Am. J. Hum. Genet. 69:1266–1270, 2001

Identification of a Major Susceptibility Locus for Restless Legs Syndrome on Chromosome 12q

Alex Desautels,[1,2] Gustavo Turecki,[2] Jacques Montplaisir,[1] Adolfo Sequeira,[2] Andrei Verner,[3] and Guy A. Rouleau[2,4]

[1]Centre d'étude du sommeil, Hôpital du Sacré-Cœur de Montréal and Centre de recherche en sciences neurologiques, Université de Montréal, [2]Research Center, Douglas Hospital, and [3]Montreal Genome Centre and [4]Centre for Research in Neurosciences, The Montreal General Hospital, McGill University, Montréal

Restless legs syndrome (RLS) is a neurological disorder characterized by leg paresthesia associated with an irresistible urge to move that often interferes with nocturnal sleep, leading to chronic sleep deprivation. To map genes that may play a role in the vulnerability to RLS, a genomewide scan was conducted in a large French-Canadian family. Significant linkage was established on chromosome 12q, for a series of adjacent microsatellite markers with a maximum two-point LOD score of 3.42 (recombination fraction .05; $P = 6 \times 10^{-4}$; autosomal recessive mode of inheritance), whereas multipoint linkage calculations yielded a LOD score of 3.59. Haplotype analysis refined the genetic interval, positioning the RLS-predisposing gene in a 14.71-cM region between D12S1044 and D12S78. These findings represent the first mapping of a locus conferring susceptibility to RLS.

Introduction

Restless legs syndrome (RLS) is a neurological condition characterized by paresthesia of the lower limbs associated with an imperative urge to move (Montplaisir et al. 2000). These symptoms occur particularly during rest, with at least a partial and temporary relief by movement. There is a significant worsening of the symptoms in the evening or during the night, which leads to nocturnal sleep disruption, resulting in daytime somnolence. It is postulated that this periodic oscillation in symptom intensity may reflect a substantial involvement of the circadian system (Hening et al. 1999; Trenkwalder et al. 1999).

Although the exact prevalence is uncertain, RLS may afflict, with varying degrees of severity, >5% of the general population (Ekbom 1945; Lavigne and Montplaisir 1994; Phillips et al. 2000). Numerous studies have suggested a substantial genetic contribution in the etiology of the primary form of RLS. Familial aggregation has been repeatedly reported since its original clinical description (Ekbom 1944), with >40% of the idiopathic cases showing a positive family history (Walters et al. 1996; Montplaisir et al. 1997; Winkelmann et al. 2000). A recent twin study reported that ~83% of twin pairs were concordant for RLS (Ondo et al. 2000), suggesting that a significant portion of the familial aggregation may be due to genetic factors, which have been thought to be transmitted in an autosomal dominant mode of inheritance (Trenkwalder et al. 1996; Lazzarini et al. 1999; Winkelmann et al. 2001). Thus far, few molecular-genetic studies have been undertaken attempting to identify genes that may predispose to this condition (Johnson et al. 1992; Dichgans et al. 1996; Zai et al. 2000; Desautels et al. 2001), and no significant finding has been reported.

In this study, we report on the mapping of an RLS locus on the short arm of chromosome 12, in a well-documented French-Canadian family, using linkage analysis of microsatellite markers spanning the entire genome.

Subjects, Material, and Methods

Subjects

One large French-Canadian family was investigated, from which 25 individuals were sampled for linkage (fig. 2). Of these individuals, 14 were considered to be affected and 4 (II:2, II:5, II:6, and II:10) were designated as having uncertain status. The family was ascertained through the Center for Sleep Disorders in Montréal, and diagnoses were made by one experienced clinician skilled in the assessment of sleep disorders (J.M.), according to the criteria of the International Restless Legs Syndrome Study Group (Walters 1995): leg paresthesia, associated with an urge to move; motor restlessness; worsening of symptoms during rest, with partial relief by movement; and worsening of symptoms during the evening and night.

Received August 1, 2001; accepted for publication September 27, 2001; electronically published November 6, 2001.

