Restless Legs Syndrome: Diagnosis, Treatment and Pathophysiology

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Stephany Fulda
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Erstgutachter: Prof. Dr. Jürgen Zulley

Zweitgutachter: Prof. Dr. Mark W. Greenlee

Content

INTRODUCTION

The restless legs syndrome (RLS) is a neurological disorder characterized by the urge to move the extremities associated with paresthesias, which are partially or totally relieved by movement, a worsening of symptoms at rest and in the evening or at night and, as a consequence, sleep disturbances[1]. RLS is a common but often under-diagnosed sensorimotor disorder of sleep/wake motor regulation with prevalence rates estimated from population surveys between 1 and 10%, increasing with age and considerably more prevalent in females than males[2]. There exist many forms regarding the clinical course of the disease, the severity and circadian expression of symptoms as well as associated features.

The first documented description of restless legs associated with severe sleep disturbances dates back to the 17th century and was reported by the English physician Sir Thomas Willis. Originally published in Latin in 1672[3] it was later published in English in the London Practice of Physick[4]:

> *"Wherefore to some, when being a Bed they betake themselves to sleep, presently in the Arms and Legs Leapings and Contractions to the Tendons, and so great a Restlessness and Tossing of their Members ensue, that the diseased are no more able to sleep, than if they were in a Place of the greatest Torture" (p. 404).*

In the 19th and 20th century several other names were given to the disorder such as *anxietas tibiarum* by Wittmaack[5] and *leg jitters* by Allison[6]. Karl Axel Ekbom was the first to provide a detailed description of the clinical features of the disorder[7] and first named it *asthenia crurum paraesthetica*. In 1945[8] he coined the term *restless legs syndrome* (RLS) to distinguish it from other similar conditions and already reported that the syndrome may cluster in families and that there might be a secondary form of RLS in anaemia or pregnancy. In recognition of Ekbom's major contribution to the understanding of this condition, RLS has also been referred to as *Ekbom syndrome*. Alternate names include *focal akathisia* of the legs[9], although this term is used very infrequently nowadays. Scientific interest was slow to respond to RLS in earlier years but picked up considerably during the 1980s when Akpinar reported that RLS was treated successfully with levodopa[10] which remained first line treatment for nearly two decades. Scientific developments were further helped along by the foundation of the International RLS Study Group (IRLSSG) that in 1995 defined uniform and internationally accepted criteria for the diagnosis of RLS[11] which were updated in 2003[1].

Today, most authors agree that RLS has its origin in the central nervous system, however, complex interactions between central and peripheral structures may contribute to the disorder. Based on the knowledge of the efficacy of dopaminergic and opioidergic drugs and the provocation or exacerbation of RLS symptoms following treatment with dopamine receptor blocking agents, there is evidence of the involvement of the dopaminergic and opioid system in the pathogenesis of RLS. Recent PET and SPECT studies revealed some controversial results of the nigrostriatal dopaminergic neurotransmission probably reflecting a dysfunction of the central dopaminergic system[12]. The aetiology, however, remains unclear, despite what is known about the conditions that may induce the syndrome[13,14].

Diagnosis of RLS

In 1995, the International RLS Study Group developed standardized criteria for the diagnosis of RLS[11] which have been recently modified[1] and correspond to the criteria of the revised

Table 1. Diagnostic criteria for RLS (from reference [1])

	Essential diagnostic criteria for RLS (adults)
1	An urge to move the legs, usually accompanied or caused by uncomfortable and unpleasant sensations in the legs
2	The urge to move or unpleasant sensations begin or worsen during periods of rest or inactivity such as lying or sitting
3	The urge to move or unpleasant sensations are partially or totally relieved by movement, such as walking or stretching, at least as long as the activity continues
4	The urge to move or unpleasant sensations are worse in the evening or night than during the day or only occur in the evening or night
	Supportive clinical features of RLS
1	*Family history* The prevalence of RLS among first-degree relatives of people with RLS is 3 to 5 times greater than in people without RLS.
2	*Response to dopaminergic therapy* Nearly all people with RLS show at least an initial positive therapeutic response to either L-dopa or a dopamine-receptor agonist at doses considered to be very low in relation to the traditional doses of these medications used for the treatment of Parkinson disease. This initial response is not, however, universally maintained.
3	*Periodic limb movements (during wakefulness or sleep)* Periodic limb movements in sleep (PLMS) occur in at least 85% of people with RLS; however, PLMS also commonly occur in other disorders and in the elderly. In children, PLMS are much less common than in adults.
	Associated features of RLS
1	*Natural clinical course* The clinical course of the disorder varies considerably, but certain patterns have been identified that may be helpful to the experienced clinician. When the age of onset of RLS symptoms is less than 50 years, the onset is often more insidious; when the age of onset is greater than 50 years, the symptoms often occur more abruptly and more severely. In some patients, RLS can be intermittent and may spontaneously remit for many years.
2	*Sleep disturbance* Disturbed sleep is a common major morbidity for RLS and deserves special consideration in planning treatment. This morbidity is often the primary reason the patient seeks medical attention.
3	*Medical evaluation/physical examination* The physical examination is generally normal and does not contribute to the diagnosis except for those conditions that may be comorbid or secondary causes of RLS. Iron status, in particular, should be evaluated because decreased iron stores are a significant potential risk factor that can be treated. The presence of peripheral neuropathy and radiculopathy should also be determined because these conditions have a possible, although uncertain, association and may require different treatment.

international classification of sleep disorders[9] (ICSD-2). RLS is characterized by: 1) an imperative desire to move the extremities which is 2) at least temporarily relieved with movement, and 3) worse or exclusively present at rest and 4) in the evening or at night (Table 1). Supportive clinical features for RLS are a positive family history for RLS, an initial response to dopaminergic therapy and the presence of periodic limb movements during sleep (PLMS). The clinical course of the disorder varies considerably and in some patients RLS can be intermittent and may spontaneously remit for many years[15,16]. To diagnose RLS in pediatric patients, the child meets four essential adult criteria for RLS and is in addition

either able to relate an indicative description in his or her own words or at least two of the following criteria are met: a sleep disturbance, a biological sibling or parent with definite RLS, or more than five periodic leg movements per hour of sleep, documented by polysomnography. Diagnostic criteria in other special populations such as the cognitively impaired elderly have also been proposed[1].

