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Autosomal dominant restless legs syndrome maps on chromosome 14q

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Summary

Restless legs syndrome (RLS) is a common neurological disorder characterized by an irresistible desire to move the extremities associated with paraesthesia/dysaesthesia. These symptoms occur predominantly at rest and worsen at night, resulting in nocturnal insomnia and chronic sleep deprivation. In this paper, we show significant evidence of linkage to a new locus for RLS on chromosome 14q13-21 region in a 30-member, three-generation Italian family affected by RLS and periodic leg movements in sleep (PLMS). This is the second RLS locus identified so far and the first consistent with an autosomal dominant inheritance pattern. The new RLS critical region spans 9.1 cM, between markers D14S70 and D14S1068. The maximum two-point log of odds ratio score value, of 3.23 at θ = 0.0, was obtained for marker D14S288. The accurate clinical evaluation of RLS-affected, as well as unaffected, family members allowed for the configuring of RLS as a phenotypic spectrum ranging from PLMS to RLS. Motor component, both while awake and during sleep, was an important aspect of the phenotype in the family analysed. The complementary clinical and genetic studies on multiplex families are likely to be of the utmost importance in unfolding the complete expressivity of RLS phenotype spectrum.

Keywords: restless legs syndrome; linkage analysis; sleep disorders; gene identification

Abbreviations: cM = centiMorgan; EMG = electromyography; IRLS-RS = International Restless Legs Syndrome Rating Scale; LOD = log of odds ratio; Mb = megabases; NPL = non-parametric multipoint analysis; PLMS = periodic leg movements in sleep; RLS = restless legs syndrome

Introduction

Restless legs syndrome (RLS; MIM 102300) is a neurological sleep/wake disorder characterized by uncomfortable and unpleasant sensations in the legs that appear at rest, with a typical circadian pattern, which induces an irresistible urge to move the legs. RLS symptoms are usually more prominent at bedtime or after 6 p.m.; only in severely affected patients do they start earlier or occur all day long (Walters, 1995; Allen and Earley, 2001). RLS is almost always (80–90%) associated with periodic leg movements during sleep (PLMS) (Montplaisir *et al*., 1997; Picchietti *et al*., 1999). PLMS are brief (1–5 s) and repetitive (every 20–40 s); muscular jerks of the lower limbs include dorsiflexion of the foot and flexion of the leg at the knee and hip (Coleman *et al*., 1980).

The unpleasant sensations in the legs can be severe enough to interfere with the onset of sleep, while sleep disruption results from PLMS. Therefore, RLS patients usually complain of insomnia, which results mainly from difficulty in getting to sleep and in sleep maintenance. RLS is a common syndrome, the adult prevalence reported to range from 2–5% of the Caucasian population (Ekbom, 1960; Montplaisir, 1994; Strang, 1967) up to 10–15% (Lavigne and Montplaisir, 1994; Phillips *et al*., 2000; Rothdach *et al*., 2000). Higher reported prevalence seems to reflect older populations studied and, probably, the use of standard diagnostic criteria (Walters, 1995). RLS symptoms worsen with increasing age and both sexes are equally affected.

RLS familial aggregation is frequent, accounting for up to 60–65% of reported cases; inheritance follows an autosomal dominant pattern in at least one-third of familial cases (Bornstein, 1961; Ambrosetto *et al*., 1965; Montagna, 1983; Jacobsen *et al*., 1986; Trenkwalder *et al*., 1996; Montplaisir *et al*., 1997; Chokroverty and Jankovic, 1999; Lazzarini *et al*., 1999; Ondo *et al*., 2000; Walters *et al*., 1990, 1994, 1996). Some families show possible anticipation (Trenkwalder *et al*.,

