Published in final edited form as:
Neurogenetics. 2009 October ; 10(4): 347. doi:10.1007/s10048-009-0187-z.

Lrrk2 R1441G-related Parkinson's disease: evidence of a common founding event in the seventh century in Northern Spain

Ignacio F. Mata,
Geriatric Research Education and Clinical Center S-182, Veterans Affairs Puget Sound Health Care System, 1660 South Columbian Way, Seattle, WA 98108, USA

Department of Neurology, University of Washington, Seattle, WA, USA

Carolyn M. Hutter,
Department of Epidemiology, University of Washington, Seattle, WA, USA

María C. González-Fernández,
Servicio General de Investigación Genómica: Banco de ADN, Universidad del País Vasco, Vitoria-Gasteiz, Spain

Marian M. de Pancorbo,
Servicio General de Investigación Genómica: Banco de ADN, Universidad del País Vasco, Vitoria-Gasteiz, Spain

Elena Lezcano,
Unidad de trastornos del movimiento, Hospital de Cruces, Baracaldo, Spain

Cecilia Huerta,
Genética Molecular-Instituto de Investigacion Nefrológica, Hospital Universitario Central de Asturias, Oviedo, Spain

Marta Blazquez,
Servicio de Neurología, Hospital Universitario Central de Asturias, Oviedo, Spain

Renee Ribacoba,
Servicio de Neurología, Hospital Alvarez-Buylla, Mieres, Spain

Luis M. Guisasola,
Servicio de Neurología, Hospital Universitario Central de Asturias, Oviedo, Spain

Carlos Salvador,
Servicio de Neurología, Hospital Universitario Central de Asturias, Oviedo, Spain

Juan C. Gómez-Esteban,
Unidad de trastornos del movimiento, Hospital de Cruces, Baracaldo, Spain

Juan J. Zarranz,
Unidad de trastornos del movimiento, Hospital de Cruces, Baracaldo, Spain

Jon Infante,
Servicio de Neurología, Hospital Universitario “Marqués de Valdecilla”, Universidad de Cantabria, Santander, Spain

Joseph Jankovic,

Correspondence to: Cyrus P. Zabetian.

Ignacio F. Mata and Carolyn M. Hutter contributed equally to this work

Department of Neurology, Baylor College of Medicine, Houston, TX, USA

Hao Deng,
Department of Neurology, Baylor College of Medicine, Houston, TX, USA

Center for Experimental Medicine, The Third Xiangya Hospital, Central South University, Changsha, China

Karen L. Edwards,
Department of Epidemiology, University of Washington, Seattle, WA, USA

Victoria Alvarez, and
Genética Molecular-Instituto de Investigacion Nefrológica, Hospital Universitario Central de Asturias, Oviedo, Spain

Cyrus P. Zabetian
Geriatric Research Education and Clinical Center S-182, Veterans Affairs Puget Sound Health Care System, 1660 South Columbian Way, Seattle, WA 98108, USA zabetian@u.washington.edu

Department of Neurology, University of Washington, Seattle, WA, USA

Abstract

Mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene together represent the most common genetic determinant of Parkinson's disease (PD) identified to date. The vast majority of patients with *LRRK2*-related PD reported in the literature carry one of three pathogenic substitutions: G2019S, R1441C, or R1441G. While G2019S and R1441C are geographically widespread, R1441G is most prevalent in the Basque Country and is rare outside of Northern Spain. We sought to better understand the processes that have shaped the current distribution of R1441G. We performed a haplotype analysis of 29 unrelated PD patients heterozygous for R1441G and 85 wild-type controls using 20 markers that spanned 15.1 Mb across the *LRRK2* region. Nine of the patients were of Basque origin and 20 were non-Basques. We inferred haplotypes using a Bayesian approach and utilized a maximum-likelihood method to estimate the age of the most recent common ancestor. Significant but incomplete allele sharing was observed over a distance of 6.0 Mb and a single, rare ten-marker haplotype 5.8 Mb in length was seen in all mutation carriers. We estimate that the most recent common ancestor lived 1,350 (95% CI, 1,020–1,740) years ago in approximately the seventh century. We hypothesize that R1441G originated in the Basque population and that dispersion of the mutation then occurred through short-range gene flow that was largely limited to nearby regions in Spain.

Keywords

Parkinson disease; *LRRK2*; R1441G; Founder effect; Basques

Introduction

Parkinson's disease (PD; MIM 168600) is the second most common neurodegenerative disorder and affects approximately 1–2% of the population over 60 years of age. It is characterized clinically by bradykinesia, resting tremor, rigidity, and postural instability, and pathologically by loss of dopamine neurons in the substantia nigra and Lewy body formation [1–3]. Only one in five PD patients report a family history of the disease and in most instances, PD is thought to result from a complex interaction between genetic and environmental factors [4,5]. However, studies of rare multigenerational pedigrees in which PD segregates in a Mendelian pattern have yielded five “causal” genes: *PARK2* (MIM 600116 and 602544), *PINK1* (MIM 605909), *PARK7* (MIM 606324), *SNCA* (MIM 163890), and *LRRK2* (MIM

609007). Of these five genes, mutations in *LRRK2* are the most prevalent in PD patients of European origin [6].

The majority of patients with *LRRK2*-related PD reported in the literature carry pathogenic variants within one of two mutational hotspots: codon 1441 in exon 31 (R1441C/G/H), and codon 2019 in exon 41 (G2019S). R1441C, R1441H, and G2019S have each arisen from at least three separate founding events and are widely geographically distributed [7–10]. All three mutations occur in Asians and in multiple European subpopulations. In contrast, R1441G is largely limited to Northern Spain. Originally, discovered in four families in the Basque Country [11], R1441G is found in approximately 20% of Basque patients with familial PD [12]. It was also identified at lower frequencies in patients from nearby provinces in Spain who did not report Basque ancestry [13,14]. Previous work suggests that these patients might share the same background haplotype, but interpretation of these data is limited by a lack of overlap in the markers analyzed across studies [11,12,14,15]. In this study, we sought to further explore whether PD patients who carry R1441G share a common founder, and if so to estimate the age of the founding event.