Subjects with RLS typically complain about disturbed sleep and in particular about disturbed sleep onset due to the restless legs symptoms occurring in the evening and at times of inactivity. Most RLS patients will be able to describe clearly the urge to move and the associated paresthesias although the clinician is bound to hear a broad range of creative descriptions of the symptomatology. Typical examples are “crawling ants”, “jittery legs”, “moving worms”, or “soda bubbling in the veins”[1]. In unclear cases, the suggested immobilization test (SIT), which may elicit and quantify the motor symptoms (involuntary leg movements) of RLS, may be helpful[17]. Improvement of symptoms with a single dose of levodopa has a high sensitivity and specificity in subjects with RLS and this response is considered as a supportive feature[18].

A laboratory evaluation including serum ferritin, electrolytes, and renal parameters can rule out potentially secondary forms of RLS such as iron deficiency anemia[19,20] or renal failure[21]. In an atypical presentation or when symptoms resemble peripheral neuropathy, nerve conduction velocities and electromyogram should be performed. Polysomnography is generally reserved for patients where other or additional sleep disorders are suspected or where the degree of sleep disturbances needs to be quantified, e.g., for judicial purposes. In addition, because opioids can worsen pre-existent sleep related breathing disorders (SRBD) polysomnography may be warranted to rule out SRBD before treatment with opioids is initiated[22,23].

A number of conditions other than RLS must be considered in the differential diagnosis of altered sensations in the legs. These include disorders of the peripheral nervous system such as peripheral neuropathies[24] and syndromes owing to irritation of the nerve root or compression of peripheral nerves[25], and vascular conditions such as peripheral arterial disease. Altered sensations in the legs and motor restlessness are also reported in patients with antipsychotic-induced akathisia[26], anxiety disorders and attention deficit hyperactivity disorder. In addition, several drugs can induce RLS and in particular antidepressants[27] and antipsychotics[28] have been associated with RLS.

Chronic RLS is associated with significantly reduced quality of life and within the different domains of quality of life the areas “energy / sleepiness” and “performance” are particularly impaired[29-31]. Investigations in different populations such as the general population or sleep lab populations revealed that about one third of RLS subjects perceive themselves as being excessively sleepy during the daytime[32]. Considerably less is known about performance deficits in subjects with RLS. So far, there have been four fully published studies on cognitive functioning in RLS patients[33-36]. Taken together, these studies suggest cognitive deficits in in the area of attention and executive functioning in subjects with clinically significant RLS. We have contributed to a further characterisation these deficits by assessing a broad range of cognitive functions in unmedicated RLS subjects and explored potential determinants of cognitive deficits in this patient group (see **CHAPTER II**).

Pathophysiology of RLS

The underlying pathogenesis of RLS is currently unknown[37]. Major hypotheses centre around dopamine and iron while some evidence also implicates the opioid system, spinal cord mechanisms, sexual steroid hormones, peripheral neuropathy, or a possible vascular

genesis. Very recently, results from the first genome-wide association studies have added to the complex picture of RLS pathophysiology.

There is evidence for a role of iron in RLS, mostly based on the involvement of iron insufficiency in cases of secondary RLS (e.g. end stage renal disease, pregnancy and iron deficiency)[38]. In addition, studies using CSF measurements[39], MRI[40] or autopsy material[41] to determine the brain iron status in RLS subjects indicate the influence of a low brain iron content in RLS. Most interestingly, iron is a co-factor of tyrosine hydroxylase, the rate-limiting enzyme for the dopamine synthesis. Thus, iron is needed for dopamine synthesis and in case of deficiency may impair the normal production of dopamine.

The striking pharmacological response to low-dose dopaminergic medications[42] and the worsening of symptoms with dopamine release blocker[43] argues for a primary role of dopamine in the pathophysiology of RLS. However, functional neuroimaging of nigrostriatal dopaminergic dysfunction in patients with idiopathic RLS has produced conflicting results and overall no obvious dopaminergic deficit in RLS[37]. This is supported by pathological examinations in RLS patients where no dopaminergic cell loss was found[41]. Neuroendocrine responses to challenges with dopaminergic agents (inhibition of prolactin, increase in human growth hormone) or dopamine-blocking substances revealed a normal response in the afternoon to a dopamine antagonist[44]. However, neuroendocrine response to a levodopa challenge was more pronounced during the night in comparison to the morning in RLS subjects[45]. This might suggest a hypersensitivity of dopamine receptors at night, the time of maximal expression of RLS symptoms. In summary, the response to dopaminergic agents is probably one of the most closely associated features of RLS. Finding a marker for an altered dopamine system in RLS has proven to be more difficult, and it seems likely that the dopamine system is predominantly involved in the circadian expression of restless legs symptoms.

