each population, major allele frequencies are bolded).

Microsatellites/ alleles	Moroccans n = 49	Moroccan Berbers n = 78	Moroccan Jews n = 57
D13S2194			
249	12 (0.24)	31 (0.39)	13 (0.23)
253	12 (0.24)	24 (0.31)	18 (0.32)
255	9 (0.18)	1 (0.01)	4 (0.07)
257	6 (0.13)	13 (0.17)	11 (0.19)
261	6 (0.13)	6 (0.08)	4 (0.07)
263	0	1 (0.01)	2 (0.03)
265	4 (0.08)	2 (0.03)	5 (0.09)
D12S2514			
285	14 (0.29)	15 (0.19)	9 (0.16)
291	21 (0.43)	34 (0.43)	25 (0.44)
294	10 (0.20)	21 (0.27)	22 (0.39)
297	4 (0.08)	2 (0.01)	0
300	0	6 (0.08)	1 (0.01)
D12S2516			
252	20 (0.41)	29 (0.37)	18 (0.32)
253	0	1 (0.01)	0
254	29 (0.59)	48 (0.62)	39 (0.68)
D12S2518			
154	24 (0.49)	44 (0.56)	49 (0.86)
168	23 (0.47)	30 (0.38)	8 (0.14)
170	2 (0.04)	4 (0.06)	0
D12S2519			
132	2 (0.04)	19 (0.24)	19 (0.33)
134	12 (0.24)	16 (0.21)	11 (0.20)
138	15 (0.31)	14 (0.18)	15 (0.26)
140	17 (0.35)	29 (0.37)	12 (0.21)
152	3 (0.06)	0	0
D12S2520			
251	9 (0.19)	10 (0.13)	7 (0.12)
254	7 (0.14)	11 (0.14)	1 (0.02)
257	26 (0.53)	37 (0.47)	39 (0.68)
260	7 (0.14)	20 (0.26)	10 (0.18)
D12S1048			
211	23 (0.47)	31 (0.39)	33 (0.58)
214	18 (0.37)	24 (0.31)	5 (0.09)
217	2 (0.04)	9 (0.12)	13 (0.23)
220	1 (0.02)	0	0
223	1 (0.02)	0	0
226	4 (0.08)	14 (0.18)	6 (0.10)

Discussion

Most of the *LRRK2* G2019S in our study shared a core haplotype 243 kb in length. This same haplotype, named "haplotype 1" (Zabetian et al., 2006a), has been observed in the vast majority of G2019S carriers of European or Middle- Eastern-North African origins (Kachergus et al., 2005; Lesage et al., 2005; Ozelius et al., 2006; Zabetian et al., 2006a; Warren et al., 2008).

An original result, already obtained in our previous work (Chang eet al., 2008), was the constatation that the proportions of shorter haplotypes found in G2019S carriers North African subjects were more important in Moroccan Berbers than in other North African carriers. Using shortest plausible haplotypes, we estimate that Berber carriers with haplotype 1 share a common ancestor who lived 128 generations ago (95% CI 107-180). If a generation is defined as 25 years (Fenner, 2005), this corresponds to 3,200 (95% CI 2,675-4,500) years ; if a 30-years interval is a better approximation of generation time (Tremblay and Vézina, 2000), this most recent ancestor lived 3,840 (95% CI 3,210 – 5,400) years ago.

Three previous studies calculated the age of the most recent common founder for G2019S carriers with haplotype 1 (Lesage et al., 2005; Zabetian et al., 2006a; Warren et al., 2008), and the estimates ranged from 29 to 104 generations; the study most similar in design to ours is that of Warren and colleagues (2008), which reported an age estimate of 2,600 (95% CI 1,950-3,850) or 3,120 (95% CI 2,340-4,650) years, based on data from Tunisian and US families. Variation between studies was likely due to difference in the origin of the study populations, analytical methods used and distribution, numbers and nature of the markers included (Table 3).

While microsatellite are more informative, on average, SNPs have much lower on less variable mutation rates; thus, shared haplotypes within one genic region based only on microsatellites might be expected to be shorter than those based on SNPs alone. Use of the shortest plausible haplotypes among individuals to calculate the number of generations produced generally a substantially older estimate of the number of years from the common founder.

The frequency of *LRRK2* G2019S in patients with idiopathic Parkinson's disease is highest in North Africa (Lesage et al., 2006; Ishihara et al., 2007) and in Western Europe (Bras et al., 2005; Infante et al., 2006), 4-6% in Portugal and Spain. The first

North African Arab-Berber study (Warren et al., 2008) estimated that the common founder lived 2,600 years ago, and an older age of 3,200 years ago is found in the present study in Berbers. In contrast, Farrer et al. (2008) hypothesized that *LRRK2* G2019S might be of Phoenician origin –rather than Arabic-Berber- dating from the founding of the ancient city of Carthage (in 814 B.C.).

G2019S has been observed on two additional background haplotypes, one in European-Americans and one in Japanese (Zabetian et al., 2006a,b), other than the one studied here. This suggests that G2019S has arisen independently, on at least three separate occasions, during history.

It is now established that the frequency of the G2019S mutation in PD patients depends mainly on their geographic origin: it accounts for 2-3% of sporadic PD in Europe, with a North-South gradient of distribution (Guedes et al., 2010; Diéterlen and Lucotte, 2010). Very rare in East Asia, it accounts for about 15% in Ashkenazi Jews (Ozélius et al., 2006) and for 4% in North African Arabs (Lesage et al., 2006; Change et al., 2008; Benamer and De Silva, 2010). The marked ethic differences in risk conferred by the G2019S mutation might result from the frequency of G2019S, or (/and) from different ethnic-related genetic backgrounds. Recently (Bar-Shira et al., 2009), using 15 flanking microsatellites and 1 SNP, it was estimated that Ashkenazi Jews carriers with haplotype 1 shared a common ancestor that corresponds (generations being defined as 30 years) to 1, 830 (95% CI 1,560-2,160) years ago, an estimation very similar to our own.

Table 3. Origins of the populations studied, numbers of markers (SNPs and microsatellites) used, and ages of the most recent common founder estimates in the four studies.

	Lesage et al (2005)	**Zabetan et al. (2006)**	**Warren et al. (2008)**	**Present study**
Population origins	French and North Africa	Europe and Jews	USA and Tunisia	Europe, Arabs, Berbers and Sephardi Jews
Marker numbers:				
SNPs (S) microsatellites (M)	4 S 12 M	12 S 13 M	39 S 2 M	1 S 7 M
Generation times / age estimates	25 years: 725 (375-1,375)	25 years: 1,875 (1,375-2,600) 30 years: 2,250 (1,650-3,120)	25 years: 2,600 (1,950-3,850) 30 years: 3,120 (2,340-4,620)	25 years: 3,200 (2,675-4,500) 30 years: 3,840 (3,210-5,400)

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