Materials and methods

Study participants

The study population was comprised of 29 unrelated PD patients who carried R1441G (Table 1), nine relatives of the patients, and 85 healthy mutation-negative controls from Northern Spain. Limited haplotype data on 14 of these patients have been published elsewhere [12,14]. Twenty-eight of the patients were recruited from hospitals in three neighboring regions of Northern Spain (Asturias, n=15; Cantabria, n=1; Basque Country, n=12) and one (a Hispanic patient who did not report Basque ancestry) was ascertained from a movement disorder clinic in North America [16]. All patients met UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria for PD [17]. The nine relatives came from three families (PJ68, FAM6, and FAM8); eight were affected and one was unaffected.

In Spain, an individual carries both of their parent's surnames throughout life and their name does not change with marriage. Thus, a great deal of information about recent ancestry can be gained by simply examining an individual's name. PD patients were classified as “Basque” if they had one or two Basque surnames and “non-Basque” if neither of their surnames was of Basque origin. Among the 29 PD patients included in the study, nine were categorized as Basque, and of these, four (FAM1, FAM3, FAM5, and FAM9) possessed two Basque surnames.

The controls were derived from two sources. Forty-two of the controls were autochthonous individuals from the Basque Country whose parents each had two Basque surnames (referred to hereafter as “Basque Controls”; mean age, 36.1±10.8 years; age range, 28–73 years; male, 47.6%). The remaining controls (n=43) were blood donors at one of two hospitals in Asturias, Spain (mean age, 40.9±11.5 years; age range, 20–62 years; male, 71.4%). Ancestral classification by surname was not possible for these individuals because data were collected anonymously.

The study was approved by the local ethics authorities at each institution and written informed consent was obtained from all participants.

Marker selection and genotyping

We selected a total of 20 markers (15 microsatellites and five single nucleotide polymorphisms [SNPs]) spanning a distance of 15.1 Mb across the *LRRK2* region for genotyping in all study participants. We began with a set of 15 markers which have been used in several previous

haplotype analyses of *LRRK2* [11,12,14,15]. We then added two microsatellites (D12S345 and D12S1713) and a SNP (rs1511547) chosen from the MAP-O-MAT database (http://compgen.rutgers.edu/mapomat/) to fill large gaps between markers. In genotyping rs1511547 (by sequencing) we identified two novel SNPs (rs55917927 and rs56260627) for which the minor allele was rare (frequency <13%) among controls but present in all patients carrying R1441G. These two potentially informative SNPs were also added to the marker set.

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 Analyzer and GeneMapper 4.0 software (Applied Biosystems). Centre d'Etude du Polymorphisme Humain (CEPH) samples 1331-01, 1331-02, 1347-13, 1362-14, 1413-18, and 1416-12 (Coriell Cell Repositories, Camden NJ, USA) were used as a reference for microsatellite allele size determination. All PCR primer sequences and assay conditions are available on request.

Data analysis

We used PHASE software v2.1.1 (http://www.stat.washington.edu/stephens/software.html) to infer haplotypes when marker phase could not be resolved by pedigree data [18]. We estimated the age of the most recent common ancestor for individuals who carried R1441G using the program Estiage [19]. This maximum-likelihood algorithm uses information on the recombination fractions between the mutation and each marker, the frequencies of the shared allele at 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) elapsed since the most recent common ancestor introduced the mutation into the population. We defined a marker as shared if a single allele was included in all disease haplotypes or in at least half and at significantly greater frequency (Fisher's exact test) than in 170 inferred control haplotypes (collapsing all other alleles into a single bin). Genetic map positions for each marker were derived from the linkage-mapping server MAP-O-MAT, and physical positions were taken from the National Center for Biotechnology Information (NCBI) human genome assembly Build 36 [20].

Results

All 29 R1441G carriers shared a ten-marker background haplotype, bounded by D12S2080 and D12S2519, which spanned a distance of 5.8 Mb across the *LRRK2* region (Fig. 1). The haplotype included alleles at two markers, rs55917927 and rs34591826 (M1646T), that were present at a frequency of only 3.0% and 0.6%, respectively, in control subjects. This haplotype was not observed among 170 control chromosomes. This strongly suggests that the mutation carriers in our study population originated from a single founder.

The telomeric boundary of the haplotype shared by all mutation carriers was delimited by a single subject (PE139). In this individual, both alleles at D12S2520 and D12S2521 were divergent from the ones shared in all other carriers (Fig. 1). This suggests a previous recombination event between D12S2519 and D12S2520 for the disease chromosome carried by PE139, rather than recurrent mutation at two consecutive markers or an error in assigning phase. Among the disease chromosomes analyzed in the study, incomplete but significant allele sharing was seen across an interval of 6.0 Mb, from D12S2080 to D12S1048 (Fig. 1).

Because many of the markers in our dataset were tightly linked, genetic distances between them could not be accurately estimated from available genetic maps. Instead, we used the genetic length and physical distance between D12S2080 and D12S1301 to calculate an average of 0.30 cM/Mb across the entire region and then computed recombination fractions using the Kosambi mapping function as previously described [9]. We then estimated the age of the most

recent common ancestor, designating D12S345 and D12S1301 as the first unshared markers in each direction. This yielded an age estimate of 45 (95% CI, 34–58) generations. Using the most widely published intergenerational interval, 25 years [21], our data indicate that the patients in our study shared a common ancestor 1,125 (95% CI, 850–1,450) years ago. However, several recent studies have concluded that 30 years is a better approximation of generation time for the period of human history in question [21–23]. Substituting a 30-year interval increases the age of the founding event to 1,350 (95% CI, 1,020–1,740) years ago. Using only Basque controls in the analysis yielded a very similar age estimate of 48 (95% CI, 37–63) generations. Excluding the subject (PE139) with the shortest shared haplotype also had little effect on the estimated age (45 generations; 95% CI, 35–58).