The most convincing evidence regarding an involvement of the opiate system, is also based on the effectiveness of opioidergic treatment in RLS[46,47]. Challenges of the opiate system in RLS patients[48] showed that administration of naloxone to opiate-treated patients reactivates RLS symptoms, while it has no consistent effect in subjects treated with dopaminergic agents. The challenge of untreated RLS patients with naloxone seems to have no adverse effects on RLS symptoms[48]. Furthermore, untreated RLS patients showed a normal hormonal response (increases in hGH, cortisol, adrenocorticotropic hormone (ACTH)) following naloxone challenge[44]. Current definitions[1] do not include a painful component of RLS sensory symptoms but they recognize that painful sensations can be part of RLS and in independent studies the percentage of RLS patients that described their symptoms as painful ranged from 56%[49] to 85%[50]. Increased pain sensitivity, i.e. static mechanical hyperalgesia, was shown in RLS patients[51]. Interestingly, this increased pain sensitivity was significantly reduced after long-term (1 year) but not short-term levodopa treatment. However, pain sensitivity is also associated with poor sleep and depression[52] and slow wave sleep deprivation[53], all factors present to a certain degree in RLS. In addition, a study with [^{11}C]diprenorphine PET found no difference of opioid binding between RLS subjects and controls, but within the group of RLS subjects opioid receptor binding correlated with RLS severity and questionnaire-based pain scores[54]. Overall, RLS responds to opioidergic agents and it is associated with painful medical conditions. Similar to the dopamine system a specific biomarker for an altered endogenous opiate system in RLS has not been identified.

The involvement of the spinal cord in the pathophysiology of RLS is based on the fact that sensory and motor symptoms are bilateral and segmentally localized in most cases. Possibly either a sensory signal from the periphery to the sensory cortex is affected at the level of the spinal cord or the abnormal input itself is generated at that level. There are several case reports describing a new onset of RLS in close temporal association with spinal pathologies

such as lumbosacral radiculopathy[55], borrelia-induced myelitis[56], transverse myelitis[57], vascular injury of the spinal cord[58], traumatic lesions or cervical spondylotic myelopathy[59]. Interestingly, most of them responded to dopaminergic treatment[56-58]. After spinal anesthesia 9% of 161 patients developed transient new onset RLS[60] although this could not be confirmed in an independent study[61]. Given the high prevalence of RLS the scarcity of the case reports does not argue convincingly in favour of a spinal generator of sensory RLS symptoms. Even in "pure" spinal pathologies such as syringomyelia or syringobulbia where 62% of unselected patients showed PLM none of them had symptoms of RLS[62].The evidence for a role of spinal mechanisms is stronger for PLMS than RLS. In particular, even in completely paraplegic patients PLMS have been observed[63-65], strongly suggesting a spinal origin of PLMS. Interestingly, the known lower occurrence of PLMS during rapid eye movement (REM) sleep is maintained in patients with spinal pathologies and only abolished in patients with complete spinal cord transsections[63,64]. PLMS have been likened to the Babinski sign[66], which in healthy persons is absent during wake and REM-sleep but can be elicited during non-REM (NREM)-sleep[67]. More recently, it has been shown that the elicited flexor reflex and especially its late components are disinhibited in idiopathic RLS patients compared to controls during sleep and wakefulness[68], which has been confirmed in uremic patients[69]. Taken together, there is evidence of a spinal hyperexcitability, which, however, might be more specific to PLMD as opposed to RLS. Whether this phenomenon is specifically located at the spinal cord level or reflects a loss of supraspinal inhibitory influences has not yet been resolved.

Epidemiological studies have shown a markedly higher prevalence of RLS in women[30,70] and this increased risk for RLS in females has been related to the number of pregnancies[70]. Prospective[71], concurrent[72] or retrospective assessment[73] of RLS occurrence during pregnancy suggests that around 25% of females will experience RLS symptoms, with the highest prevalence during the last trimester. Hormonal changes during pregnancy are primarily increases in plasma levels of estrogens, progesteron and prolactin, and Ekbom[8] favoured a hormonal hypothesis regarding the incidence of RLS in pregnancy. While iron and folate requirements during pregnancy are increased and may play a role in the etiology of RLS[71] there is, however, a recent study[74] that followed pregnant women with and without RLS from the 35th week of gestation to approximately 12 weeks postpartum, and found markedly elevated estradiol levels in pregnant women with RLS during late-term pregnancy but not after delivery when subjects were symptom-free. We have explored the hormonal hypothesis of RLS by assessing the prevalence of RLS symptoms in a group of transsexual patients treated with either testosterone or estrogens with the hypothesis that male-to-female transsexual subjects treated with estrogens would report a higher prevalence of RLS symptoms than female-to-male transsexuals treated with testosterone (see **CHAPTER IV**).

Peripheral neuropathy has been implicated as a cause for secondary RLS but the relationship with RLS seems to be complex[75]. It is thought that at the basic perceptual level sensory stimuli are distorted, possibly leading to a hypersensitization of the sensory pathway that may induce a *circulus vitiosus* maintaining restless legs symptoms. Although a greater percentage of RLS patients than previously expected may show subtle abnormalities when examined using electrophysiological or other sophisticated techniques it is also obvious that these abnormalities are not a necessary precondition for the development of RLS[76,77,50]. And although the prevalence of RLS in patients with neuropathy may be higher than expected in the general population, the majority of patients even with severe neuropathy will not develop RLS[78,79]. Whether neuropathy is a sufficient cause in selected patients to trigger or maintain RLS is still open to research.

Ekbom himself decidedly favoured a vascular pathogenesis of RLS predominantly based on the good therapeutic results obtained by the use of two vasodilatative agents (carbachol and

tolazoline) in 23 out of 29 patients[8]. This is reminiscent of the clonidine treatment which has been found to be effective in two double-blind studies in idiopathic[80] and uremic RLS[81]. However, in two large studies with 1566 primary care patients in the UK[82] and 2404 subjects in the US[83] the presence of RLS symptoms was unrelated to the venous reflux or venous obstruction determined by duplex ultrasonography. Like peripheral neuropathy, vascular disturbances can be caused by a multitude of common factors but they also have wide-ranging consequences including peripheral nerve damage. The vascular hypothesis has recently received new scientific interest due to the newly emerging association of RLS and PLMS to heart disease, hypertension and stroke although the exact nature of the relationship is unclear at present[84].

