

Clinical and Genetic Description of a Family With a High Prevalence of Autosomal Dominant Restless Legs Syndrome

JESSICA E. YOUNG, BSc; CARLES VILARIÑO-GÜELL, PhD; SIONG-CHI LIN, MD; ZBIGNIEW K. WSZOLEK, MD;
AND MATTHEW J. FARRER, PhD

OBJECTIVE: To conduct clinical and molecular genetic analyses of the members of an extended family in Central Indiana with a high prevalence of restless legs syndrome (RLS).

PARTICIPANTS AND METHODS: From February 1, 2006, through August 31, 2008, we collected data from members of this family, which is of English descent. Genealogical methods were used to expand the family tree, and family members were screened with an RLS questionnaire. Telephone interviews and personal examinations were performed at Mayo Clinic and during a field trip to Central Indiana. Blood samples were collected for molecular genetic analysis. A follow-up telephone interview was conducted 1 year later.

RESULTS: The family tree spans 7 generations with 88 living members, 30 of whom meet the criteria for diagnosis of RLS established by the International Restless Legs Syndrome Study Group. Three affected family members also have Parkinson disease or essential tremor. The mode of RLS inheritance is compatible with an autosomal dominant pattern. The affected family members do not exhibit linkage to the 5 known RLS loci or mutations in the RLS susceptibility genes *MEIS1* and *BTBD9*.

CONCLUSION: Of 88 members of this single extended family in Central Indiana, 30 were diagnosed as having RLS. Because our analysis shows that the disease is not linked to any of the known RLS loci or risk-associated genes, we postulate that members of this family may carry a gene mutation in a novel genetic locus.

Mayo Clin Proc. 2009;84(2):134-138

BTBD9 = BTB (POZ) domain containing 9; **LOD** = logarithm of the odds; **MEIS1** = Meis homeobox 1; **PD** = Parkinson disease; **PLM** = periodic leg movement; **RLS** = restless legs syndrome

Restless legs syndrome (RLS) is a neurologic disorder of combined sensory and motor dysfunction first described by Ekbom¹ in 1945. The prevalence of idiopathic RLS is estimated to be 1.2% to 15.0%.^{2,3} All studies report an increase in prevalence with age, and some suggest that RLS may occur more frequently in women.⁴ Common treatments include levodopa, dopamine agonists, opiates, benzodiazepines, anticonvulsants, and iron supplements.⁵ Symptoms can appear intermittently and later become persistent. Secondary forms of RLS have been described and are associated with other conditions, including pregnancy, iron deficiency, peripheral neuropathies, diabetes, or chronic renal failure.³

The clinical diagnosis of RLS is made on the basis of 4 essential criteria: (1) an urge to move the legs that is usually accompanied by uncomfortable and unpleasant sensations in the legs, (2) a worsening of the urge to move or the

unpleasant sensations during periods of rest or inactivity such as lying down or sitting, (3) partial or total relief of the symptoms by movements such as walking or stretching, and (4) occurrence or worsening of the unpleasant sensations in the evening or at night. A family history of RLS and response to dopaminergic agents are additional features that support a diagnosis of RLS. Most patients also present with periodic leg movements (PLMs) during sleep and quiet wakefulness, resulting in difficulty falling or staying asleep.⁶

Familial aggregation of RLS has been recognized since Ekbom¹ formally described the condition in 1945. Studies in twins have consistently shown that RLS has a genetic etiology^{7,8} and suggest that the condition might best be explained by an oligogenic, multifactorial model, with 1 or more genes of major effect.³ To date, 5 loci for RLS have been reported (Table 1). These include 1 autosomal recessive locus in a French-Canadian pedigree that was mapped to chromosome 12q12-21 (RLS1)⁹ and 4 autosomal dominant loci: 1 in a North Italian pedigree (RLS2: 14q13-21),¹⁰ 1 in 2 families from the United States (RLS3: 9p24-22),¹¹ 1 in a population isolate from South Tyrol (RLS4: 2q33),¹² and 1 in a large French-Canadian pedigree (RLS5: 20p13).¹³

We describe a large kindred (Figure) with autosomal dominant RLS and report the results of the genetic analysis of the 5 previously known RLS loci and other previously identified susceptibility genes.

PARTICIPANTS AND METHODS

Our RLS proband was identified in 2005. Because this person reported an extensive family history of RLS, we

From the Department of Neurology (J.E.Y., Z.K.W.), Department of Neuroscience (C.V.-G., M.J.F.), and Department of Psychiatry and Psychology (S.-C.L.), Mayo Clinic, Jacksonville, FL.