Discussion

R1441G displays a frequency gradient which peaks in the Basque Country (Fig. 2) and it appears to be very rare outside of Northern Spain. Among several thousand PD patients from elsewhere in Europe and North America who have been screened for R1441G, only one (patient F67) has been found to carry the mutation [13,16,24–28]. Based on this pattern of distribution and the results of the present study, we hypothesize that R1441G originated in the Basque population and that a founding event occurred in approximately the seventh century. Dispersion of the mutation then occurred through short-range gene flow, largely limited to nearby regions. This scenario is consistent with current knowledge on the origin and history of the Basque population. The Basques are unusual among modern inhabitants of Europe in that they are thought to constitute a “relic” population descended from Paleolithic Europeans and have remained relatively genetically isolated [29,30]. This view is supported by archaeological findings, linguistic studies, and analyses of both classic genetic and molecular markers [31–33]. However, examination of Y-chromosome markers also indicates that modest levels of gene flow have occurred between the Basques and neighboring populations (e.g. Catalans) over the past two millennia [34]. In addition, the distribution of mutations for other diseases that are believed to have originated among the Basques is similar to that seen for R1441G. This includes the *CAPN3* 2362AG→TCATCT mutation for limb-girdle muscular dystrophy type 2A (estimated to have arisen in the sixth to eighth century) [35] and *PRNP* D178N which results in fatal familial insomnia [36].

In contrast to R1441G, the *LRRK2* G2019S mutation has been frequently observed across Europe, the Middle East, North Africa, and the Americas [12,13,37–43]. Most G2019S carriers share a common ancestor who is estimated to have lived approximately 2,250 years ago and likely originated in the Middle East [9]. While the spread of R1441G was probably slowed by the geographic and cultural boundaries that surround the Basque region, G2019S became widely dispersed, perhaps as a result of the large-scale migrations of the Jewish Diaspora.

Paisan-Ruiz and colleagues performed a haplotype analysis of the *LRRK2* region in four extended Basque PD pedigrees with R1441G using a large set of SNP and microsatellite markers [11]. The authors later genotyped a subset of these markers (11 SNPs) that spanned 2.0 Mb across the region in 17 singleton PD cases (16 Basque and one non-Basque) who carried the mutation [15]. In both studies, they concluded that all mutation carriers shared a common founder, but did not calculate an age for the founding event. In the four Basque families and in the singleton group, complete allele sharing was observed over distances of 1.0 Mb and 170 kb, respectively. In our sample of 29 carriers, we observed complete allele sharing over a much larger distance of 5.8 Mb. Though the authors did not formally test for significant but incomplete allele sharing, at a marker (rs10876410) 1.3 Mb upstream from the mutation, only nine of the 17 singleton cases shared alleles. In contrast, in our sample we observed complete allele sharing at rs10876410 and at four markers further upstream, including D12S2080 located 5.7 Mb away from R1441G (Fig. 1). The shorter haplotypes observed by Paisan-Ruiz and

colleagues imply that the founding event for R1441G might be substantially older than the estimate derived from our dataset. The reasons for the differences between our data and theirs are not entirely clear, and direct comparisons between datasets are difficult because only one of the markers (rs10876410) overlapped between studies. One possible reason is the nature of the markers used. While microsatellites are on average more informative, SNPs have much lower and less variable mutation rates [44]. Thus, within the same region, shared haplotypes based solely on microsatellites might be expected to be shorter than those based on SNPs alone. However, large differences in haplotype size remain even if one considers only the SNP markers in each dataset. Another consideration is whether the subjects in one or more studies might have actually arisen from multiple founders. In our sample, we believe that this is highly unlikely because all mutation carriers were heterozygous for rs34591826 (located 9.7 kb downstream of R1441G) and the minor allele frequency for this SNP was only 0.6% in controls (Fig. 1). The minor allele of rs34591826 was also included within the disease haplotype in the four large Basque families [11]. However, in the 17 singleton PD cases rs34591826 was not genotyped and the four SNPs (rs4768224, rs12423567, rs1820544, and rs10784616) that constituted the core haplotype shared by all subjects in that study were less informative [15]. The shared alleles for these four SNPs had frequencies ranging from 0.28 to 0.70 in the HapMap CEU sample (http://www.hapmap.org). Thus, there is somewhat less certainty that all of the individuals in this singleton PD sample did indeed descend from a common ancestor, particularly because the analysis was conducted with phase-unknown data and statistical methods were not used to infer phase. Finally, another possibility to explain the differences between our data and those of Paisan-Ruiz and colleagues is genotyping error.

Our study also had some limitations. Because DNA from family members was available for only three mutation carriers, we used Bayesian methods to reconstruct haplotypes in most instances. This has the potential to introduce additional uncertainty into our estimates of the age of the founding event which is not taken into account with the maximum-likelihood methods we used. Also, the use of surnames to categorize mutation carriers as Basque or non-Basque is subject to potential misclassification and it is possible that the ancestors of some subjects came from both groups.

Our data provide further empirical evidence that the Basques have remained genetically isolated over the past one to two millennia. Our findings also lend further support to the idea that important genetic determinants for PD can be highly population-specific. Finally, there is little information available on the prevalence of R1441G in Central and South America where the majority of intercontinental Basque migration has occurred. We are addressing this issue in ongoing studies of the *LRRK2* gene in PD cohorts from across these regions.