In the idiopathic form of RLS a positive family history is often reported and large pedigrees with familial RLS suggest that the disorder follows a pattern of autosomal dominant inheritance with a high degree of penetrance[85]. Linkage and association studies have identified several loci (on chromosomes 12q, 14q, 9p, 2q, 16p, and 20p) for RLS but no disease-causing gene has been found as yet[85]. Genome-wide association studies have recently identified polymorphisms in three genes with no obvious relationship to dopamine that account for 70% of the population risk for RLS[86,87] (see **CHAPTER V**). A single variant in the BTBD9 gene on chromosome 6 contributes to 50% of the population risk. Although the functions of BTBD9 remain uncertain, its biological plausibility is evidenced by its dose-dependent relationship to periodic limb movements of sleep, decrements in iron stores, and ethnic differences in RLS prevalence.

Treatment of RLS

RLS tends to be a lifelong disorder. There exist many forms regarding the clinical course of the disease, the severity and circadian expression of symptoms as well as associated features, which makes it necessary to tailor RLS therapy to the individual patient. Also, in the idiopathic form, all treatment options are symptomatic and may be necessary for years or even decades. Thus, loss of effectiveness, side effects and augmentation are often encountered with long-term treatment of RLS. Because of the limited disease-specific knowledge current treatment strategies are not curative, but nevertheless may produce an effective and lasting relief of symptoms. Although clinically based treatment has focused on levodopa, opioids and benzodiazepines for a long time, evidence-based and clinical guidelines identify dopamine agonists as a first line treatment for daily restless legs symptoms.

The severity of RLS can differ widely between subjects and is distinguished by such features as frequency and intensity of sensorimotor symptoms, the timing of symptoms during the 24 h day and the association with insomnia. Insomnia might be secondary to RLS, constitute a concomitant disorder that needs specific treatment or may even be caused by the substances used to treat RLS (e.g. levodopa or dopamine agonists[88]).

There are guidelines for the treatment of RLS, in particular the most thorough and evidence-based review and guideline by the Restless Legs Syndrome Task Force of the Standards of Practice Committee of the American Academy of Sleep Medicine (AASM)[42,89]. The latest guidelines were published in 2008 and are based on studies that have been published until December 2006[90]. A recent clinical guideline from the Medical Advisory Board of the Restless Legs Syndrome Foundation has been the very first RLS specialists' consensus approach to a clinical algorithm for the treatment of RLS[91]. In this guideline the clinically useful distinction between intermittent, daily, and refractory RLS is made and different

treatment strategies are recommended. In addition, the guidelines recommend that (i) non-pharmacological approaches are to be considered in every RLS patient, (ii) levodopa constitutes a treatment option for intermittent but not daily or refractory RLS, (iii) high potency opioids are reserved for severe refractory RLS, and (iv) dopamine agonists are the first line treatment. Non-pharmacological options that should be considered include recommending of mental alerting activities at times of restless legs symptoms. Cessation of alcohol, nicotine and caffeine intake could be tried and medications taken by the patient should be critically evaluated since several substances may induce or aggravate RLS. These include dopamine antagonists but also certain antidepressants. Iron status should be considered in every patient.

Today, the established pharmacological options for RLS include levodopa, the dopamine agonists ropinirole, pramipexole, cabergoline, and pergolide and gabapentin[90]. Several other drugs such as rotigotine, bromocriptine, oxycodone, carbamazepine, valproic acid and clonidine have been shown to be efficacious in some studies[92]. The efficiency of oral iron on RLS symptoms depends on the iron status of the subject and the use of intravenous iron is considered investigational at present[90] .

As a disorder RLS is unique in the sense that it responds to both dopaminergic and opiodergic agents which are the two main systems thought to play a crucial role in the physiological response to a placebo[93]. Indeed, in recent treatment trials, a large and lasting placebo effect has been observed with up to 50% of RLS patients reporting a substantial and clinically significant improvement of RLS symptoms during placebo treatment[94-96]. To elucidate this effect, we have conducted a systematic review and meta-analysis[97] that quantified the magnitude of the placebo effect in RLS treatment studies, combining results from studies conducted during the past 25 years (see **CHAPTER III**). We have also explored whether the placebo effect differed between the various outcome modalities assessed in RLS treatment trials such as RLS severity, PLMS index, subjective and objective assessment of sleep and daytime functioning. Our main findings were a placebo effect that was large for the RLS severity measures, moderate for daytime functioning, small to moderate for subjective and objective sleep parameters, and absent for PLMS and sleep efficiency. This has led us to propose that RLS is a model disease to study the mechanisms of the placebo response.

In the following, four studies addressing open questions in the diagnosis, treatment, and pathophysiology of RLS are presented. In particular, these explore cognitive functioning in RLS (Chapter II), the placebo response in RLS treatment studies (Chapter III), the prevalence of RLS in transsexual patients (Chapter IV), and the first genome-wide association study in RLS (Chapter V). The findings of these studies are discussed in the respective chapters and in relation to more recent evidence in the final discussion (Chapter VI).