Address for correspondence and reprints: Dr. Guy A. Rouleau, Centre for Research in Neurosciences, The Montreal General Hospital, 1650 Cedar Avenue, Montréal, Québec, H3G 1A4, Canada. E-mail: mi32@musica.mcgill.ca

0002-9297/2001/6906-0012$02.00

All subjects were interviewed personally, at two different times during a 10-year follow-up interval. Follow-up is particularly useful in evaluation of RLS, considering that disease severity may fluctuate over the patient's lifetime (Walters et al. 1996). All clinical evaluations were performed blind to the genotype status. Polysomnographic recordings were performed to document periodic leg movements in sleep in some affected subjects (III:4 and III:5) and in the proband (II:4).

None of the subjects were affected with any condition known to cause nonidiopathic RLS or with any other neurologic or sleep disorder, including sleep apnea syndrome, rapid-eye-movement sleep behavior disorders, and narcolepsy. In addition, none of the subjects reported the use of medication known to affect sleep, sensory, or motor functions. The study protocol was approved by the local institutional review board, and written informed consent was obtained from all participants.

Genotyping

Genomic DNA was isolated from peripheral lymphocytes or from transformed lymphoblastoid cell lines, following standard methods (Sambrook et al. 1989). Genotyping was conducted at the Genome Centre at Montreal General Hospital, by use of a modified Multi-Probe-I (Packard) and two ABI 377 DNA sequencers. The panel of microsatellite markers was obtained from the Whitehead Institute as a modified version of the Cooperative Human Linkage Center human screening set (Dubovsky et al. 1995) and consisted of 378 polymorphic, fluorescently labeled markers, covering the entire genome, with an average intermarker spacing of 10 cM. The marker map positions were based on the sex-averaged maps from the Marshfield Medical Research Foundation. Gels were analyzed in an automated system, and, after a visual inspection, generated files were automatically transferred to a UNIX system. Lane tracking, allele binning, and inheritance checking were performed using NEWMAT, the Bass/Grace gel-analysis system, and PEDMANAGER softwares. Allele frequencies were derived from a random sample of French Canadian subjects. Furthermore, two polymorphisms were manually genotyped to increase the marker density in the region of chromosome 12q.

Linkage Analysis

Two-point LOD score was calculated using the MLINK routine of the FASTLINK software package, version 5.1 (Lathrop et al. 1984; Cottingham et al. 1993; Schäffer 1996). Keeping the pedigree structure, simulations were performed to assess empirical *P* values by use of SIMULATE (Ott 1989). The replicates were subsequently analyzed with the MSIM program of the SLINK software (Ott 1989; Weeks et al. 1990). Since the mode of inheritance of RLS is unknown, pairwise LOD scores were maximized over three major models (table 1). In the region showing significant evidence of linkage, location-score analysis and haplotyping were computed by the SIMWALK2 program (Sobel and Lange 1996).

Results

Parametric two-point linkage analysis revealed a maximum LOD score (Z_{max}) >1.0 at 12 loci on three different chromosomes (fig. 1). All significant and suggestive results were observed under the autosomal recessive mode of inheritance, with a high disease-predisposing allele frequency. The strongest evidence of linkage was detected with eight adjacent microsatellite markers genotyped between D12S398 and D12S78 on chromosome 12q13-23. In this 44-cM region, the LOD scores were within the range from 1.71 to 3.42, with a maximum LOD score for D12S1044 ($Z_{max} = 3.42$ at recombination fraction [θ] .05; $P = 6 \times 10^{-4}$). Furthermore, suggestive evidence of linkage was established with marker GGAT1A4 on locus 10q22 ($Z_{max} = 2.17$ at $\theta = 0$; $P = 2 \times 10^{-3}$) and a region spanning 19 cM on chromosome 5q31, which showed a maximum LOD score of 1.51 with marker D5S816 ($\theta = .2$; $P = 5 \times 10^{-3}$). Given the strength of the linkage result on chromosome 12, we opted to further analyze this locus. Multipoint analysis was performed on chromosome 12 under the recessive model. These calculations provided additional support for the localization of RLS-predisposing loci to chromosome 12q, yielding a maximum multipoint LOD score of 3.59 at a position 13.93 cM centromeric from marker D12S1300.

