ELSEVIER

Sleep Medicine 3 (2002) S9–S12

SLEEP MEDICINE

www.elsevier.com/locate/sleep

The genetics of restless legs syndrome

J. Winkelmann*

Department of Neurology, Max Planck Institute of Psychiatry, Kraepelinstrasse 10, 80804 Munich, Germany

Keywords: Restless legs syndrome; Sleep; Genetics; Familial

1. Introduction

The first description of restless legs syndrome (RLS) as a hereditary disorder dates back to Oppenheim in his *Textbook of Nervous Diseases* published in 1923 [1]. Another early description was published in 1940 by Mussio-Fournier and Ravak. They described a woman who experienced crawling sensations in the legs during her pregnancy and who had a mother, sister, and two daughters who also experienced these sensations. At that time, however, because the syndrome was generally unknown, the authors did not distinguish between the symptoms of RLS and an additional allergic disposition in the family [2]. Ekbom also suspected a strong familial component of the disorder and suggested an autosomal-dominant mode of inheritance [3]. Since then, it is commonly believed that RLS is an 'inherited disorder' in at least a proportion of patients, with estimates ranging from approximately one third [4] to about 90% [5]. It is important to remember that development of the currently accepted diagnostic criteria for RLS occurred only 7 years ago, in 1995 [6]. These diagnostic criteria provide the prerequisites for comparing epidemiological studies and for examining families for genetic investigations. Since then, several studies have investigated the occurrence of familial RLS in different populations of RLS patients, but only in a limited number of studies have personal interviews of relatives of the index cases been conducted to verify the diagnosis and the definite occurrence of the familial cases.

2. Frequency of familial RLS

In a study of 54 patients with RLS, Ondo and Jankovic found a positive family history in 42% of cases [5]. When subgroups of idiopathic and non-idiopathic forms were analyzed, 92% of patients with idiopathic RLS had a family history of the disease, while only 13% of those with RLS secondary to peripheral neuropathy reported a family history of RLS. In patients with a positive family history, 58 of 246 possible first-degree relatives (23.6%) were reported to have symptoms consistent with RLS. In this study, the diagnosis of secondary cases was primarily based on the family history reported by the proband. The authors state that diagnosis in secondary cases was confirmed or refuted by telephone interviews 'when possible,' but they did not mention how many relatives actually had been contacted. Montplaisir and co-workers reported that 63% of 127 consecutive clinic patients with RLS, diagnosed according to the criteria of the International RLS study group, had affected first-degree relatives ($n = 568$) [7]. Of these first-degree relatives, 39% were affected by symptoms of RLS. Again, diagnosis was not confirmed by direct interview or examination of these relatives. This was also the case in a questionnaire study conducted by Allen et al., who reported that the first-degree relatives of 96 RLS patients had a 4–6-fold increased risk of developing RLS compared to controls [8]. In a study performed by our group, 300 index patients with RLS were interviewed directly with a standardized questionnaire [9]. All subjects were classified according to their family history status and were categorized as having a 'definite positive family history' when at least one first-degree relative was affected with RLS according to the personal interview. In contrast, subjects were defined as having a 'possible positive family history' if they mentioned having affected relatives but if a confirmation of the diagnosis of RLS was not possible for various reasons. A 'definitive positive family history' was present in 42% of 182 patients with idiopathic RLS and a 'possible positive family history' was present in 12.6% of these patients. In contrast, only 12% and 6% of uremic patients were classified as having a 'definite positive family history' and a 'possible positive family history', respectively.

Clinical features (such as age-at-onset), variability of signs and symptoms, and associated features have been compared between familial and sporadic cases of RLS in a number of studies. No consistent difference in clinical

* Tel.: +49-8930-622374; fax: +49-8930-622311.
E-mail address: janew@mpipsykl.mpg.de (J. Winkelmann).

1389-9457/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S1389-9457(02)00141-7

features were found, except for a younger age-at-onset in familial cases: 25.9 vs. 29.2 years in the study by Montplaisir et al. [7], and 35.4 vs. 47.2 years in the study by Winkelmann et al. [9]. In the so-called 'Night-Walker Survey', Walters et al. subdivided 105 patients, interviewed by telephone, into two groups: patients with an age-at-onset of $<$ 20 years and patients with an age-at-onset $>$ 20 years. In the younger group, 81% reported a positive family history, in contrast to the older group, in which only 58% were aware of other affected family members [10]. Investigating the age-at onset of the disease, Allen et al. showed a bimodal age-at-onset distribution with a break between 45 years of age. The same study also demonstrated a correlation of an earlier age-at-onset and 'familial' RLS [8]. Lastly, Allen and Earley compared the effects of current age and serum ferritin on RLS severity for early- and late-onset RLS [11]. Again, two groups were classified: patients with an age-at-onset $<$ 45 years and patients with an age-at-onset $>$ 45 years. They were able to demonstrate an age effect in the early-onset form of RLS that slowly progresses with age and has a limited relation to serum iron. On the other hand, late-onset RLS appeared to occur less commonly in families, progressed rapidly with age, and had a stronger relation to iron status. Taken together, the results of these studies suggest a new concept for RLS: the phenotype of the disease may depend on the age-at-onset of the symptoms. It has been further suggested that this concept should be considered to better define the RLS phenotype in future studies [11]. However, the relevant age-at-onset of the disease at which to subdivide RLS patients in an early- and late-onset group remains to be investigated.