Ms Young and Drs Lin and Wszolek received support from The Mayo Foundation Research Committee (CR program). Drs Wszolek and Farrer received support from the Morris K. Udall National Institutes of Health/National Institute of Neurological Disorders and Stroke Parkinson Disease Center of Excellence grant awarded to Mayo Clinic's site in Jacksonville, FL (P05 NS40256) and the Pacific Alzheimer Research Foundation Centre grant (C06-01).

Individual reprints of this article are not available. Address correspondence to Zbigniew K. Wszolek, MD, Department of Neurology, Mayo Clinic, 4500 San Pablo Rd, Jacksonville, FL 32224 (wszolek.zbigniew@mayo.edu).

© 2009 Mayo Foundation for Medical Education and Research

TABLE 1. Clinical and Molecular Genetic Data on Large Families With RLS and Population Isolates^a

Ancestry	No. of families (No. of members)	No. of generations	No. of affected	Age at onset (y)	Age ranges (y)	Additional diagnosis	Locus
French-Canadian	1 (25)	3	14	NR	NR	No	12q12-21 (RLS 1)
North Italian	1 (30)	3	15	26	18-42	1 Guillain-Barré, 1 migraine	14q13-21 (RLS 2)
United States	2 (23)	3, 4	15	NR	NR	1 Neuropathy	9p24-22 (RLS 3)
South Tyrolean	Population isolate (530)	12	47	32	NA	NR	2q33 (RLS 4)
French-Canadian	1 (59)	NA	20 (17 relatives)	30	NA	NR	20p13 (RLS 5)
English (our family)	1 (88)	7	30	32	5-65	2 PD, 1 ET	NR

^a ET = essential tremor; NA = not available; NR = not reported; PD = Parkinson disease; RLS = restless legs syndrome.

performed a genealogical investigation to expand the family tree. After receiving approval for the study from the Mayo Clinic Institutional Review Board, we performed genealogical, clinical, and molecular investigations. The study was conducted from February 1, 2006, through August 31, 2008.

GENEALOGICAL STUDIES

Historical material and family and medical records were collected, and interviews with family members were conducted.

CLINICAL STUDIES

The study was designed in 4 phases. In the first phase, family members were mailed an approved, customized RLS questionnaire and the International Restless Legs Syndrome Study Group rating scale. The RLS question-

naire included information regarding patient demographics, sleep onset and duration, RLS diagnostic criteria, PLM, age at RLS onset and disease duration, past RLS diagnosis, RLS treatment and its efficacy, other sleep disorders, past sleep studies, secondary RLS conditions, current medications, and family history of RLS or neurodegenerative disease. In the second phase, family members who completed an RLS questionnaire were interviewed by telephone (by S.-C.L.) to substantiate the RLS diagnosis. In the third phase, the investigators traveled to Central Indiana to examine an available subset of family members. During the fourth phase, all affected persons were contacted by telephone 1 year later for follow-up questioning. During this interview, the affected patients were asked questions outlined in the RLS questionnaire to reconfirm the diagnosis of RLS. Blood samples were collected from 39 family members during phases 1, 2, and 3.

