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

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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 $\theta = 0.05$; $P = 6 \times 10^{-4}$; 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 (Ekborg 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 (Ekborg 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.
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 M ultiprobe I (Packard) and two AB1 377 DNA sequencers. The panel of microsatellite markers was obtained from the Whitehead Institute as a modified version of the C ooperative 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 M arsonfield M edical 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 NEW- M AT, the Bass/Grace gel-analysis system, and PEDM AN- GER software. 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 M LINK 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 SIM U LATE (Ott 1989). The replicates were subsequently analyzed with the M SIM 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 SIM WALK2 program (Sobel and Lange 1996).

Results

Parametric two-point linkage analysis revealed a maximum LOD score ($Z_{\text{max}}$, > 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 ($Z_{\text{max}} = 3.42$ at recombination fraction $\theta = 0.05$; $P = 6 \times 10^{-4}$). Furthermore, suggestive evidence of linkage was established with marker GGAT1A4 on locus 10q22 ($Z_{\text{max}} = 2.17$ at $\theta = 0.05$; $P = 2 \times 10^{-3}$) and a region spanning 19 cM on chromosome 5q31, which showed a maximum LOD score of 1.51 with marker D5S816 ($\theta = 0.2$; $P = 5 \times 10^{-3}$). 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 |

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<thead>
<tr>
<th>Model of Inheritance</th>
<th>Allele Frequency</th>
<th>Penetrance</th>
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<tr>
<td></td>
<td>$p$</td>
<td>$q$</td>
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<tr>
<td>Dominant 1</td>
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<td>.01</td>
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<tr>
<td>Dominant 2</td>
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<td>.05</td>
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<tr>
<td>Recessive</td>
<td>.75</td>
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Note.—$\theta$ 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.

**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 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.
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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 (H11001) 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:


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