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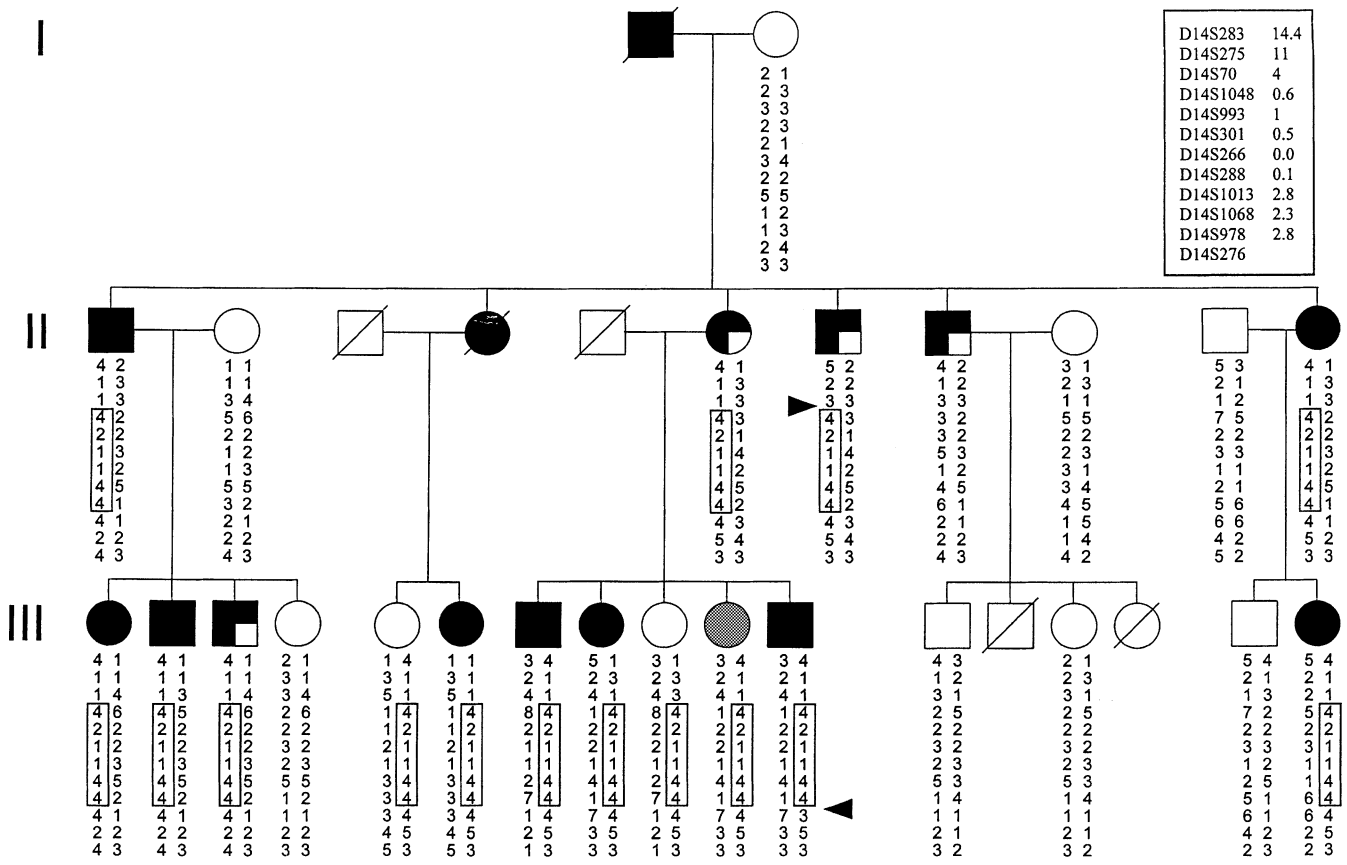

Fig. 1 Pedigree of RLS family showing 14q locus haplotypes. Marker order and distances in centiMorgans are indicated in the inset. The disease haplotype is boxed. Arrows point to recombination events defining the critical region. Filled symbols denote RLS individuals; partially filled symbols, RLS and leg cramps; grey symbols, intermediate phenotype (PLMS).

1996; Lazzarini *et al*., 1999). Up to now, only one twin study is available (Ondo *et al*., 2000), showing a high concordance rate (83.3%) between identical twins. However, age at onset, disease severity and symptom descriptions often varied within the concordant pairs. Familial and sporadic RLS cases present similar signs, symptoms and clinical course. The only differences between the two groups consist of a significantly earlier age of onset and a more frequent worsening during pregnancy in patients with hereditary RLS (Winkelmann *et al*., 2000).