3. Mode of inheritance of RLS

The pattern of inheritance has been described in single families. Therefore, it is generally assumed that RLS follows a Mendelian autosomal dominant mode of inheritance. Ekbom stated that he had seen "several families in which a dominant mode of inheritance seemed probable". Additional early descriptions of families with a pedigree structure compatible with dominant inheritance are the families of Mussio-Fournier [2], Huizenga [12], and Bornstein [13]. Recently, several large families with RLS have been described in more detail. Montagna et al. reported a family with symptoms of typical RLS over three generations. In the generation of the index patient, all nine siblings of the index patient were affected, and the authors assumed autosomal dominant inheritance [14]. Mean age-at-onset was in the second decade, considerably younger than that observed in the larger case series mentioned.

The phenomenon of 'anticipation' has been discussed in relation to familial RLS, and evidence for an earlier age-at-onset in later generations has been found in a subset of families [15,16]. Anticipation is a phenomenon that has been described in several inherited neurologic disorders. It is caused by 'unstable' mutations in the form of expanded trinucleotide repeat sequences. Classic examples are Huntington's disease [17] and spinocerebellar ataxias (SCA) [18]. Schöls et al. investigated 89 SCA patients for possible symptoms of RLS and found that 45% of SCA3 patients and 18% of SCA2 patients present symptoms of RLS [18]. Interestingly, these patients also received a good therapeutic benefit with dopaminergic agents, pointing to a possible common underlying pathophysiological mechanism, and the authors concluded that RLS in the SCA patients could possibly be due to trinucleotide expansions. In another study, symptoms of RLS were found in a total of 28% of a population of SCA patients vs. only 10% in controls. However, the occurrence of RLS was found to increase with age but not with the length of the CAG repeat or a higher age of ataxia onset [19]. So far, evidence that expanded CAG repeats in the SCA3 gene is a molecular factor involved in the etiology of RLS is lacking. Furthermore, the possibility that familial RLS may be caused by an expanded trinucleotide repeat is not obvious, as all of the disorders known to date with expanded trinucleotide repeats are neurodegenerative disorders. Up to now, no neurodegenerative changes have been documented in RLS. In addition, the patients with SCA and RLS have structural spinal as well as cerebellar 'abnormalities,' and one has to keep in mind that secondary forms of RLS can also occur in association with spinal cord lesions [20].

4. Variable expressivity of the putative RLS gene

Based on clinical observations, RLS has been defined as a disease entity, and its key features have been outlined by the RLS Study Group. Several studies of large pedigrees with RLS demonstrate that the expressivity of the symptoms of RLS within a single family is highly variable [15,16]. Walters et al. also concluded that PLM could be a 'forme fruste' of RLS [21]. The onset of the disease is usually in the second decade of life, and it often follows an intermittent, fluctuating course. At the onset of the disorder, the diagnosis is sometimes not obvious because symptoms are generally mild in the beginning, and symptom-free periods can occur. Treatment is usually initiated in the fifth decade of life; however, some patients with familial RLS need treatment early in their youth. The uncertainty complicates the search for the genetic basis of RLS, and so far there is no specific test to diagnose RLS and to specify and limit the clinical picture. In addition, diagnostic criteria have been set up for adults and may have to be modified for children.

A twin study investigating 12 pairs of twins demonstrated a high concordance rate of approximately 83% [22]. The disease severity, the age-at-onset, and the symptom description varied between the twins. Furthermore, serum ferritin did not relate to RLS severity, although the sample was rather small, making the detection of correlational relationships difficult.