Acknowledgments

We dedicate this paper to our dear colleague, Dr. Luis M. Guisasola, a superb clinician, researcher, and educator, who recently passed away. We thank the individuals who participated in the study. This work was supported by the Basque Government and University of the Basque Country (grant S-PE07UN44, M.M.P. and M.C.G-F.); the NIH (NINDS, K08 NS044138, C.P.Z.); the Department of Veterans Affairs (Merit Review Award, C.P.Z.); the Parkinson's Disease Foundation (Fellowship Award, I.F.M.); the Spanish Fondo de Investigacion Sanitaria (grant FIS PI070014, J.I; grant 05/008, V.A.); and the Veterans Integrated Service Network 20 Geriatric, Mental Illness, and Parkinson's Disease Research, Education, and Clinical Centers.

References

1. Samii A, Nutt JG, Ransom BR. Parkinson's disease. Lancet 2004;363:1783–1793. doi:10.1016/S0140-6736(04)16305-8. [PubMed: 15172778]
2. Przedborski S, Tieu K, Perier C, Vila M. MPTP as a mitochondrial neurotoxic model of Parkinson's disease. J Bioenerg Biomembr 2004;36:375–379. doi:10.1023/B:JOBB.0000041771.66775.d5. [PubMed: 15377875]

3. Jankovic J. Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 2008;79:368–376. doi:10.1136/jnnp.2007.131045. [PubMed: 18344392]
4. Payami H, Larsen K, Bernard S, Nutt J. Increased risk of Parkinson's disease in parents and siblings of patients. Ann Neurol 1994;36:659–661. doi:10.1002/ana.410360417. [PubMed: 7605419]
5. Bonifati V, Fabrizio E, Vanacore N, De Mari M, Meco G. Familial Parkinson's disease: a clinical genetic analysis. Can J Neurol Sci 1995;22:272–279. [PubMed: 8599769]
6. Farrer MJ. Genetics of Parkinson disease: paradigm shifts and future prospects. Nat Rev Genet 2006;7:306–318. doi:10.1038/nrg1831. [PubMed: 16543934]
7. Haugarvoll K, Rademakers R, Kachergus JM, Nuytemans K, Ross OA, Gibson JM, Tan EK, Gaig C, Tolosa E, Goldwurm S, Guidi M, Riboldazzi G, Brown L, Walter U, Benecke R, Berg D, Gasser T, Theuns J, Pals P, Cras P, De Deyn PP, Engelborghs S, Pickut B, Uitti RJ, Foroud T, Nichols WC, Hagenah J, Klein C, Samii A, Zabetian CP, Bonifati V, Van Broeckhoven C, Farrer MJ, Wszolek ZK. Lrrk2 R1441C parkinsonism is clinically similar to sporadic Parkinson disease. Neurology 2008;70:1456–1460. doi:10.1212/01.wnl.0000304044.22253.03. [PubMed: 18337586]
8. Ross OA, Spanaki C, Griffith A, Lin CH, Kachergus J, Haugarvoll K, Latsoudis H, Plaitakis A, Ferreira JJ, Sampaio C, Bonifati V, Wu RM, Zabetian CP, Farrer MJ. Haplotype analysis of Lrrk2 R1441H carriers with parkinsonism. Parkinsonism Relat Disord. 2008 doi:10.1016/j.parkreldis.2008.09.001.
9. Zabetian CP, Hutter CM, Yearout D, Lopez AN, Factor SA, Griffith A, Leis BC, Bird TD, Nutt JG, Higgins DS, Roberts JW, Kay DM, Edwards KL, Samii A, Payami H. LRRK2 G2019S in families with Parkinson Disease who originated from Europe and the Middle East: evidence of two distinct founding events beginning two Millennia ago. Am J Hum Genet 2006;79:752–758. doi: 10.1086/508025. [PubMed: 16960813]
10. Zabetian CP, Morino H, Ujike H, Yamamoto M, Oda M, Maruyama H, Izumi Y, Kaji R, Griffith A, Leis BC, Roberts JW, Yearout D, Samii A, Kawakami H. Identification and haplotype analysis of LRRK2 G2019S in Japanese patients with Parkinson disease. Neurology 2006;67:697–99. doi: 10.1212/01.wnl.0000227732.37801.d4. [PubMed: 16728648]
11. Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, de Munain AL, 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. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 2004;44:595–600. doi:10.1016/j.neuron. 2004.10.023. [PubMed: 15541308]
12. Gonzalez-Fernandez MC, Lezcano E, Ross OA, Gomez-Esteban JC, Gomez-Busto F, Velasco F, Alvarez-Alvarez M, Rodriguez-Martinez MB, Ciordia R, Zarranz JJ, Farrer MJ, Mata IF, de Pancorbo MM. Lrrk2-associated parkinsonism is a major cause of disease in Northern Spain. Parkinsonism Relat Disord 2007;13:509–515. doi:10.1016/j.parkreldis.2007.04.003. [PubMed: 17540608]
13. Gaig C, Ezquerra M, Marti MJ, Munoz E, Valldeoriola F, Tolosa E. LRRK2 mutations in Spanish patients with Parkinson disease: frequency, clinical features, and incomplete penetrance. Arch Neurol 2006;63:377–382. doi:10.1001/archneur.63.3.377. [PubMed: 16533964]
14. Mata IF, Taylor JP, Kachergus J, Hulihan M, Huerta C, Lahoz C, Blazquez M, Guisasola LM, Salvador C, Ribacoba R, Martinez C, Farrer M, Alvarez V. LRRK2 R1441G in Spanish patients with Parkinson's disease. Neurosci Lett 2005;382:309–311. doi:10.1016/j.neulet.2005.03.033. [PubMed: 15925109]
15. Simon-Sanchez J, Marti-Masso JF, Sanchez-Mut JV, Paisan-Ruiz C, Martinez-Gil A, Ruiz-Martinez J, Saenz A, Singleton AB, Lopez de Munain A, Perez-Tur J. Parkinson's disease due to the R1441G mutation in Dardarin: a founder effect in the Basques. Mov Disord 2006;21:1954–1959. doi:10.1002/ mds.21114. [PubMed: 16991141]
16. Deng H, Le W, Guo Y, Hunter CB, Xie W, Huang M, Jankovic J. Genetic analysis of LRRK2 mutations in patients with Parkinson disease. J Neurol Sci 2006;251:102–106. doi:10.1016/j.jns. 2006.09.017. [PubMed: 17097110]
17. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51:745–752. doi:10.1136/jnnp.51.6.745. [PubMed: 2841426]
18. Stephens M, Scheet P. Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. Am J Hum Genet 2005;76:449–462. doi:10.1086/428594. [PubMed: 15700229]