The same RLS symptoms may originate from reduced iron stores, folate and B12 deficiency, neuropathy, renal failure, diabetes, pregnancy, carcinoma, amyloidosis, lesions of the lumbosacral plexus and possibly rheumatological diseases (secondary or symptomatic RLS forms). RLS has been reported to be associated with attention deficit, hyperactivity disorder in children (Picchietti *et al*., 1999), familial essential tremor (Larner and Allen, 1997) and spinocerebellar ataxia type 3/Machado–Joseph disease (SCA3) (van Alfen *et al*., 2001).

In this paper, a genome-wide linkage analysis performed on an Italian RLS family allowed for the identification of a new RLS locus on chromosome 14q13-21.

Subjects and methods

Subjects

A large Family with idiopathic RLS from Northern Italy was investigated. A detailed family pedigree (Fig. 1) was constructed by collecting their clinical histories and individually interviewing all putatively affected, as well as unaffected, family members and spouses. Patients who spontaneously reported RLS complaints were asked to define their sensory symptoms and to describe their motor ones. In particular, questions were asked about jerks, dyskinesias and cramps, as well as about the urge to move. Age at onset of symptoms, clinical RLS course, therapy and therapeutic results were recorded. Women were asked about symptoms during pregnancy. Congenital as well as early-onset RLS forms were assessed by asking parents about their children's daytime motor restlessness, attention deficit and oppositional behaviours (Picchietti *et al*., 1999), sleep onset and sleep maintenance problems, and repetitive leg movements in sleep (Montplaisir *et al*., 1985). For deceased subjects, affection status, including age at onset, was ascertained from histories provided by living family members.

The affection status was set according to the diagnostic clinical criteria of the International RLS Study Group

(Walters, 1995). Four minimal criteria must be present to diagnose RLS. These are: (i) an intense, irresistible urge to move the legs, usually associated with sensory complaints (paraesthesia or dysaesthesia); (ii) motor restlessness; (iii) worsening of symptoms at rest and relief with motor activation; and (iv) increased severity in the evening or at night. However, the final diagnosis was made after undertaking a physical and neurological examination, electromyography (EMG) and nerve conduction studies, polysomnographic recordings and PLMS scoring, blood chemistry including full blood count, and creatinine, urea, glucose, iron, ferritin, transferrin, magnesium, vitamin B12 and folate levels. Blood potassium and calcium were also evaluated in RLS patients complaining of leg cramps (individuals II-6, II-7, II-8 and III-3). There were 15 affected family members (13 of whom are still living) over a span of three generations. Individuals III-13 and III-15 died of hereditary lymphohistocytosis when they were 15 months and 3 years old, respectively.

A standardized International Restless Legs Syndrome Rating Scale (IRLS-RS) questionnaire (Hening, 2001; Walters *et al*., 2003) was submitted to RLS affected members at two different times during a 2-year follow-up period. The questionnaire, compiled by a physician interviewing the patient, is a 10-point scale for subjective rating of RLS symptoms severity. It covers the following topics: severity of RLS syndrome sensory and motor components; relief with movement; sleep disturbance; daytime drowsiness and tiredness; severity of RLS syndrome as a whole; frequency of RLS symptoms throughout a week; length of RLS symptoms for a whole day; quality of daytime performances; and RLS symptoms consequences on behaviour and mood. Each answer varies numerically from 0 to 4, with 0 representing the absence of a problem and 4 a very severe problem.

Polysomnography was performed in all but three (II-7, III-6 and III-8) affected subjects and in compliant healthy individuals (III-4, III-9, III-10, III-12 and III-14). PLMS were detected by EMG recordings of the tibialis anterior muscle and scored according to the American Sleep Disorder Association rules (Atlas Task Force, 1993). A PLMS index >5 is considered abnormal.

Genotyping and linkage analysis

Venous blood samples were obtained from 24 individuals (all living affected and unaffected members and four spouses). All family members signed an appropriate consent form. Genomic DNA was isolated from peripheral lymphocytes following standard methods (Sambrook, 1989). A genome-wide linkage analysis was carried out on affected family members using a set of 382 fluorescence-labelled polymorphic microsatellites (Dye Set 2; P-E ABI PRISM, Foster City, CA, USA) covering the 22 autosomes at an average reciprocal distance of ~10 cM (centiMorgans).