5. Linkage studies in RLS

Several approaches have been used to elucidate further the underlying genetic basis of the disease. As a first step, candidate genes were studied. Candidate genes are those genes that can be hypothesized to be involved in the etiology of the inherited disease based on our knowledge of the pathology, pathophysiology, or pharmacology of the disease. Because of the excellent therapeutic benefit of dopaminergic and opioidergic agents [23–27], it is possible that genes coding for proteins involved in the central neurotransmission of these neurotransmitters are involved in the pathophysiology of RLS. There is further evidence that drugs acting on $GABA_A$ receptor subunits improve the symptoms of RLS [28]. Our group studied polymorphic markers surrounding 22 candidate genes [29]. Linkage analyses using markers either within or surrounding the genes for tyrosine hydroxylase (TH); GTP-cyclohydrolase; the dopamine transporter (DAT); the dopamine receptors (D1–D5); $GABA_A$ receptor subunits (α1–6, β1–3, χ1–3, p1–2); and the α1 subunit of the glycine receptor (chromosome 5, q31) were performed. No evidence for linkage could be found for any of these chromosomal regions in the investigated family [30]. Furthermore, Desautels et al. performed a large population-based case-control study focusing on the French Canadian population [30]. Eight candidate genes involved in dopaminergic transmission (D1–D5 receptors, DAT, TH, and dopamine β-hydroxylase) were investigated. A comparison of the allele and genotype frequencies between patients and controls found that these loci have no major effect on the vulnerability to RLS. Additionally, a stratification analysis according to age-at-onset and PLMS-index disclosed no significant association.

There are several difficulties in determining genetic linkages. To date, there are no objective measurements that specify and clearly define the phenotype of RLS, resulting in difficulties in diagnosis. Another possibility for a failure to determine a genetic linkage is the misspecification of the underlying genetic model of the disease. Genetic studies are based on an accurate assumption of the mode of inheritance and an accurate assumption of, for example, penetrances and rate of phenocopies: not all of these parameters are known in RLS. Our group performed a formal, complex segregation analysis for the first time to investigate the most likely mode of inheritance of RLS [31]. We investigated 238 RLS patients and as many of their first-degree relatives as possible. Assessments were based on direct, personal, standardized diagnostic interviews. Respondents were classified as RLS 'affected' or 'non-affected'. Complex segregation analysis was performed with the families stratified into two groups according to the mean age-at-onset of the disease within the families: age-at-onset of ≤ 30 years and of > 30 years. In the younger group, segregation analysis strongly favored a major gene acting as autosomal dominant with a multifactorial component. In contrast, no evidence for a major gene could be elucidated in the older group, in which the most likely mode of inheritance was a free transmission probability [30]. The segregation pattern found in these families argues for an autosomal allele acting dominantly in RLS families with an early age-at-onset of symptoms and suggests that RLS is an etiologically heterogeneous disease. Similar patterns of inheritance have been documented in other diseases with complex inheritance like Parkinson's disease, Alzheimer's disease, and breast cancer.

Very recently, Montplaisir's group published the first identification of a major susceptibility locus for RLS on chromosome 12q in a French Canadian family [32]. Possible candidate genes within the suspected chromosome region of interest are the timeless gene and the gene encoding the neuropeptide neurotensin (NTS), which is reported to act as a neuromodulator of dopaminergic transmission [32]. Their results suggested a 'pseudodominant pattern of inheritance' in which the true mode of inheritance is autosomal recessive, but because of the high disease-carrier frequency, there is frequent homozygote–heterozygote mating and consequent disease diversity. If this mode of inheritance, as well as the susceptibility locus on chromosome 12, only accounts for a subgroup of French-Canadian RLS families or for families with different origins as well is an interesting, open question that needs to be investigated in future genetic studies. This first identification of a susceptibility locus in RLS hopefully opens new insight to the pathophysiology of the disorder: however, it is possible that there is heterogeneity.

Considering the difficulties of using the results of linkage studies to assign the appropriate affection status, further studies should investigate the clinical picture of RLS and clearly define the exact phenotype of the disease. A further approach includes linkage studies that can be performed in large populations using methods such as the affected sib-pair method or the transmission disequilibrium test. It can be expected that all of these investigations will provide important new information on the molecular and cellular basis of RLS.

References

[1] Oppenheim H. Lehrbuch der Nervenkrankheiten, 7th ed. Berlin: Karger, 1923. p. 1774.
[2] Mussio-Fournier JC, Rawak F. Familiäres Auftreten von Pruritus. Urtikaria und parästhetischer Hyperkinese der unteren Extremitäten. Confin Neurol 1940;3:110–115.
[3] Ekbom KA. Restless legs syndrome. Neurology 1960;10:868–873.
[4] Sachdev P. Akathisia and restless legs. New York: Cambridge University Press, 1995.
[5] Ondo W, Jankovic J. Restless legs syndrome: clinicoetiologic correlates. Neurology 1996;47:1435–1441.
[6] Walters AS. and the International Restless Legs Syndrome Study Group. Toward a better definition of the restless legs syndrome. Mov Disord 1995;10:634–642.
[7] Montplaisir J, Boucher S, Poirier G, Lavigne G, et al. Clinical, polysomnographic, and genetic characteristics of restless legs syndrome: a