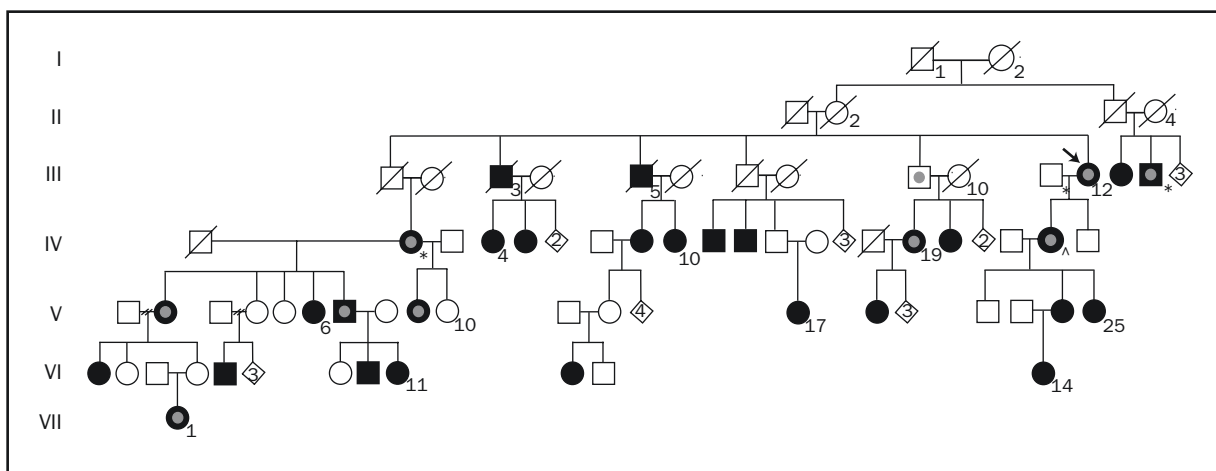


FIGURE. Abbreviated pedigree of the Central Indiana restless legs syndrome family. Square = male. Circle = female. Diamonds with numbers inside indicate a number of descendants regardless of sex. A diagonal line through a square or circle indicates a deceased person. An arrow indicates proband. Phenotypes of affected individuals: * = Parkinson disease; \wedge = essential tremor. Fully darkened symbols indicate restless legs syndrome; darkened symbols outlined with inner light gray circle indicate restless legs syndrome and periodic leg movement while awake; and symbol with inner gray only indicates periodic movement while awake.

MOLECULAR GENETIC INVESTIGATIONS

Genomic DNA was extracted from peripheral blood lymphocytes using standard protocols. To interrogate the segregation of the chromosomal regions linked to RLS, simple tandem repeat, microsatellite markers providing coverage at approximately 2.2-cM resolution were genotyped in a subset of samples collected in phases 1 and 2 and large enough to discriminate linkage to known RLS loci (estimated maximum logarithm of the odds [LOD] score = 2.26). This subset consisted of 9 affected persons (III-12, IV-2, IV-5, IV-9-12, IV-19, V-17), 2 unaffected persons (III-9, IV-13) and 1 person who had married into the family (IV-14). Fluorescent-labeled forward primers flanked informative dinucleotide, trinucleotide, or tetranucleotide repeats with heterozygosity greater than 0.75. Polymerase chain reactions included 20 pmol of each primer, 15 ng of DNA template, 5 mM of nucleoside triphosphates (deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate and deoxythymidine triphosphate), 1x Qiagen buffer (Qiagen, Valencia, CA), 1x Q solution, and 0.02 units Taq polymerase (Qiagen) per reaction. Thermocycling was performed on a Hybaid MultiBlock Satellite Thermal Cycler System (Thermo Fisher Scientific, Waltham, MA) with a 1-cycle denaturation step at 93°C for 2 minutes, followed by 35 cycles at 93°C for 20 seconds, touchdown from 57°C to 52°C from cycles 3 through 15 (30 seconds each) and 72°C (45 seconds), with a final elongation step at 72°C for 10 minutes. Single tandem repeat microsatellites were then paneled into groups of products having compatible allele sizes and fluorescent dyes and were analyzed on an Applied Biosystems 3730 capillary array system using GeneMapper Software 4.0 (Applied Biosystems, Foster City, CA). The DNA genotyping standards CEPH 1331-01 and 1331-02 (for more information on genotyping standards, see <http://www.cephb.fr/>) were genotyped as size controls and included on every plate to ensure consistency on allele calls. Data from the GeneMapper files were exported for analysis.

Parametric 2-point LOD scores were calculated using MLINK from the FASTLINK package, and haplotypes were generated using SIMWALK.¹⁴⁻¹⁶ The disease was assumed to have an autosomal dominant inheritance, a gene frequency of 0.0001, and penetrance of 100%. The genetic distances of the markers were obtained from the Marshfield sex-averaged map, and allele frequencies were estimated from within the family.

Polymerase chain reaction was used to sequence BTB (POZ) domain containing 9 (*BTBD9*) and Meis homeobox 1 (*MEIS1*) exons and exon-intron boundaries in 2 affected persons (III-12 and IV-10) (primer pair sequences are available on request). These 2 persons were selected because of their affected status and genealogical distance.

Products of polymerase chain reaction were purified from unincorporated nucleotides using Solid Phase Reversible Immobilization technology (Agencourt Bioscience, Beverly, MA) with a Biomek FX Laboratory Automation Workstation (Beckman Coulter, Fullerton, CA). Sequence analysis was performed as previously described.³

RESULTS

GENEALOGICAL INVESTIGATIONS

The family is most likely of English ancestry and traceable to the early 19th century. The pedigree contains 88 persons spanning 7 generations with 30 members affected with RLS (28 alive, 2 deceased). Historical records of the 2 deceased affected family members confirmed their RLS diagnosis.