For linkage calculations, RLS was modelled as an autosomal dominant trait with a variable penetrance, identifying two liability classes: 0.95 penetrance for individuals with RLS symptoms (the reported value varies from 0.86 to full penetrance in most families; Trenkwalder *et al*., 1996; Lazzarini *et al*., 1999), either with or without PLMS; 0.70 penetrance for members showing PLMS without reporting RLS symptoms (Walters *et al*., 1990; Picchietti *et al*., 1999). Additional parameters were marker mutation rate, 10^{-5}, and disease allele and phenocopy rate, 10^{-3} (Winkelmann *et al*., 2002). Recombination frequencies for males and females were assumed to be equal. Alleles were considered equally frequent in the preliminary screening, whereas published allele frequencies were used for candidate loci (Dib *et al*., 1996).

Two-point and non-parametric multipoint log of odds ratio (LOD) scores were calculated by MLINK (LINKAGE software package; Lathrop *et al*., 1985) and GENEHUNTER 2.1 (Kruglyak and Lander, 1998).

Electronic database information

The accession number and URLs for data in this article are: Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for RLS; MIM 102300); Human Genome Browser UCSC, http://genome.cse.ucsc.edu (for physical mapping).

Results

Clinical and polysomnographic data

In Table 1, age at follow-up, age at RLS onset, disease duration, RLS severity assessments, clinical course of the syndrome, sleep disturbance, co-morbid conditions and polysomnographic findings of affected, as well as of unaffected, family members are shown. The presence of PLMS was also investigated in unaffected family members, since asymptomatic individuals with PLMS, who were obligate carriers of the RLS gene, have been reported (Boghen and Peyronnard, 1976; Walters *et al*., 1990; Lazzarini *et al*., 1999). On the basis of these data, we assumed individuals with PLMS to be RLS-intermediate or RLS-milder phenotype. On this assumption, the PLMS score allowed us to characterize individual III-10 as a milder phenotype. The inheritance was consistent with an autosomal dominant pattern with high and age-dependent penetrance, according to reported data (Trenkwalder *et al*., 1996; Lazzarini *et al*., 1999; Ondo *et al*., 2000; Winkelmann *et al*., 2000)

RLS sensory symptoms and motor phenomena

RLS-affected family members (Fig. 1) complained of leg paraesthesias/burning sensation, with leg restlessness beginning in the evening (I-1, II-1, II-4, II-6, II-7, II-8, II-11, III-3, III-6, III-7, III-8, III-11, III-17), or of a warm legs sensation while lying down, and relief with changing temperature (III-

Table 1 *Clinical and polysomnographic findings family A members*

Family members		RLS							Other illnesses	Polysomnography recording	PLMS† index
Pedigree reference	Age at follow-up (years)	Affection status	Age at onset (years)	Duration (years)	Course	Age at onset of chronic RLS (years)	SSS*	Insomnia			
II-1	72	Yes	27	45	Progression	30	14	Mild	Guillain–Barrè syndrome; ischaemic cardiopathy	Yes	82.9
II-6	67	Yes	28	39	Progression	60	18	Severe	Primary hypertension	Yes	88.0
II-7	65	Yes	25	40	Stability	No	–	Mild	Dyslipidosis, gout	No	–
II-8	61	Yes	28	33	Stability	No	12	Moderate	No	Yes	59.0
II-11	54	Yes	23	31	Progression	31	25	Severe	Retina detachment	Yes	29.9
III-1	46	Yes	20	26	Progression	40	10	Mild	No	Yes	9.1
III-2	44	Yes	25	19	Stability	No	9	Mild	No	Yes	40.7
III-3	38	Yes	20	18	Progression	30	9	Mild	Chron disease	Yes	30.7
III-4	32	No	–	–	–	–	–	No	No	Yes	4.3
III-6	39	Yes	26	4	Regression	–	–	Mild	Atopic asthma	No	–
III-7	42	Yes	42	0	–	–	9	No	No	Yes	24.3
III-8	39	Yes	27	12	Progression	30	24	Moderate	Mitral prolapse	No	–
III-9	37	No	–	–	–	–	–	No	No	Yes	1.4
III-10	32	No	–	–	–	–	–	No	No	Yes	17.3
III-11	27	Yes	18	9	Progression	26	23	Moderate	Glaucoma	Yes	25.7
III-12	26	No	–	–	–	–	–	No	No	Yes	0.0
III-14	18	No	–	–	–	–	–	No	No	Yes	0.0
III-17	28	Yes	25	3	Stability	no	7	Mild	Migraine	Yes	9.5

*RLS-SSS: restless legs syndrome severity scale score. †PLMS: periodic legs movement in sleep; a pathological value for PLMS index is defined as more than five periodic legs movements per hour of sleep.