References

1. Allen, R.P. et al. 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.* **4**, 101-119 (2003).
2. Garcia-Borreguero, D., Egatz, R., Winkelmann, J. & Berger, K. Epidemiology of restless legs syndrome: the current status. *Sleep Med. Rev.* **10**, 153-167 (2006).
3. Willis, T. *De Animae Brutorum*. (Wells and Scott: London, 1672).
4. Willis, T. *The London practice of physick: or the whole practical part of physick contained in the works of Dr. Willis. Faithfully made English, and printed together for the publick good.* (Basset and Crooke: London, 1685).
5. Wittmaack, T. *Pathologie und Therapie der Sensibiliät-Neurosen*. (E. Schäfer: Leipzig, 1861).
6. Allison, F. Obscure pains in the chest, back or limbs. *Can. Med. Assoc. J.* **48**, 36-38 (1943).
7. Ekbom, K. Asthenia crurum paraesthetica ('irritable legs'). New syndrome consisting of weakness, sensation of cold and nocturnal paresthesia in legs, responding to certain extent to treatment with Priscol and Doryl; note on paresthesia in general. *Acta Med.Scand.* **118**, 197-209 (1944).
8. Ekbom, K.A. Restless legs: clinical study of hitherto overlooked disease in legs characterized by peculiar paresthesia ('anxietas tibiarum'), pain and weakness and occuring in two main forms, asthenia crurum paresthetica and asthenia crurum dolorosa; short review of paresthesias in general. *Acta Med. Scand.* **158**, 1-123 (1945).
9. American Academy of Sleep Medicine *The international classification of sleep disorders: diagnostic and coding manual*. (American Academy of Sleep Medicine: Westchester, Illinois, 2005).
10. Akpinar, S. Treatment of restless legs syndrome with levodopa plus benserazide. *Arch. Neurol.* **39**, 739 (1982).
11. Walters, A.S. Toward a better definition of the restless legs syndrome. The International Restless Legs Syndrome Study Group. *Mov. Disord.* **10**, 634-642 (1995).
12. Wetter, T.C., Eisensehr, I. & Trenkwalder, C. Functional neuroimaging studies in restless legs syndrome. *Sleep Med.* **5**, 401-406 (2004).
13. Trenkwalder, C. & Paulus, W. Why do restless legs occur at rest?--pathophysiology of neuronal structures in RLS. Neurophysiology of RLS (part 2). *Clin. Neurophysiol.* **115**, 1975-1988 (2004).
14. Allen, R.P. & Earley, C.J. Restless legs syndrome: a review of clinical and pathophysiologic features. *J. Clin. Neurophysiol.* **18**, 128-147 (2001).
15. Walters, A.S. et al. A questionnaire study of 138 patients with restless legs syndrome: the 'Night-Walkers' survey. *Neurology* **46**, 92-95 (1996).
16. Ondo, W. & Jankovic, J. Restless legs syndrome: clinicoetiologic correlates. *Neurology* **47**, 1435-1441 (1996).
17. Michaud, M., Paquet, J., Lavigne, G. & Desautels, A. Sleep laboratory diagnosis of restless legs syndrome. *Eur. Neurol.* **48**, 108-113 (2002).
18. Stiasny-Kolster, K., Kohnen, R., Möller, J., Trenkwalder, C. & Oertel, W. Validation of the "L-DOPA test" for diagnosis of restless legs syndrome. *Mov.Disord.* **21**, 1333-1339 (2006).
19. Nordlander, N. Therapy in restless legs. *Acta Med.Scand.* **145**, 453-457 (1953).
20. O'Keeffe, S., Gavin, K. & Lavan, J. Iron status and restless legs syndrome in the elderly. *Age Ageing* **23**, 200-203 (1994).
21. Collado-Seidel, V. et al. Clinical and biochemical findings in uremic patients with and without restless legs syndrome. *Am.J.Kidney Dis.* **31**, 324-328 (1998).
22. Wang, D. & Teichtahl, H. Opioids, sleep architecture and sleep-disordered breathing. *Sleep Med. Rev.* **11**, 35-46 (2007).
23. Walters, A. et al. Long-term follow-up on restless legs syndrome patients treated with opioids. *Mov.Disord.* **16**, 1105-1109 (2001).
24. Gemignani, F. et al. Restless legs syndrome and polyneuropathy. *Mov. Disord.* **21**, 1254-1257 (2006).

25. Walters, A.S., Wagner, M. & Hening, W.A. Periodic limb movements as the initial manifestation of restless legs syndrome triggered by lumbosacral radiculopathy. *Sleep* **19**, 825-826 (1996).
26. Walters, A.S., Hening, W., Rubinstein, M. & Chokroverty, S. A clinical and polysomnographic comparison of neuroleptic-induced akathisia and the idiopathic restless legs syndrome. *Sleep* **14**, 339-345 (1991).
27. Rottach, K.G. et al. Restless legs syndrome as side effect of second generation antidepressants. *J. Psychiatr. Res.* **43**, 70-75 (2008).
28. Hoque, R. & Chesson, A.L. Pharmacologically induced/exacerbated restless legs syndrome, periodic limb movements of sleep, and REM behavior disorder/REM sleep without atonia: literature review, qualitative scoring, and comparative analysis. *J. Clin. Sleep Med.* **6**, 79-83 (2010).
29. Abetz, L. et al. Evaluating the quality of life of patients with restless legs syndrome. *Clin. Ther.* **26**, 925-935 (2004).
30. Allen, R.P. et al. Restless legs syndrome prevalence and impact: REST general population study. *Arch. Intern. Med.* **165**, 1286-1292 (2005).
31. Happe, S. et al. Assessing health-related quality of life in patients with restless legs syndrome. *Sleep Med.* **10**, 295-305 (2009).
32. Fulda, S. & Wetter, T.C. Is daytime sleepiness a neglected problem in patients with restless legs syndrome? *Mov. Disord.* **22**, S409-413 (2007).
33. Saletu, B. et al. EEG mapping, psychometric, and polysomnographic studies in restless legs syndrome (RLS) and periodic limb movement disorder (PLMD) patients as compared with normal controls. *Sleep Med.* **3**, S35-42 (2002).
34. Pearson, V.E. et al. Cognitive deficits associated with restless legs syndrome (RLS). *Sleep Med.* **7**, 25-30 (2006).
35. Driver-Dunckley, E. et al. No evidence for cognitive dysfunction or depression in patients with mild restless legs syndrome. *Mov. Disord.* **24**, 1843-1847 (2009).
36. Celle, S. et al. Prevalence and clinical correlates of restless legs syndrome in an elderly French population: the synapse study. *J. Gerontol. A Biol. Sci. Med. Sci.* **65**, 167-173 (2010).
37. Barrière, G., Cazalets, J., Bioulac, B., Tison, F. & Ghorayeb, I. The restless legs syndrome. *Prog. Neurobiol.* **77**, 139-165 (2005).
38. Allen, R.P. & Earley, C.J. The role of iron in restless legs syndrome. *Mov. Disord.* **22**, S440-S448 (2007).
39. Earley, C.J. et al. Abnormalities in CSF concentrations of ferritin and transferrin in restless legs syndrome. *Neurology* **54**, 1698-1700 (2000).
40. Allen, R., Barker, P., Wehrl, F., Song, H. & Earley, C. MRI measurement of brain iron in patients with restless legs syndrome. *Neurology* **56**, 263-265 (2001).
41. Connor, J. et al. Neuropathological examination suggests impaired brain iron acquisition in restless legs syndrome. *Neurology* **61**, 304-309 (2003).
42. Hening, W.A., Allen, R.P., Earley, C.J., Picchietti, D.L. & Silber, M.H. An update on the dopaminergic treatment of restless legs syndrome and periodic limb movement disorder. *Sleep* **27**, 560-583 (2004).
43. Kraus, T., Schuld, A. & Pollmacher, T. Periodic leg movements in sleep and restless legs syndrome probably caused by olanzapine [5]. *J. Clin. Psychopharmacol.* **19**, 478-479 (1999).
44. Winkelmann, Schadrack, Wetter, Zieglgänsberger & Trenkwalder Opioid and dopamine antagonist drug challenges in untreated restless legs syndrome patients. *Sleep Med.* **2**, 57-61 (2001).
45. Garcia-Borreguero, D., Larrosa, O. & de la Llave, Y. Circadian aspects in the pathophysiology of the restless legs syndrome. *Sleep Med.* **3**, S17-21 (2002).
46. Kaplan, P.W., Allen, R.P., Buchholz, D.W. & Walters, J.K. A double-blind, placebo-controlled study of the treatment of periodic limb movements in sleep using carbidopa/levodopa and propoxyphene. *Sleep* **16**, 717-723 (1993).
47. Walters, A.S. et al. Successful treatment of the idiopathic restless legs syndrome in a randomized double-blind trial of oxycodone versus placebo. *Sleep* **16**, 327-332 (1993).
48. Walters, A.S. Review of receptor agonist and antagonist studies relevant to the opiate system in restless legs syndrome. *Sleep Med.* **3**, 301-304 (2002).