study of 133 patients diagnosed with new standard criteria. Mov Disord 1997;12:61–65.
[8] Allen RP, LaBuda MC, Becker PM, Earley CJ. Family history study of RLS patients from two clinical populations. Sleep Res 1997;26:309.
[9] Winkelmann J, Wetter TC, Collado-Seidel V, Gasser T, et al. Clinical characteristics and frequency of the hereditary restless legs syndrome in a population of 300 patients. Sleep 2000;23:597–602.
[10] Walters AS, Hickey K, Maltzman J, Verrico T, et al. A questionnaire study of 138 patients with restless legs syndrome: the 'Night-Walkers' survey. Neurology 1996;46:92–95.
[11] Allen RP, Earley CJ. Defining the phenotype of the restless legs syndrome (RLS) using age-of-symptom-onset. Sleep Med 2000;1:11–19.
[12] Huizenga J. Hereditary acromyalgia or "restless legs". Acta Genet 1957;7:121–123.
[13] Bornstein B. Restless legs. Psychiatr Neurol Neurochir 1961;141:165–201.
[14] Montagna P, Coccagna G, Cirignotta F, Lugaresi E. Familial restless legs-syndrome: long-term follow-up. In: Guilleminault C, Lugaresi E, editors. Sleep/wake disorders: natural history, epidemiology, and long-term evolution, New York: Raven, 1983. pp. 231–235.
[15] Trenkwalder C, Collado-Seidel V, Gasser T, Oertel WH. Clinical symptoms and possible anticipation in a large kindred of familial restless legs syndrome. Mov Disord 1997;11:389–394.
[16] Lazzarini A, Walters AS, Hickey K, Coccagna G, et al. Studies of penetrance and anticipation in five autosomal-dominant restless legs syndrome pedigrees. Mov Disord 1999;14:111–116.
[17] Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993;72:971–983.
[18] Schöls L, Haan J, Riess O, Amoiridis G, Przuntek H. Sleep disturbance in spinocerebellar ataxias: is the SCA3 mutation a cause of restless legs syndrome? Neurology 1998;51:1603–1607.
[19] Abele M, Bürk K, Laccone F, Dichgans J, Klockgether TH. Restless legs syndrome in spinocerebellar ataxia types 1, 2, and 3. J Neurol 2001;248:311–314.
[20] Winkelmann J, Wetter TC, Trenkwalder C, Auer DP. Periodic limb movements in syringomyelia and syringobulbia. Mov Disord 2000;15:752–753.
[21] Walters AS, Picchietti D, Hening W, Lazzarini A. Variable expressivity in familial restless legs syndrome. Arch Neurol 1990;47:1219–1220.
[22] Ondo WG, Vuong KV, Wang Q. Restless legs syndrome in monozygotic twins: clinical correlates. Neurology 2000;55:1404–1406.
[23] Trenkwalder C, Stiasny K, Pollmacher TL-dop. pa therapy of uremic and idiopathic restless legs syndrome: a double-blind, crossover trial. Sleep 1995;18:681–688.
[24] Wetter TC, Stiasny K, Winkelmann J, Buhlinger A, et al. A randomized controlled study of pergolide in patients with restless legs syndrome. Neurology 1999;52:944–950.
[25] Walters A, Hening W, Cote L, Fahn S. Dominantly inherited restless legs with myoclonus and periodic movements of sleep: a syndrome related to the endogenous opiates? Adv Neurol 1986;43:309–319.
[26] Walters AS, Wagner ML, Hening WA, Grasing K, et al. A double blind randomized controlled trial of oxicodone versus placebo. Sleep 1993;16:327–332.
[27] Hening WA, Allen R, Earley C, Kushida C, et al. The treatment of restless legs syndrome and periodic limb movement disorder. An American Academy of Sleep Medicine Review. Sleep 1999;22:970–998.
[28] Boghen D, Lamothe L, Elie R, Godbout R, et al. The treatment of the restless legs syndrome with clonazepam: a prospective controlled study. Can J Neurol Sci 1986;13:245–247.
[29] Dichgans M, Walther E, Collado-Seidel V, Trenkwalder C, et al. Autosomal dominant restless legs syndrome: genetic model and evaluation of 22 candidate genes. Mov Disord 1996;11:87–90.
[30] Desautels A, Turecki G, Montplaisir J, Ftouhi-Paquin N, et al. Dopaminergic neurotransmission and restless legs syndrome: a genetic association analysis. Neurology 2001;57:1304–1306.
[31] Winkelmann J, Müller-Myhsok B, Wittchen HU, et al. Segregation analysis of restless legs syndrome provides evidence for a single gene and autosomal dominant mode of inheritance. Ann Neurol 2002;52:297–302.
[32] Desautels A, Turecki G, Montplaisir J, Sequeira A, et al. Identification of a major susceptibility locus for restless legs syndrome on chromosome 12 q. Am J Hum Genet 2001;69:1266–1270.