Table 2 *Two-point LOD score values for 14q markers*

Marker	θ						
	0.00	0.01	0.05	0.1	0.2	0.3	0.4
D14S283	–1.41	–0.58	–0.05	0.16	0.38	0.35	0.18
D14S275	–1.31	–0.42	0.23	0.54	0.68	0.52	0.24
D14S70	0.50	0.51	0.59	0.78	0.88	0.70	0.35
D14S1048	1.58	2.15	2.49	2.43	1.97	1.30	0.56
D14S993	0.99	1.06	1.17	1.15	0.92	0.58	0.24
D14S301	1.58	2.15	2.49	2.43	1.97	1.30	0.56
D14S266	3.05	2.99	2.75	2.44	1.80	1.15	0.51
D14S288	3.23	3.17	2.91	2.58	1.90	1.20	0.52
D14S1013	1.58	2.15	2.49	2.43	1.97	1.30	0.56
D14S1068	–1.11	0.07	0.99	1.24	1.16	0.80	0.35
D14S978	0.20	0.20	0.39	0.57	0.62	0.47	0.23
D14S276	–0.62	–0.59	–0.48	–0.35	–0.18	–0.08	–0.02

1, III-2). Individuals II-11, the proband, and III-11 complained of leg restlessness at rest also during the day. Some (II-6, II-7, II-8 and III-3) complained of additional cramp-like leg motor symptoms during the day, especially with heightened exercise (shooting, running), and II-7 reported this also at night. III-1 also reported a worsening of RLS sensory symptoms with heightened exercise. Localization of symptoms were in the calves and bilateral. II-1 also reported arm motor restlessness while awake during the last 3 years.

Age at onset

The age at onset was determined as the first occurrence of clinical symptoms of the legs according to the patient's memory. Age at onset in this family ranged from 18 (III-11) to 28 (II-6 and II-8) years. Only in one individual (III-7) was onset delayed, at 42 years. The mean age at onset was 26 ± 5.87 years. Age at RLS onset was 26 years for the deceased II-4, while it was not known for the founder I-1. No evidence of apparent anticipation was observed in our pedigree.

Variable expressivity and clinical progression

Variable expressivity for RLS symptoms in this family was established by evaluating the diagnostic criteria (Walters, 1995) and administering the IRLS-RS for severity. Individual II-7 refused to be interviewed. The interview of individual III-6 was inconsistent, due to the recent onset of RLS. The score obtained did not correlate to RLS duration and course, nor to sleep disturbances and PLMS index. On the other hand, severity of PLMS index seemed to be correlated to the presence of additional (other than leg restlessness resulting from the sensation) limb movements while awake, i.e. both cramp-like motor symptoms and arm motor restlessness. Chronic RLS is defined when symptoms occur on a daily basis. Progression refers to increasing symptom severity and frequency. In fact, RLS course is typically chronic and progressive (Walters *et al*., 1996). In this family, 58% of the affected members reported the typical course of the disease (Table 1). Individuals II-11 and III-8 sought medical attention because of a severe lack of sleep. RLS occurred in proband (II-11) initially, just after the first childbirth, while it was exacerbated during pregnancy in III-8. Individual III-6 complained of RLS symptoms for 4 years, from age 26 to 30 years, her age at the birth of her only child. She has been symptom-free for 9 years. Progression for individual III-7 cannot yet be established, since symptoms appeared only a few months before this study was undertaken.

Routine RLS investigations

Neurological examination, routine EMG and nerve conduction studies were normal in all members of the pedigree. Ferritin level was low (<45 μg/100 ml) in III-2, III-3 and III-11. Oral iron supplement was recommended, but normal ferritin level did not improve RLS symptoms in the three subjects, as already reported for other RLS patients (Ondo *et al*., 2000).

Linkage analysis

A previously reported susceptibility locus for RLS on chromosome 12q (Desautels *et al*., 2001) was excluded from linkage in our family by assuming both the recessive genetic model hypothesized by the authors and the dominant model described in the Subjects and methods section (data not shown).