19. Genin E, Tullio-Pelet A, Begeot F, Lyonnet S, Abel L. Estimating the age of rare disease mutations: the example of Triple-A syndrome. J Med Genet 2004;41:445–449. doi:10.1136/jmg.2003.017962. [PubMed: 15173230]
20. Kong X, Matise TC. MAP-O-MAT: internet-based linkage mapping. Bioinformatics 2005;21:557–559. doi:10.1093/bioinformatics/bti024. [PubMed: 15374870]
21. Fenner JN. Cross-cultural estimation of the human generation interval for use in genetics-based population divergence studies. Am J Phys Anthropol 2005;128:415–423. doi:10.1002/ajpa.20188. [PubMed: 15795887]
22. Sigurgardottir S, Helgason A, Gulcher JR, Stefansson K, Donnelly P. The mutation rate in the human mtDNA control region. Am J Hum Genet 2000;66:1599–1609. doi:10.1086/302902. [PubMed: 10756141]
23. Tremblay M, Vezina H. New estimates of intergenerational time intervals for the calculation of age and origins of mutations. Am J Hum Genet 2000;66:651–658. doi:10.1086/302770. [PubMed: 10677323]
24. Farrer M, Stone J, Mata IF, Lincoln S, Kachergus J, Hulihan M, Strain KJ, Maraganore DM. LRRK2 mutations in Parkinson disease. Neurology 2005;65:738–740. doi:10.1212/01.WNL.0000169023.51764.b0. [PubMed: 16157908]
25. Goldwurm S, Di Fonzo A, Simons EJ, Rohe CF, Zini M, Canesi M, Tesei S, Zecchinelli A, Antonini A, Mariani C, Meucci N, Sacilotto G, Sironi F, Salani G, Ferreira J, Chien HF, Fabrizio E, Vanacore N, Dalla Libera A, Stocchi F, Diroma C, Lamberti P, Sampaio C, Meco G, Barbosa E, Bertoli-Avella AM, Breedveld GJ, Oostra BA, Pezzoli G, Bonifati V. 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 2005;42:e65. doi:10.1136/jmg.2005.035568. [PubMed: 16272257]
26. Nichols WC, Elsaesser VE, Pankratz N, Pauciulo MW, Marek DK, Halter CA, Rudolph A, Shults CW, Foroud T. LRRK2 mutation analysis in Parkinson disease families with evidence of linkage to PARK8. Neurology 2007;69:1737–1744. doi:10.1212/01.wnl.0000278115.50741.4e. [PubMed: 17804834]
27. Berg D, Schweitzer K, Leitner P, Zimprich A, Lichtner P, Belcredi P, Brussel T, Schulte C, Maass S, Nagele T. Type and frequency of mutations in the LRRK2 gene in familial and sporadic Parkinson's disease*. Brain 2005;128:3000–3011. [PubMed: 16251215]
28. Zabetian CP, Samii A, Mosley AD, Roberts JW, Leis BC, Yearout D, Raskind WH, Griffith A. A clinic-based study of the LRRK2 gene in Parkinson disease yields new mutations. Neurology 2005;65:741–744. doi:10.1212/01.WNL.0000172630.22804.73. [PubMed: 16157909]
29. Cavalli-Sforza LL, Piazza A. Human genomic diversity in Europe: a summary of recent research and prospects for the future. Eur J Hum Genet 1993;1:3–18. [PubMed: 7520820]
30. Gonzalez AM, Garcia O, Larruga JM, Cabrera VM. The mitochondrial lineage U8a reveals a Paleolithic settlement in the Basque country. BMC Genomics 2006;7:124. doi:10.1186/1471-2164-7-124. [PubMed: 16719915]
31. Alfonso-Sanchez MA, Cardoso S, Martinez-Bouzas C, Pena JA, Herrera RJ, Castro A, Fernandez-Fernandez I, De Pancorbo MM. Mitochondrial DNA haplogroup diversity in Basques: a reassessment based on HVI and HVII polymorphisms. Am J Hum Biol 2008;20:154–164. doi:10.1002/ajhb.20706. [PubMed: 18172868]
32. de Pancorbo MM, Lopez-Martinez M, Martinez-Bouzas C, Castro A, Fernandez-Fernandez I, de Mayolo GA, de Mayolo AA, de Mayolo PA, Rowold DJ, Herrera RJ. The Basques according to polymorphic Alu insertions. Hum Genet 2001;109:224–233. doi:10.1007/s004390100544. [PubMed: 11511929]
33. Perez-Miranda AM, Alfonso-Sanchez MA, Kalantar A, Pena JA, Pancorbo MM, Herrera RJ. Allelic frequencies of 13 STR loci in autochthonous Basques from the province of Vizcaya (Spain). Forensic Sci Int 2005;152:259–262. doi:10.1016/j.forsciint.2004.09.118. [PubMed: 15978353]
34. Hurles ME, Veitia R, Arroyo E, Armenteros M, Bertranpetit J, Perez-Lezaun A, Bosch E, Shlumukova M, Cambon-Thomsen A, McElreavey K, Lopez De Munain A, Rohl A, Wilson IJ, Singh L, Pandya A, Santos FR, Tyler-Smith C, Jobling MA. Recent male-mediated gene flow over a linguistic barrier in Iberia, suggested by analysis of a Y-chromosomal DNA polymorphism. Am J Hum Genet 1999;65:1437–1448. doi:10.1086/302617. [PubMed: 10521311]