49. Bassetti, C.L., Mauerhofer, D., Gugger, M., Mathis, J. & Hess, C.W. Restless legs syndrome: a clinical study of 55 patients. *Eur. Neurol.* **45**, 67-74 (2001).
50. Winkelmann, J. et al. Clinical characteristics and frequency of the hereditary restless legs syndrome in a population of 300 patients. *Sleep* **23**, 597-602 (2000).
51. Stiasny-Kolster, K., Magerl, W., Oertel, W.H., Möller, J.C. & Treede, R. Static mechanical hyperalgesia without dynamic tactile allodynia in patients with restless legs syndrome. *Brain* **127**, 773-782 (2004).
52. Chiu, Y.H. et al. Poor sleep and depression are independently associated with a reduced pain threshold. Results of a population based study. *Pain* **115**, 316-321 (2005).
53. Onen, S.H., Alloui, A., Gross, A., Eschallier, A. & Dubray, C. The effects of total sleep deprivation, selective sleep interruption and sleep recovery on pain tolerance thresholds in healthy subjects. *J. Sleep Res.* **10**, 35-42 (2001).
54. von Spiczak, S. et al. The role of opioids in restless legs syndrome: an [11C]diprenorphine PET study. *Brain* **128**, 906-917 (2005).
55. Walters, A.S., Wagner, M. & Hening, W.A. Periodic limb movements as the initial manifestation of restless legs syndrome triggered by lumbosacral radiculopathy. *Sleep* **19**, 825-826 (1996).
56. Hemmer, B., Riemann, D., Glocker, F.X., Lücking, C.H. & Deuschl, G. Restless legs syndrome after a borrelia-induced myelitis. *Mov. Disord.* **10**, 521-522 (1995).
57. Brown, L.K., Heffner, J.E. & Obbens, E.A. Transverse myelitis associated with restless legs syndrome and periodic movements of sleep responsive to an oral dopaminergic agent but not to intrathecal baclofen. *Sleep* **23**, 591-594 (2000).
58. Tings, T., Baier, P.C., Paulus, W. & Trenkwalder, C. Restless Legs Syndrome induced by impairment of sensory spinal pathways. *J. Neurol.* **250**, 499-500 (2003).
59. Hartmann, M., Pfister, R. & Pfadenhauer, K. Restless legs syndrome associated with spinal cord lesions. *J. Neurol. Neurosurg. Psychiatr.* **66**, 688-689 (1999).
60. Högl, B., Frauscher, B., Seppi, K., Ulmer, H. & Poewe, W. Transient restless legs syndrome after spinal anesthesia: a prospective study. *Neurology* **59**, 1705-1707 (2002).
61. Crozier, T.A., Karimdadian, D. & Happe, S. Restless legs syndrome and spinal anesthesia. *N. Engl. J. Med.* **359**, 2294-2296 (2008).
62. Nogués, M. et al. Periodic limb movements in syringomyelia and syringobulbia. *Mov. Disord.* **15**, 113-119 (2000).
63. Dickel, M.J., Renfrow, S.D., Moore, P.T. & Berry, R.B. Rapid eye movement sleep periodic leg movements in patients with spinal cord injury. *Sleep* **17**, 733-738 (1994).
64. Yokota, T., Hirose, K., Tanabe, H. & Tsukagoshi, H. Sleep-related periodic leg movements (nocturnal myoclonus) due to spinal cord lesion. *J. Neurol. Sci.* **104**, 13-18 (1991).
65. de Mello, M.T., Lauro, F.A., Silva, A.C. & Tufik, S. Incidence of periodic leg movements and of the restless legs syndrome during sleep following acute physical activity in spinal cord injury subjects. *Spinal Cord* **34**, 294-296 (1996).
66. Smith, R.C. Relationship of periodic movements in sleep (nocturnal myoclonus) and the Babinski sign. *Sleep* **8**, 239-243 (1985).
67. Fujiki, A., Shimizu, A., Yamada, Y., Yamamoto, J. & Kaneko, Z. The Babinski reflex during sleep and wakefulness. *Electroencephalogr. Clin. Neurophysiol.* **31**, 610-613 (1971).
68. Bara-Jimenez, W., Aksu, M., Graham, B., Sato, S. & Hallett, M. Periodic limb movements in sleep: state-dependent excitability of the spinal flexor reflex. *Neurology* **54**, 1609-1616 (2000).
69. Aksu, M. & Bara-Jimenez, W. State dependent excitability changes of spinal flexor reflex in patients with restless legs syndrome secondary to chronic renal failure. *Sleep Med.* **3**, 427-430 (2002).
70. Berger, K., Luedemann, J., Trenkwalder, C., John, U. & Kessler, C. Sex and the risk of restless legs syndrome in the general population. *Arch. Intern. Med.* **164**, 196-202 (2004).
71. Lee, K.A., Zaffke, M.E. & Baratte-Beebe, K. Restless legs syndrome and sleep disturbance during pregnancy: the role of folate and iron. *J. Womens Health Gend. Based Med.* **10**, 335-341 (2001).