The genome-wide scan performed on the affected family members allowed for the identification of five potential candidate loci, on chromosomes 2q24, 9p12, 14q21, 15q11 and 22q12. By increasing the local marker density and including all the pedigree members, all but one candidate loci on chromosome 14 were excluded. Chromosome 14 locus was significantly confirmed to be linked to RLS, reaching a maximum two-point LOD score value of 3.23 (P = 0.029; LOD score values >3 are considered significant) for D14S288 at θ = 0.0 (Table 2). The same parameters used for two-point genetic linkage calculation were used in a non-parametric multipoint analysis (NPL), which identified a region covering 14q13-21 bands as associated with RLS with a maximum NPL score of 3.47 (P = 0.013; Fig. 2).

A common haplotype (4_2_1_1_4_4; Fig. 1) shared by all the affected individuals was identified with markers D14S1048, D14S993, D14S301, D14S266, D14S288 and D14S1013. Crossing-over events in individuals II-7 and III-11 allowed for the defining of the upper and lower limit, respectively, of the RLS-critical region. It extends from marker D14S70 to D14S1068, spanning a region of 9.1 cM, corresponding to 12.8 Mb (magabases), on chromosome 14q13-21 region. Individual II-8 seemed to share a part of the affected haplotype (markers D14S266 and D14S288). Two hypotheses can explain individual II-8's phenotype, as we excluded mis-paternity by several hundred genotyping data.

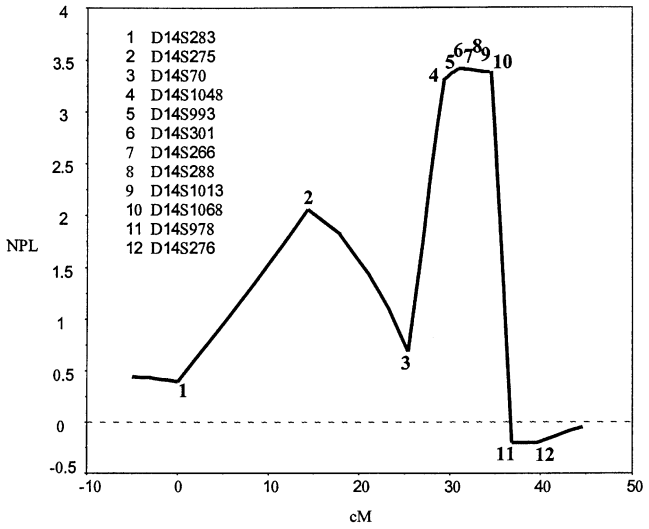

Fig. 2 NPL multipoint graph of 14q RLS locus. The inset shows the markers surrounding the 14q RLS locus.

In a first scenario, a double recombination event took place in the II-8 paternal meiosis, between markers D14S301 and D14S1013. Therefore, individual II-8 would be the carrier of the minimal affected haplotype spanning only 1.23 Mb on physical map (Kent *et al*., 2002), an improbably small distance for a double recombination event. However, owing to the non-availability of I-1 blood sample, the double crossing-over could not be completely excluded. A second hypothesis is that patient II-8 is a phenocopy. This *a posteriori* assumption (i.e. based on the genotype determination) did not justify the exclusion of II-8 from the analysis; indeed, individual II-8 was considered affected and assigned to liability class 1 (attributed penetrance 0.001, 0.95, 0.95). Three non-symptomatic members (III-5, III-9 and III-10) aged 43, 37 and 32 years, respectively, at the time of the last follow-up, carried the affected haplotype. The PLMS score allowed for the identification of individual III-10 as an RLS-milder phenotype, as suggested previously (Walters *et al*., 1990; Lazzarini *et al*., 1999; see Table 1). Since polysomnographic recording was not available for individual III-5, the question of whether she is a non-penetrant or an age-dependent penetrant, or shows an RLS-milder phenotype, remains unsolved. Conversely, individual III-9 showed no PLMS. PLMS might be missed during a single night of recording, since variability in detecting PLMS is a well-known problem (Chokroverty and Jankovic, 1999). Negative two-point LOD scores were obtained for 14q markers under a recessive model of inheritance (data not shown).