CLINICAL STUDIES

The mean \pm SD age of patients at RLS onset was 32 \pm 16.32 years (range, 5-65 years). The mean \pm SD disease duration was 18.63 \pm 16.78 years (range, 1-54 years).

The disease was present in both sexes, with a male-to-female ratio of 8:22. Male-to-male transmission was present 1 time; female-to-female, 9 times; and male-to-female, 5 times. Of the 28 living affected patients, 10 were personally examined by study investigators and the remaining 18 were contacted by telephone to confirm their self-reported RLS diagnosis. All 28 family members with RLS were contacted a second time for a 1-year follow-up interview that confirmed their diagnosis of RLS. Each affected patient who was either personally examined or interviewed over the telephone fulfilled the criteria for diagnosis of RLS of the International Restless Legs Syndrome Study Group. Two family members with RLS (III-14, IV-2) had Parkinson disease (PD), 1 (IV-24) had RLS and essential tremor, and 2 (V-7, VI-1) had sleep apnea. Of the affected patients, 10 reported symptoms of PLM in addition to RLS, and 1 reported PLM without RLS (III-9); 7 reported RLS symptoms affecting their upper extremities and torso (III-12, III-14, IV-2, IV-9, IV-24, V-2, and VII-1).

Six of the family members were diagnosed as having RLS alone by their primary care physician before the study was initiated and were taking RLS medication with beneficial response. Of those, 3 were treated with pramipexole, 1 with ropinirole, 1 with clonazepam, and 1 with zolpidem. Of the 2 family members previously diagnosed as having RLS and PD, 1 (IV-2) was treated with carbidopa-levodopa and pramipexole with benefit, and 1 (III-14) was treated for RLS but not for PD. The family member previously diagnosed as having RLS and essential tremor (IV-24) was treated with klonopin with benefit. Additional family members were diagnosed as having RLS after enrolling in the study.

TABLE 2. LOD Scores for Known RLS Loci^a

Locus	Marker	Position ^b (cM)	LOD score	
			Parametric	Nonparametric
RLS1	D12S1064	95.03	-∞	0.55
	D12S101	100.92	-∞	0.69
	D12S1300	104.12	-∞	0.67
	D12S346	104.65	-∞	0.67
	D12S1059	105.18	-∞	0.67
	D12S1030	109.47	-∞	0.52
	D12S353	115.18	-∞	0.49
	D12S84	117.81	0.59	0.49
	D12S811	120.70	-∞	0.47
	D12S2070	125.31	-∞	0.60
	D12S1720	133.33	-∞	0.97
	D12S1349	139.61	-∞	0.89
	RLS2	D14S70	40.11	-2.16
D14S599		40.68	-∞	0.17
D14S1014		42.81	-∞	0.12
D14S306		44.06	-3.27	0.13
D14S579		45.12	-∞	0.13
D14S583		48.32	-∞	0.25
D14S1027		49.90	-∞	0.23
D14S541		50.50	-∞	0.19
D14S976		51.09	-∞	0.20
RLS3		D9S54	6.92	-2.36
	D9S288	9.83	-∞	0.19
	D9S1810	12.78	-∞	0.18
	D9S935	15.38	-∞	0.17
	D9S286	18.06	-∞	0.07
	D9S144	21.56	-∞	0.06
	D9S269	24.07	-∞	0.04
	D9S285	29.52	-∞	0.21
	D9S162	34.42	1.04	0.49
	D9S1870	37.58	-2.20	0.64
	D9S171	42.73	-2.67	0.22
	D9S259	47.19	-∞	0.21
	D9S1853	53.60	0.57	0.22
	D9S2149	54.50	-∞	0.21
	D9S1817	59.34	-∞	0.20
RLS4	D2S311	196.85	-2.95	0.42
	D2S2392	196.86	-∞	0.42
	D2S116	198.65	-2.47	0.41
	D2S2289	199.18	-2.90	0.41
	D2S1384	200.43	-3.35	0.41
	D2S248	201.83	-∞	0.47
	D2S369	202.92	-1.14	0.47
	D2S325	204.53	-∞	0.47
	D2S2208	205.06	-1.23	0.47
	RLS5	D20S1155	0.01	-∞
D20S103		2.13	-∞	0.68
D20S117		2.83	-∞	0.68
D20S199		6.25	-∞	0.69
D20S906		7.61	-2.97	0.69
D20S57		8.81	-∞	0.92
D20S842		8.97	-∞	0.92
D20S867		10.12	-2.94	0.90
D20S889		11.20	-∞	0.90
D20S482		12.12	-∞	0.89
D20S835		15.05	-∞	0.80

^a LOD = logarithm of the odds; RLS = restless legs syndrome.