35. Cobo AM, Saenz A, Poza JJ, Urtasun M, Indakoetxea B, Urtizberea JA, Lopez de Munain A, Calafell F. A common haplotype associated with the Basque 2362AG –> TCATCT mutation in the muscular calpain-3 gene. Hum Biol 2004;76:731–741. doi:10.1353/hub.2005.0002. [PubMed: 15757244]

36. Rodriguez-Martinez AB, Alfonso-Sanchez MA, Pena JA, Sanchez-Valle R, Zerr I, Capellari S, Calero M, Zarranz JJ, de Pancorbo MM. Molecular evidence of founder effects of fatal familial insomnia through SNP haplotypes around the D178N mutation. Neurogenetics 2008;9:109–118. doi:110.1007/s10048-10008-10120-x. [PubMed: 18347820]

37. Kay DM, Zabetian CP, Factor SA, Nutt JG, Samii A, Griffith A, Bird TD, Kramer P, Higgins DS, Payami H. Parkinson's disease and LRRK2: frequency of a common mutation in U.S. movement disorder clinics. Mov Disord 2006;21:519–523. doi:10.1002/mds.20751. [PubMed: 16250030]

38. 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. Identification of a novel LRRK2 mutation linked to autosomal dominant parkinsonism: evidence of a common founder across European populations. Am J Hum Genet 2005;76:672–680. doi:10.1086/429256. [PubMed: 15726496]

39. Ozelius LJ, Senthil G, Saunders-Pullman R, Ohmann E, Deligtisch A, Tagliati M, Hunt AL, Klein C, Henick B, Hailpern SM, Lipton RB, Soto-Valencia J, Risch N, Bressman SB. LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. N Engl J Med 2006;354:424–425. doi:10.1056/NEJMc055509. [PubMed: 16436782]

40. Orr-Urtreger A, Shifrin C, Rozovski U, Rosner S, Bercovich D, Gurevich T, Yagev-More H, Bar-Shira A, Giladi N. The LRRK2 G2019S mutation in Ashkenazi Jews with Parkinson disease: is there a gender effect. Neurology 2007;69:1595–1602. doi:10.1212/01.wnl.0000277637.33328.d8. [PubMed: 17938369]

41. Lesage S, Durr A, Tazir M, Lohmann E, Leutenegger AL, Janin S, Pollak P, Brice A. LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. N Engl J Med 2006;354:422–423. doi:10.1056/NEJMc055540. [PubMed: 16436781]

42. 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. A common LRRK2 mutation in idiopathic Parkinson's disease. Lancet 2005;365:415–416. [PubMed: 15680457]

43. 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. Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. Lancet 2005;365:410–412. [PubMed: 15680455]

44. Tishkoff SA, Verrelli BC. Patterns of human genetic diversity: implications for human evolutionary history and disease. Annu Rev Genomics Hum Genet 2003;4:293–340. doi:10.1146/annurev.genom.4.070802.110226. [PubMed: 14527305]

Position	Marker	F67	PE8	PE73	PE139	PE155	PE230	PE362	PE420	PE526	PJ50	PJ68	PJ76	PJ89	PJ99	PJ124
31075611	D12S1648	**106**/128	**114**/110	**114**/122	**134**/126	**114**/136	**114**/134	**110**/114	**114**/134	**110**/114	**110**/114	**114**	**114**/130	**114**/114	**106**/124	**110**/114
32216079	D12S345	**218**/214	**214**/232	**212**/214	**224**/224	**214**/224	**214**/210	**214**/214	**210**/214	**210**/214	**214**/224	**214**/224	**224**/230	**214**/214	**218**/230	**214**/236
33305760	D12S2080	**188**/188	**188**/196	**188**/196	**188**/188	**188**/188	**188**/188	**188**/188	**188**/196	**188**/188	**188**/188	**188**	**188**/188	**188**/188	**188**/192	**188**/192
34142287	rs1511547	**T**/C	**T**/C	**T**/C	**T**/T	**T**/T	**T**/C	**T**/C	**T**/T	**T**/C	**T**/C	**T**/C	**T**/C	**T**/C	**T**/C	**T**/C
34142413	rs55917927	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T
34142467	rs56260627	**A**/G	**A**/G	**A**/G	**A**/G	**A**/A	**A**/G	**A**/G	**A**/G	**A**/G	**A**/G	**A**/G	**A**/G	**A**/G	**A**/G	**A**/G
37708307	rs10876410	**T**/A	**T**/A	**T**/T	**T**/T	**T**/T	**T**/A	**T**/A	**T**/A	**T**/T	**T**/T	**T**	**T**/A	**T**/T	**T**/A	**T**/A
38738007	D12S2194	**249**/253	**249**/249	**249**/249	**249**/249	**249**/253	**249**/253	**249**/249	**249**/257	**249**/257	**249**/253	**249**	**249**/253	**249**/261	**249**/249	**249**/265
38989339	D12S2516	**252**/252	**252**/253	**252**/254	**252**/252	**252**/252	**252**/253	**252**/254	**252**/254	**252**/252	**252**/252	**252**/244	**252**/252	**252**/254	**252**/252	**252**/252
38990396	R1441G	**G**/C	**G**/C	**G**/C	**G**/C	**G**/C	**G**/C	**G**/C	**G**/C	**G**/C	**G**/C	**G**	**G**/C	**G**/C	**G**/C	**G**/C
39000059	rs34591826	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**	**C**/T	**C**/T	**C**/T	**C**/T
39034922	D12S2518	**154**/154	**154**/170	**154**/154	**154**/168	**154**/154	**154**/170	**154**/154	**154**/154	**154**/168	**154**/168	**154**	**154**/168	**154**/154	**154**/168	**154**/154
39116885	D12S2519	**138**/140	**138**/132	**138**/132	**138**/134	**138**/140	**138**/134	**138**/132	**138**/138	**138**/132	**138**/138	**138**	**138**/138	**138**/134	**138**/138	**138**/140
39120098	D12S2520	**248**/260	**248**/257	**248**/260	**254**/257	**248**/260	**248**/260	**248**/260	**248**/257	**248**/260	**248**/248	**248**	**248**/248	**248**/251	**248**/248	**248**/257
39128754	D12S2521	**323**/319	**323**/363	**323**/363	**319**/367	**323**/319	**323**/367	**323**/371	**323**/315	**323**/359	**323**/331	**323**	**323**/227	**323**/359	**323**/331	**323**/319
39132380	D12S2522	**281**/283	**281**/297	**281**/297	**281**/297	**281**/283	**281**/301	**281**/297	**281**/295	**281**/297	**281**/281	**281**	**281**/281	**281**/301	**281**/281	**281**/283
39176380	D12S2517	**184**/206	**184**/196	**184**/188	**204**/186	**184**/188	**184**/198	**184**/186	**184**/180	**184**/190	**184**/192	**184**	**184**/188	**184**/200	**184**/192	**184**/190
39312730	D12S1048	**211**/217	**211**/211	**211**/223	**217**/226	**211**/211	**211**/223	**211**/214	**211**/214	**211**/214	**211**/211	**211**	**226**/211	**211**/223	**211**/211	**226**/211
42348911	D12S1301	**100**/100	**108**/100	**108**/116	**120**/100	**108**/104	**112**/100	**120**/112	**100**/108	**108**/100	**108**/116	**100**	**120**/108	**108**/116	**108**/112	**120**/100
44845572	D12S1713	**183**/169	**187**/179	**175**/169	**175**/175	**175**/175	**175**/179	**179**/175	**183**/175	**175**/175	**185**/179	**175**	**183**/185	**175**/175	**175**/183	**179**/175
46208211	D12S1701	**95**/91	**99**/101	**99**/93	**101**/95	**101**/99	**91**/99	**99**/101	**95**/95	**99**/97	**101**/95	**95**	**101**/95	**99**/93	**99**/95	**101**/101
		Non-Basques														