Haplotype construction and analysis of recombination events between the RLS phenotype and the markers that span the region of interest were used to define the smallest cosegregating region (SCR) in this family (fig. 2). In this pedigree, the disease-causing gene is telomeric to D12S1044 and centromeric to D12S78, defining a 14.71-cM interval within chromosome 12q. Consistent with the autosomal recessive model of inheritance with a high carrier frequency, which reflects a high disease-allele frequency in the population, several haplotypes

Table 1

Genetic Models Used in the Parametric Analysis

Model of Inheritance	Allele Frequency		Penetrance		
	p	*q*	f_1	f_2	f_0
Dominant 1	.99	.01	.9	0	0
Dominant 2	.95	.05	.5	0	.005
Recessive	.75	.25	.005	.005	.8

NOTE.—θ between males and females was considered to be equal.

cosegregate with the affected phenotype. The most common haplotypes shared by affected subjects are 3-2-5 and 4-3-2 for markers D12S1044 through D12S78, inherited from founder I:2, as well as haplotype 3-3-2, inherited from individual I:1.

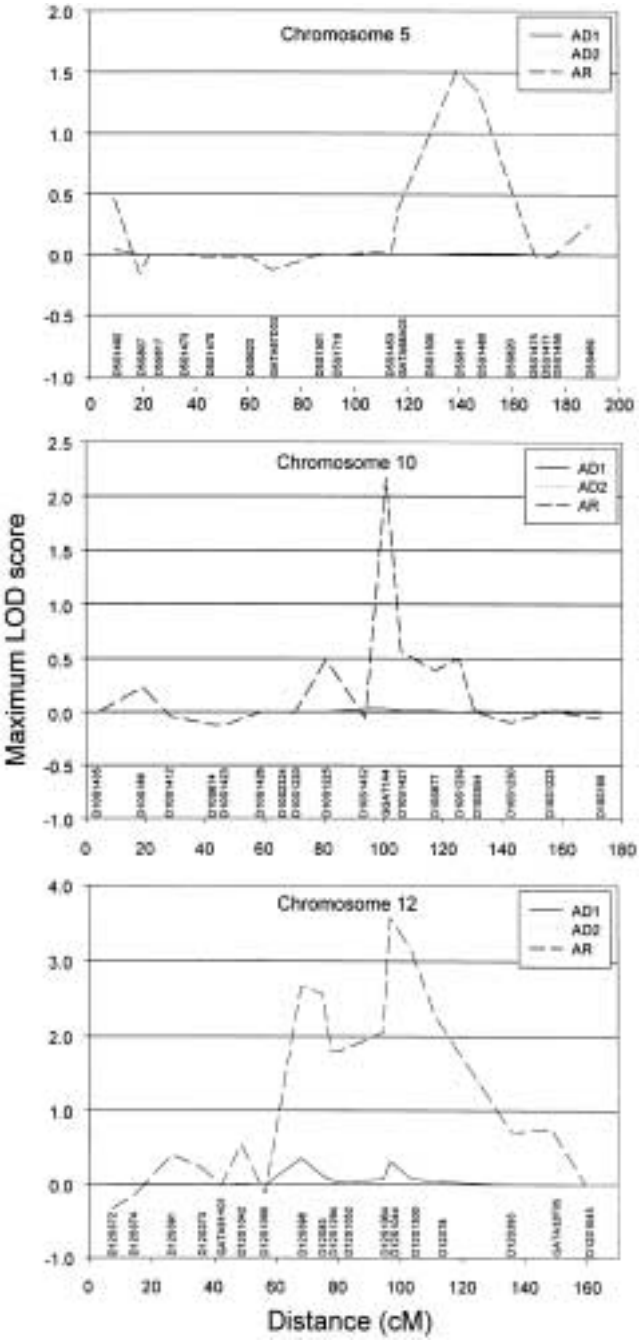

Figure 1 Maximum two-point LOD scores, plotted against genetic distance along chromosomes containing regions that provided two-point LOD scores >1.0. "AD" denotes that an autosomal dominant model was used; "AR" denotes that an autosomal recessive model was used.

Discussion

This finding represents the first identification of a major genetic locus for the RLS phenotype. By use of a genomewide approach, the mapping of a large French-Canadian kindred provided significant evidence for a susceptibility locus on chromosome 12q, supported by a multipoint LOD score of 3.59. Haplotype analysis revealed that, in this family, the disease gene is likely to be located within a 14.71-cM region between markers D12S1044 and D12S78.