Discussion

Here we report the mapping of a new locus for RLS with PLMS to chromosome 14q13-22 in a 9.1 cM region. The exclusion of the previously reported RLS locus in 12q, besides our family, was recently confirmed in two additional Italian RLS pedigrees (Kock *et al*., 2002). A true dominant, rather than a recessive (Desautels *et al*., 2001), model of inheritance was assumed for linkage calculations. In addition, the occurrence of intermediate phenotypes is consistent with an autosomal dominant pattern of inheritance, where disease diversity in severity of symptoms and signs is due to variable expressivity. Moreover, statistical evidence for a single RLS gene and autosomal dominant mode of inheritance have recently been proposed (Winkelmann *et al*., 2002).

However, the assumption of an autosomal recessive inheritance pattern with a high disease-carrier frequency and a remarkable allelic heterogeneity was instrumental to the 12q locus identification (Desautels *et al*., 2001). On this basis, the affected family members are supposed to be compound heterozygotes. For instance, it has been reported that in Quebec, where the French-Canadian 12q-associated family (Desautels *et al*., 2001) are resident, there is a high proportion of familial cases and a higher prevalence of RLS (Lavigne and Montplaisir, 1994).

Although a high frequency of RLS resulting in bilinear transmission was also reported (Ondo *et al*., 2000), both the prevalence and proportion of familial cases seem to vary widely according to the geographical origin of the studied population, reflecting either founder effects or the influence of environmental factors.

The best fitting inheritance model seems to be autosomal dominant with a common disease allele in RLS families showing early age at onset (mean age at onset >30 years) and intrafamilial phenocopy (Winkelmann *et al*., 2002). Our family seems to fit this genetic model and may represent the first actual example of a major gene acting dominantly.

Since polysomnography registrations were obtained only after the recent recruitment of the family, we could not detect individuals with PLMS who later developed RLS. Nonetheless, PLMS seem to act as a precursor of RLS, as previously reported (Boghen and Peyronnard, 1976; Walters *et al*., 1990; Lazzarini *et al*., 1999). In fact, we considered PLMS to be an intermediate phenotype, a forme fruste of RLS, associated with a reduced penetrance value (as for individual III-10).

Age-dependent RLS penetrance was shown to reach full value by age 60 years (Trenkwalder *et al*., 1996). Variable age at onset was documented even in identical twins; in particular, in two twin pairs the age at onset varied by 42 years (Ondo *et al*., 2000).

Iron store reduction is a well-recognized condition mimicking idiopathic RLS. However, whether iron deficiency plays a primary role in causing RLS or whether it aggravates or triggers RLS symptoms in predisposed subjects (Sun *et al*., 1998) remains to be determined. Earley and colleagues demonstrated that it is cerebrospinal fluid ferritin that could potentially affect RLS phenotype (Earley *et al*., 2000). However, three patients showing low blood ferritin level failed to improve RLS symptoms by iron supplementation, thus confirming the diagnosis of familial RLS.

Our results also suggest a phenotypic continuum from limb movements during wakefulness to PLMS. It has been supposed that they may be the same clinical entity; the difference in temporal distribution (frequency and periodicity) may result from physiological changes of the same neuronal system across the sleep–wake cycle (Montplaisir *et al*., 1985).

In previous reports, RLS candidate genes were chosen from those encoding enzymes and receptors of the dopaminergic, enkephalinergic and GABAergic systems (Dichgans, 1996); the alpha1 subunit of the glycine receptor was also considered (Lazzarini *et al*., 1999), but all were excluded. Using a genome-wide linkage approach, Desautels and colleagues identified the first major genetic locus for RLS on chromosome 12q22-23, within a 14.71 cM region between markers D12S1044 and D12S78 (Desautels *et al*., 2001).

The 12 Mb of the 14q RLS critical region includes more than 60 genes, and selecting RLS positional candidates is quite difficult. Nonetheless, among them we recognized the gene coding for ninein (GSK3B-interacting protein), the glia maturation factor beta (GMF-β); the survival of motoneuron-interacting protein 1 (SIP1); and somatostatin receptor 1 (SSTR1).

In conclusion, our study provides evidence for a new RLS-associated locus, the first acting in an autosomal dominant mode. Besides the major gene contribution, additional modifier genes may account for specific phenotype traits such as late age at onset, severity and motor involvement.

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