Position	Marker	PJ141	St1	FAM2	FAM4	FAM12	FAM1	FAM3	FAM5	FAM6	FAM7	FAM8	FAM9	FAM10	FAM11	% Controls
31075611	D12S1648	**106**/128	**106**/126	**110**/110	**122**/122	**106**/110	**134**/122	**106**/128	**106**/134	**110**	**130**/108	**106**	**122**/130	**106**/122	**122**/114	
32216079	D12S345	**218**/240	**214**/218	**210**/210	**210**/218	**218**/224	**224**/234	**218**/214	**212**/218	**218**	**218**/210	**218**	**218**/210	**210**/218	**210**/218	
33305760	D12S2080	**188**/196	**188**/196	**188**/188	**188**/188	**188**/172	**188**/184	**188**/180	**188**/172	**188**	**188**/188	**188**	**188**/184	**188**/172	**188**/192	**47.1**
34142287	rs1511547	**T**/T	**T**/C	**T**/C	**T**/T	**T**/T	**T**/C	**T**/C	**T**/T	**T**/C	**T**/C	**T**	**T**/T	**T**/T	**T**/C	**18.1**
34142413	rs55917927	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**3.0**
34142467	rs56260627	**A**/A	**A**/G	**A**/G	**A**/G	**A**/A	**A**/G	**A**/G	**A**/A	**A**/G	**A**/G	**A**/G	**A**/A	**A**/A	**A**/G	**12.7**
37708307	rs10876410	**T**/T	**T**/T	**T**/T	**T**/A	**T**/A	**T**/A	**T**/T	**T**/T	**T**	**T**/T	**T**	**T**/T	**T**/T	**T**/A	**57.8**
38738007	D12S2194	**249**/249	**249**/257	**249**/249	**249**/257	**249**/249	**249**/261	**249**/253	**249**/249	**249**	**249**/253	**249**	**249**/245	**249**/257	**249**/261	**32.5**
38989339	D12S2516	**252**/254	**252**/254	**252**/254	**252**/252	**252**/254	**252**/254	**252**/254	**252**/254	**252**	**252**/252	**252**	**252**/252	**252**/254	**252**/254	**32.9**
38990396	R1441G	**G**/C	**G**/C	**G**/C	**G**/C	**G**/C	**G**/C	**G**/C	**G**/C	**G**	**G**/C	**G**	**G**/C	**G**/C	**G**/C	
39000059	rs34591826	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**/T	**C**	**C**/T	**C**	**C**/T	**C**/T	**C**/T	**0.6**
39034922	D12S2518	**154**/154	**154**/154	**154**/154	**154**/154	**154**/154	**154**/154	**154**/154	**154**/154	**154**	**154**/154	**154**	**154**/154	**154**/154	**154**/154	**86.5**
39116885	D12S2519	**138**/132	**138**/132	**138**/134	**138**/134	**138**/138	**138**/136	**138**/132	**138**/132	**138**	**138**/140	**138**	**138**/132	**138**/132	**138**/132	**28.8**
39120098	D12S2520	**248**/257	**248**/257	**248**/254	**248**/254	**248**/260	**248**/257	**248**/257	**248**/260	**248**	**248**/260	**248**/260	**248**/257	**248**/257	**248**/257	**10.0**
39128754	D12S2521	**323**/371	**323**/363	**323**/379	**323**/375	**323**/315	**323**/315	**323**/367	**323**/371	**323**/319	**323**/359	**323**	**323**/371	**323**/363	**323**/359	**4.1**
39132380	D12S2522	**281**/297	**281**/297	**281**/297	**281**/301	**281**/295	**281**/295	**281**/297	**281**/297	**281**	**281**/297	**281**	**281**/297	**281**/297	**281**/297	**10.7**
39176380	D12S2517	**184**/186	**184**/182	**184**/188	**184**/186	**184**/180	**184**/180	**184**/182	**184**/184	**184**	**184**/186	**184**	**184**/184	**184**/188	**184**/182	**11.3**
39312730	D12S1048	**211**/211	**211**/211	**226**/211	**211**/214	**211**/214	**211**/214	**211**/211	**211**/211	**211**/217	**211**/217	**211**/217	**211**/211	**211**/214	**211**/214	**40.0**
42348911	D12S1301	**108**/100	**108**/108	**120**/100	**112**/112	**108**/112	**108**/116	**116**/116	**112**/104	**108**	**108**/104	**108**	**108**/112	**112**/120	**108**/108	
44845572	D12S1713	**175**/179	**175**/175	**187**/175	**175**/179	**175**/175	**175**/175	**175**/175	**175**/179	**175**	**175**/175	**175**	**175**/175	**175**/179	**187**/175	
46208211	D12S1701	**95**/99	**95**/95	**95**/97	**95**/97	**95**/101	**99**/95	**97**/95	**95**/95	**99/95**	**95**/99	**95**	**99**/101	**95**/97	**99**/89	
		Non-Basques					**Basques**									