72. Suzuki, K. et al. The prevalence of restless legs syndrome among pregnant women in Japan and the relationship between restless legs syndrome and sleep problems. *Sleep* **26**, 673-677 (2003).
73. Manconi, M. et al. Restless legs syndrome and pregnancy. *Neurology* **63**, 1065-1069 (2004).
74. Dzaja, A., Wehrle, R., Lancel, M. & Pollmächer, T. Elevated estradiol plasma levels in women with restless legs during pregnancy. *Sleep* **32**, 169-174 (2009).
75. Pourfar, M. & Feigin, A. The numb and the restless: Peripheral neuropathy and RLS. *Neurology* **72**, 950-951 (2009).
76. Polydefkis, M. et al. Subclinical sensory neuropathy in late-onset restless legs syndrome. *Neurology* **55**, 1115-1121 (2000).
77. Machtey, I. Epidemiology of restless legs syndrome. *Arch. Intern. Med.* **161**, 483-484 (2001).
78. Rutkove, S.B., Matheson, J.K. & Logigian, E.L. Restless legs syndrome in patients with polyneuropathy. *Muscle Nerve* **19**, 670-672 (1996).
79. Hattan, E., Chalk, C. & Postuma, R.B. Is there a higher risk of restless legs syndrome in peripheral neuropathy? *Neurology* **72**, 955-960 (2009).
80. Wagner, M.L. et al. Randomized, double-blind, placebo-controlled study of clonidine in restless legs syndrome. *Sleep* **19**, 52-58 (1996).
81. Ausserwinkler, M. & Schmidt, P. Erfolgreiche Behandlung des "restless legs"-Syndroms bei chronischer Niereninsuffizienz mit Clonidin. *Schweiz. Med. Wochenschr.* **119**, 184-186 (1989).
82. Bradbury, A. et al. The relationship between lower limb symptoms and superficial and deep venous reflux on duplex ultrasonography: The Edinburgh Vein Study. *J. Vasc. Surg.* **32**, 921-931 (2000).
83. Langer, R.D. et al. Relationships between symptoms and venous disease: the San Diego population study. *Arch. Intern. Med.* **165**, 1420-1424 (2005).
84. Walters, A.S. & Rye, D.B. Review of the relationship of restless legs syndrome and periodic limb movements in sleep to hypertension, heart disease, and stroke. *Sleep* **32**, 589-597 (2009).
85. Winkelmann, J. et al. Genetics of restless legs syndrome (RLS): State-of-the-art and future directions. *Mov. Disord.* **22**, S449-458 (2007).
86. Winkelmann, J. et al. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. *Nat. Genet.* **39**, 1000-1006 (2007).
87. Stefansson, H. et al. A genetic risk factor for periodic limb movements in sleep. *N. Engl. J. Med.* **357**, 639-647 (2007).
88. Fulda, S. & Wetter, T.C. Dopamine agonists for the treatment of restless legs syndrome. *Expert Opin. Pharmacother.* **6**, 2655-2666 (2005).
89. Littner, M.R. et al. Practice parameters for the dopaminergic treatment of restless legs syndrome and periodic limb movement disorder. *Sleep* **27**, 557-559 (2004).
90. Trenkwalder, C. et al. Treatment of restless legs syndrome: an evidence-based review and implications for clinical practice. *Mov. Disord.* **23**, 2267-2302 (2008).
91. Silber, M.H. et al. An algorithm for the management of restless legs syndrome. *Mayo Clin. Proc.* **79**, 916-922 (2004).
92. Fulda, S. & Wetter, T.C. Emerging drugs for restless legs syndrome. *Expert Opin. Emerg. Drugs* **10**, 537-552 (2005).
93. Colloca, L., Lopiano, L., Benedetti, F. & Lanotte, M. Placebo response in conditions other than pain. *Semin. Pain Med.* **3**, 43-47 (2005).
94. Allen, R. et al. Ropinirole decreases periodic leg movements and improves sleep parameters in patients with restless legs syndrome. *Sleep* **27**, 907-914 (2004).
95. Trenkwalder, C. et al. Ropinirole in the treatment of restless legs syndrome: results from the TREAT RLS 1 study, a 12 week, randomised, placebo controlled study in 10 European countries. *J. Neurol. Neurosurg. Psychiatr.* **75**, 92-97 (2004).
96. Walters, A. et al. Ropinirole is effective in the treatment of restless legs syndrome. TREAT RLS 2: a 12-week, double-blind, randomized, parallel-group, placebo-controlled study. *Mov.Disord.* **19**, 1414-1423 (2004).
97. Fulda, S. & Wetter, T.C. Where dopamine meets opioids: a meta-analysis of the placebo effect in restless legs syndrome treatment studies. *Brain* **131**, 902-917 (2008).