Although parametric linkage analysis does not allow the determination of the true underlying mode of inheritance of RLS, the genetic parameters that provide the highest LOD score may approximate the real genetic model, given that there is linkage and the result is not spurious (Hodge and Elston 1994). If this is correct, our results suggest a pseudodominant pattern, in which the true mode of inheritance is autosomal recessive but, because of a high disease-carrier frequency, there are frequent homozygote-heterozygote matings and consequent disease diversity.

We cannot exclude the possibility that other genes may influence the occurrence of the phenotype. This seems particularly likely for RLS, considering the high prevalence rate and the substantial variability in the severity of symptoms observed in the general population. It would not be unexpected that several genetic loci, along with greater or smaller contributions from environmental factors, determine the ultimate phenotype of each gene carrier.

Several plausible candidate genes have been mapped within the region of interest (i.e., between markers D12S1044 and D12S78). Among these are the putative orthologue of the *timeless* gene (*TIM*) in *Drosophila* (Koike et al. 1998) and the gene encoding the tridecapeptide neurotensin (*NTS*) (Marondel et al. 1996). Among other things, NTS is reported to act as a neuromodulator of the dopaminergic (DA) transmission (Ervin et al. 1981; Kalivas et al. 1983), and autoradiographic analyses have shown a dense localization of NTS receptors on DA-containing neurons (Quirion et al. 1985; Szigethy and Beaudet 1989). This is particularly relevant, since several lines of evidence implicate the DA system in the pathogenesis of RLS (Montplaisir et al. 1999; Turjanski et al. 1999).

We are continuing our efforts toward the identification of RLS genes, through recruitment and analysis of new affected families, to confirm this result and to refine the genetic interval to a size more suitable for positional cloning. The identification of a susceptibility gene for RLS potentially will lead to a better clinical characterization, as well as to the development of diagnostic tests and new modalities for treatment.