Fig. 1.

Genotypes for 20 markers surrounding the *LRRK2* R1441G mutation. A single ten-marker haplotype (indicated by *dark gray shading*) was shared by all 29 mutation carriers. Incomplete but significant allele sharing is denoted by *light gray shading* and the frequency of each shared allele in 170 control chromosomes is displayedinthe *lower right-hand margin.* In instances in which phase could not be unambiguously determined, both alleles are shown. Disease haplotypes inferred using PHASE are indicated in *bold* type

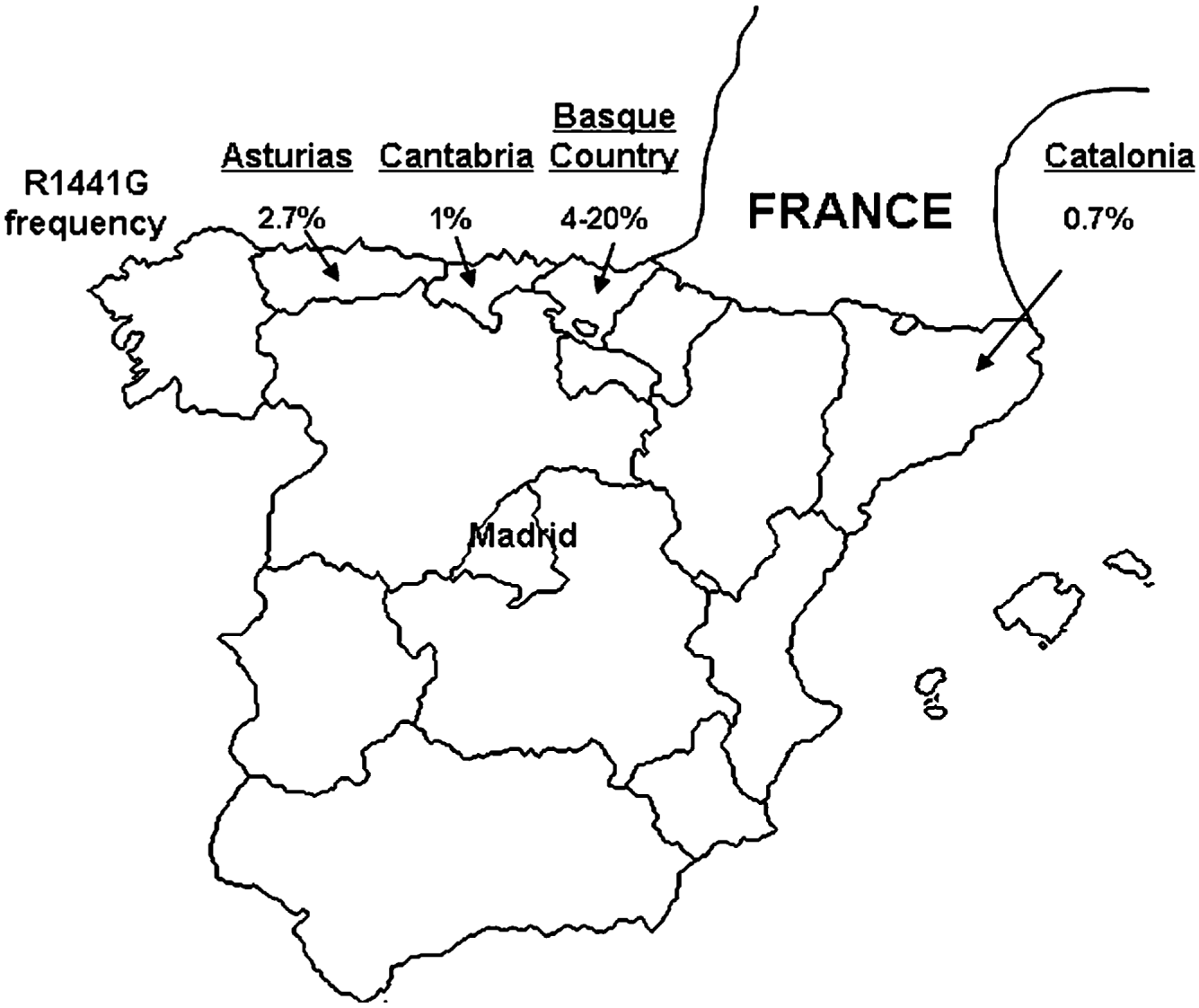

Fig. 2.
R1441G distribution in Spain. Mutation carrier frequencies based on previous studies [12–14] are shown for four different regions in Northern Spain

Table 1

Characteristics of R1441G carriers

No. of subjects	**29**
Age (mean±SD)	62.4±11.3
Age at onset (mean±SD)	52.0±13.0
Disease duration (mean±SD)	11.8±7.1
Family history of PD (%)	65.5
Male, *n* (%)	15 (51.7)