^b Genetic distances were obtained from the Marshfield sex-averaged map.

Of the family members, 7 reported having additional illnesses at the onset of their RLS: 2 reported fibromyalgia (IV-2, V-2); 1, neuropathy (III-14); 1, iron deficiency (VI-12); and 1, unspecified anemia (IV-2). Symptoms of RLS during pregnancy were reported by 2 family members (V-4, V-2). Although V-4 had no RLS symptoms after childbirth, V-2 reported that the RLS became chronic after childbirth. None of the family members reported kidney failure, low back injury, chronic heart failure, or poor leg circulation at the onset of their RLS symptoms. No autopsies were performed in deceased family members with RLS.

MOLECULAR GENETIC INVESTIGATIONS

Parametric and nonparametric analyses did not reveal any significant or suggestive LOD score for known RLS loci (Table 2). Haplotype segregation did not identify a common haplotype segregating with RLS disease. Direct sequencing of *MEIS1* and *BTBD9* did not identify any unknown variant in coding regions or within 100 bases from the exon-intron boundaries.

DISCUSSION

The Central Indiana kindred reported here comprises 88 family members spanning 7 generations. The family is white and most likely of English descent, but we were unable to confirm their genealogy. Of these 88 family members, 30 have been diagnosed as having RLS; of these, 7 have severe RLS involving the upper extremities and torso and 6 have been treated with RLS medication with positive response. The pattern of RLS disease inheritance is compatible with an autosomal dominant model. All affected family members fulfilled diagnostic criteria for RLS. Because there are no confirmed pathognomonic biomarkers for RLS, our 4-phase study design was used to substantiate the diagnosis of RLS. The family was initially screened using an RLS questionnaire. Family members were then interviewed over the telephone or personally examined and were later contacted again for a 1-year follow-up telephone interview.

Although our study design attempted to distinguish family members with primary RLS from those with secondary RLS, our study had its limitations. An inherent limitation in using the questionnaire is the potential unreliability of self-report. The diagnosis of RLS is challenging because of the lack of objective measures (ie, tests, pathology, imaging) to prove a person has RLS, the subjective nature of the RLS diagnostic criteria, the fluctuating nature of RLS onset, the subjective interpretation and reporting of RLS criteria, and the lack of pathognomonic biomarkers. To further substantiate diagnosis, polysomnography could have been included to assess sleep and motor symptoms.

Genetic analysis in this pedigree excluded all known RLS loci through linkage analysis and haplotype segregation. Therefore, a novel locus harboring a gene with mutations leading to the RLS phenotype may be present in members of this family. Data from 39 family members have been collected (22 affected with RLS). Power analysis has shown that a maximum 2-point LOD score of 5.02 may be obtained assuming a disease penetrance of 80%, a phenocopy rate of 5%, and a disease allele frequency of 0.01 (more realistic parameters than those used in most studies). Data derived from this RLS kindred are sufficiently powerful to map a novel gene for RLS.

Pathogenic and susceptibility variants for a disease have been found to be present in the same gene. For example, the leucine-rich repeat kinase 2 gene (*LRRK2*) was found to harbor pathogenic mutations resulting in PD^{17,18} and was later identified as a risk factor for that disease.^{19,20} Variants in 3 loci (*MEIS1*, *BTBD9*, and mitogen-activated protein kinase kinase 5 [*MAP2K5*]/*LBXCOR1* homolog (mouse) [*LBXCOR1*]) have been identified recently as risk factors for the development of RLS^{21,22}; however, only the results for *MEIS1* and *BTBD9* have been replicated.²³ To assess the presence of pathogenic mutations in *MEIS1* and *BTBD9*, all exon and exon-intron boundaries were sequenced, but novel variants were not identified.

CONCLUSION

In a large family with 30 members diagnosed as having RLS, linkage to all known RLS loci was excluded, and mutations in the susceptibility genes *MEIS1* and *BTBD9* were not detected. This family highlights the genetic heterogeneity of RLS and may carry a novel RLS locus.

We thank the family members, whose participation made this research project possible.