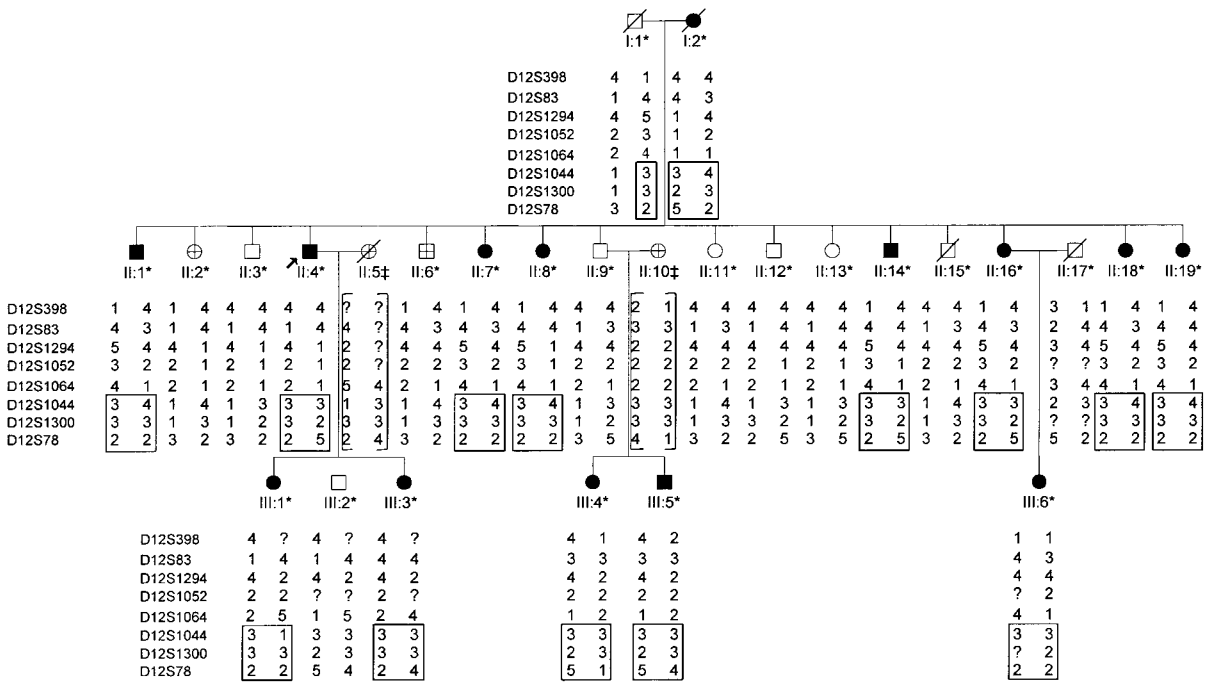

Figure 2 Pedigree drawing of the family analyzed, showing haplotypes for six markers located on chromosome 12q. The marker order, from centromere to telomere, is given to the left of each generation. Inferred haplotypes are bracketed. Individuals whose DNA sample was genotyped are indicated by asterisks (*), and unblackened symbols containing a cross (+) denote subjects of unknown disease status (e.g., II:2 and II:6). Double daggers (‡) indicate subjects who are probably affected (II:5 and II:10) but who were not clinically assessed (they were considered as having unknown phenotypes for the linkage analyses). A question mark (?) denotes unknown alleles. The haplotype segregating with the RLS phenotype is boxed.

Acknowledgments

We are very grateful to the participating family for their kindness and their precious collaboration in this study. We also thank Thomas Hudson for access to the Genome Centre facilities, Corine Darmond-Zwaig for technical assistance, and Carl Brewer for binning of raw genotypes. We furthermore acknowledge G. Poirier for his valuable help with subject recruitment. A.D. is the recipient of a Canadian Institutes of Health Research (CIHR) studentship. J.M., G.T., and G.A.R. are supported by the CIHR. This research is partly supported by the National Institutes of Health (grant 1R01NS37754-01A1) and by the CIHR (grant MGP-6472).

Electronic-Database Information

URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/
Cooperative Human Linkage Center, http://www.chlc.org/

References

Cottingham RW, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53: 252–263

Desautels A, Turecki G, Montplaisir J, Ftouhi-Paquin N, Michaud M, Chouinard VA, Rouleau GA (2001) Dopaminergic neurotransmission and restless legs syndrome: a genetic association analysis. Neurology 57:1304–1306

Dichgans M, Walther E, Collado-Seidel V, Trenkwalder C, Müller-Myhsok B, Oertel W, Gasser T (1996) Autosomal dominant restless legs syndrome: genetics model and evaluation of 22 candidate genes. Mov Disord 11:87

Dubovsky J, Sheffield VC, Duyk GM, Weber JL (1995) Sets of short tandem repeat polymorphisms for efficient linkage screening of the human genome. Hum Mol Genet 4:449–452

Ekbom KA (1944) Asthenia crurum paraesthetica ("irritable legs"). Acta Med Scand 118:197–209

Ekbom KA (1945) Restless legs syndrome. Acta Neurol Scand Suppl 158:1–122

Ervin GN, Birkemo LS, Nemeroff CB, Prange AJ Jr (1981) Neurotensin blocks certain amphetamine-induced behaviours. Nature 291:73–76

Hening WA, Walters AS, Wagner M, Rosen R, Chen V, Kim S, Shah M, Thai O (1999) Circadian rhythm of motor restlessness and sensory symptoms in the idiopathic restless legs syndrome. Sleep 22:901–912

Hodge SE, Elston RC (1994) Lods, wrods, and mods: the interpretation of lod scores calculated under different models. Genet Epidemiol 11:329–342

Johnson W, Walters A, Lehner T, Coccagna G, Ehrenberg B,

Lazzarini A, Stenross E, Lugaresi E, Hickey K, Pichietti D, Hening W, Gilliam C, Gold B, Brin M, Fazzini E, McCormack M, Fahn S, Ott J, Chokroverty S (1992) Affected only linkage analysis of autosomal dominant restless legs syndrome. Sleep Res 21:214–214

Kalivas PW, Burgess SK, Nemeroff CB, Prange AJ Jr (1983) Behavioral and neurochemical effects of neurotensin microinjection into the ventral tegmental area of the rat. Neuroscience 8:495–505

Koike N, Hida A, Numano R, Hirose M, Sakaki Y, Tei H (1998) Identification of the mammalian homologues of the *Drosophila timeless* gene, *Timeless1*. FEBS Lett 441:427–431

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Lavigne GJ, Montplaisir JY (1994) Restless legs syndrome and sleep bruxism: prevalence and association among Canadians. Sleep 17:739–743