REFERENCES

1. Ekbom KA. Restless legs: a clinical study. *Acta Med Scand.* 1945; 158(suppl):1-123.
2. Allen RP, Walters AS, Montplaisir J, et al. Restless legs syndrome prevalence and impact: REST General Population Study. *Arch Intern Med.* 2005;165(11):1286-1292.

3. Mata IF, Bodkin CL, Adler CH, et al. Genetics of restless legs syndrome. *Parkinsonism Relat Disord.* 2006;12(1):1-7.
4. Winkelmann J. Genetics of restless legs syndrome. *Curr Neurol Neurosci Rep.* 2008;8(3):211-216.
5. Thorpy MJ. New paradigms in the treatment of restless legs syndrome. *Neurology.* 2005;64(12, suppl 3):S28-S33.
6. Allen RP, Picchietti D, Hening WA, Trenkwalder C, Walters AS, Montplaisir J. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology: a report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. *Sleep Med.* 2003;4(2):101-119.
7. Desai AV, Cherkas LF, Spector TD, Williams AJ. Genetic influences in self-reported symptoms of obstructive sleep apnoea and restless legs: a twin study. *Twin Res.* 2004;7(6):589-595.
8. Ondo WG, Vuong KD, Wang Q. Restless legs syndrome in monozygotic twins: clinical correlates. *Neurology.* 2000;55(9):1404-1406.
9. Desautels A, Turecki G, Montplaisir J, Sequeira A, Verner A, Rouleau GA. Identification of a major susceptibility locus for restless legs syndrome on chromosome 12q. *Am J Hum Genet.* 2001 Dec;69(6):1266-1270. Epub 2001 Nov 6.
10. Bonati MT, Ferini-Strambi L, Aridon P, Oldani A, Zucconi M, Casari G. Autosomal dominant restless legs syndrome maps on chromosome 14q. *Brain.* 2003;126(part 6):1485-1492.
11. Chen S, Ondo WG, Rao S, Li L, Chen Q, Wang Q. Genomewide linkage scan identifies a novel susceptibility locus for restless legs syndrome on chromosome 9p. *Am J Hum Genet.* 2004 May;74(5):876-885. Epub 2004 Apr 7.
12. Pichler I, Marroni F, Volpato CB, et al. Linkage analysis identifies a novel locus for restless legs syndrome on chromosome 2q in a South Tyrolean population isolate. *Am J Hum Genet.* 2006 Oct;79(4):716-723. Epub 2006 Aug 14.
13. Levchenko A, Provost S, Montplaisir JY, et al. A novel autosomal dominant restless legs syndrome locus maps to chromosome 20p13. *Neurology.* 2006;67(5):900-901.
14. Cottingham RW Jr, Idury RM, Schäffer AA. Faster sequential genetic linkage computations. *Am J Hum Genet.* 1993;53(1):252-263.
15. Schäffer AA, Gupta SK, Shriram K, Cottingham RW Jr. Avoiding recomputation in linkage analysis. *Hum Hered.* 1994;44(4):225-237.
16. Sobel E, Lange K. Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. *Am J Hum Genet.* 1996;58(6):1323-1337.
17. Paisán-Ruiz C, Jain S, Evans EW, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron.* 2004;44(4):595-600.
18. Zimprich A, Biskup S, Leitner P, et al. Mutations in *LRRK2* cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron.* 2004;44(4):601-607.
19. Di Fonzo A, Wu-Chou YH, Lu CS, et al. A common missense variant in the *LRRK2* gene, Gly2385Arg, associated with Parkinson's disease risk in Taiwan. *Neurogenetics.* 2006 Jul;7(3):133-138. Epub 2006 Apr 22.
20. Ross OA, Wu YR, Lee MC, et al. Analysis of *Lrrk2* R1628P as a risk factor for Parkinson's disease. *Ann Neurol.* 2008;64(1):88-92.
21. Stefansson H, Rye DB, Hicks A, et al. A genetic risk factor for periodic limb movements in sleep. *N Engl J Med.* 2007 Aug 16;357(7):639-647. Epub 2007 Jul 18.
22. Winkelmann J, Schormair B, Lichtner P, et al. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. *Nat Genet.* 2007 Aug;39(8):1000-1006. Epub 2007 Jul 18.
23. Vilarinho-Güell C, Farrer MJ, Lin SC. A genetic risk factor for periodic limb movements in sleep [letter]. *N Engl J Med.* 2008;358(4):425-427.