Lazzarini A, Walters AS, Hickey K, Coccagna G, Lugaresi E, Ehrenberg BL, Picchietti DL, Brin MF, Stenroos ES, Verrico T, Johnson WG (1999) Studies of penetrance and anticipation in five autosomal-dominant restless legs syndrome pedigrees. Mov Disord 14:111–116

Marondel I, Renault B, Lieman J, Ward D, Kucherlapati R (1996) Physical mapping of the human neurotensin gene (NTS) between markers D12S1444 and D12S81 on chromosome 12q21. Genomics 38:243–245

Montplaisir J, Boucher S, Poirier G, Lavigne G, Lapierre O, Lesperance P (1997) Clinical, polysomnographic, and genetic characteristics of restless legs syndrome: a study of 133 patients diagnosed with new standard criteria. Mov Disord 12:61–65

Montplaisir J, Nicolas A, Denesle R, Gomez-Mancilla B (1999) Restless legs syndrome improved by pramipexole: a double-blind randomized trial. Neurology 52:938–943

Montplaisir J, Nicolas A, Godbout R, Walters AS (2000) Restless legs syndrome and periodic movements during sleep. In: Kryger MH, Roth T, Dement WC (eds) Principles and practice of sleep medicine. 3d edition. W. B. Saunders, Philadelphia, pp 742–752

Ondo WG, Vuong KD, Wang Q (2000) Restless legs syndrome in monozygotic twins: clinical correlates. Neurology 55: 1404–1406

Ott J (1989) Computer-simulation methods in human linkage analysis. Proc Nat Acad Sci USA 86:4175–4178

Phillips B, Young T, Finn L, Asher K, Hening WA, Purvis C (2000) Epidemiology of restless legs symptoms in adults. Arch Intern Med 160:2137–2141

Quirion R, Chiueh CC, Everist HD, Pert A (1985) Comparative localization of neurotensin receptors on nigrostriatal and mesolimbic dopaminergic terminals. Brain Res 327: 385–389

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. 2d ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Schäffer AA (1996) Faster linkage analysis computations for pedigrees with loops or unused alleles. Hum Hered 46: 226–235

Sobel E, Lange K (1996) Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. Am J Hum Genet 58:1323–1337

Szigethy E, Beaudet A (1989) Correspondence between high affinity 125I-neurotensin binding sites and dopaminergic neurons in the rat substantia nigra and ventral tegmental area: a combined radioautographic and immunohistochemical light microscopic study. J Comp Neurol 279:128–137

Trenkwalder C, Hening WA, Walters AS, Campbell SS, Rahman K, Chokroverty S (1999) Circadian rhythm of periodic limb movements and sensory symptoms of restless legs syndrome. Mov Disord 14:102–110

Trenkwalder C, Seidel VC, Gasser T, Oertel WH (1996) Clinical symptoms and possible anticipation in a large kindred of familial restless legs syndrome. Mov Disord 11:389–394

Turjanski N, Lees AJ, Brooks DJ (1999) Striatal dopaminergic function in restless legs syndrome: 18F-dopa and 11C-raclopride PET studies. Neurology 52:932–937

Walters AS (1995) Toward a better definition of the restless legs syndrome. Mov Disord 10:634–642

Walters AS, Hickey K, Maltzman J, Verrico T, Joseph D, Hening WA, Wilson V, Chokroverty S (1996) A questionnaire study of 138 patients with restless legs syndrome: the "night-walkers" survey. Neurology 46:92–95

Weeks DE, Ott J, Lathrop GM (1990) SLINK: a general simulation program for linkage analysis. Am J Hum Genet Suppl 47:A204

Winkelmann J, Wetter TC, Collado-Seidel V, Gasser T, Dichgans M, Yassouridis A, Trenkwalder C (2000) Clinical characteristics and frequency of the hereditary restless legs syndrome in a population of 300 patients. Sleep 23:597–602

Winkelmann J, Mueller-Myhsok B, Wittchen HU, Hock B, Prager M, Pfister H, Dichgans M, Gasser T, Trenkwalder C (2001) Segregation analysis of families with restless legs syndrome provides evidence for a single gene and autosomal-dominant mode of inheritance. Sleep Suppl 24:A102

Zai C, Wigg KG, Barr CL (2000) Genetics and sleep disorders. Semin Clin Neuropsychiatry 5:33–43