II. Short-term attention and verbal fluency is decreased in restless legs syndrome patients*

This is a pre-copy-editing, author-produced PDF of an article accepted for publication in Movement Disorders *following peer review. The definitive publisher-authenticated version [Short-term attention and verbal fluency is decreased in restless legs syndrome patients. Stephany Fulda, Marie E. Beitinger, Simone Reppermund, Juliane Winkelmann, Thomas C. Wetter. Movement Disorders 2010; DOI 10.1002/mds.23353*] *is available at: www3.interscience.wiley.com*

Abstract

Restless legs syndrome (RLS) is a frequent sleep-related movement disorder with disturbed sleep and quality of life. RLS patients complain about increased daytime sleepiness but there are only few and inconsistent reports about cognitive functioning in this group. We compared cognitive performance of 23 unmedicated RLS patients to that of 23 healthy controls matched individually for age, gender, and educational level. Cognitive tasks were chosen to assess short term attention, working memory, learning and memory, verbal fluency, and executive functioning. RLS patients performed worse than controls in the area of attention and verbal fluency and performance in these tasks was associated with RLS severity, sleep quality, depression scores, and iron status. There was no difference for working memory, memory, learning, cognitive flexibility, and abstract reasoning. We conclude that there is evidence for deficits in short-term attention and verbal fluency in RLS patients.

Introduction

The restless legs syndrome (RLS) is a neurological disorder characterized by the urge to move the extremities associated with paraesthesias which are partially or totally relieved by movement, a worsening of symptoms at rest and in the evening or at night and, as a consequence, sleep disturbances[1,2]. RLS is a frequent disorder and epidemiological studies show a rather consistent prevalence of 3 to 10% for lifetime occurrence of RLS in European and North-American populations[3]. Commonly distinguished within the population exhibiting RLS symptoms are so-called RLS sufferers in whom RLS symptoms occur on a frequent basis (e.g. at least two times a week) and with at least moderate distress[4,5]. Indeed, several studies have shown that RLS is associated with a significantly reduced quality of life, and within the different domains of quality of life the areas “energy / sleepiness” and “performance” are particularly impaired[5-8]. Independent investigations in different populations such as the general population or sleep lab populations revealed that about one third of RLS

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subjects perceive themselves as being excessively sleepy during the daytime (reviewed in[9]). Considerably less is known about performance deficits in subjects with RLS. So far, there have been four published studies on cognitive functioning in RLS patients[10-13], although several other research groups, including ours, have reported preliminary data at scientific meetings[14-17]. Taken together, these studies showed cognitive deficits in the area of attention and executive functioning in subjects with clinically significant RLS[10,11,13] but not in subjects with mild RLS[12]. The aim of the present study was to further characterize cognitive deficits in unmedicated RLS subjects by assessing a broad range of cognitive functions and to increase the specificity of findings by carefully matching healthy controls from a general population sample on a one-to-one basis according to age, gender, and educational level. In addition, we explored potential determinants of cognitive deficits in this patient group.

Methods

Subjects

We included consecutive unmedicated subjects with RLS visiting the RLS outpatient clinic at the Max Planck Institute of Psychiatry, Munich. RLS was unequivocally diagnosed according to established criteria[1] by an experienced RLS expert. Polysomnography was conducted in seven patients according to clinical need. Exclusion criteria were any psychiatric disorder, any neurological or brain disorder or other sleep disorder suspected to interfere with the cognitive performance, and any severe and untreated medical conditions. Patients had to be free of any RLS medication, hypnotics or narcotics for at least two weeks; stable medication intake for chronic conditions such as hypertension were allowed.

Control subjects were recruited from a general population study in which participants were randomly drawn from community registries of all residents aged between 18 and 75 years living in the metropolitan area of Munich. Inclusion criteria for the general population study were (1) European descent, (2) no professional psychological help-seeking at any time, and (3) no severe somatic disorder. Subjects were rated in a face-to-face interview using a computer-assisted diagnostic interview (modified version of the Munich-Composite-International-Diagnostic Interview, DIA-X/M-CIDI[18]). All persons with a lifetime history of alcohol dependence, drug abuse or dependence, possible psychotic disorder, mood disorder, anxiety disorder including obsessive-compulsive disorder and post-traumatic stress disorder, somatoform disorder, dissociative disorder not otherwise specified, and eating disorder according to the M-CIDI interview were excluded. Subjects older than 65 years were additionally tested using the Mini Mental State Examination (MMSE), and were excluded from further procedures if having a score of less than 27. Controls were selected from this sample according to age (± 3 years), gender, and educational level to match individual RLS subjects. The study was approved by the local ethics committee and all participants gave informed written consent.

Procedure

All subjects completed the Pittsburgh Sleep Quality Index (PSQI[19]), the Epworth Sleepiness Scale (ESS[20]), the symptom check list SCL-90-R[21,22], the Beck Depression Inventory (BDI[23]), and the state-trait anxiety inventory (STAI[24]). Subjects with RLS also completed the international RLS study group scale (IRLS[25,26]).

Table 1. Description of neuropsychological tasks

Name (Short name)	Cognitive function / Description
Attention	
D2 Cancellation test (d2)[27] [*Aufmerksamkeits-Belastungstest d2*]	Selective visual attention Paper-and-pencil speed cancellation test where subjects are asked to search for the target stimulus among non-targets. The performance score is calculated as the number of correctly canceled items within 4:40 minutes.
Trail-Making Test (ZVT)[28] [*Zahlen-Verbindungs-Test*]	Focused attention Paper-and-pencil test similar to the Trail-MakingTest A[28]. The subject has to connect as quickly as possible the numbers 1 to 90 which are arranged pseudo-randomly on a sheet of paper. Two matrices were administered (ZVT-A, ZVT-B), and the average time of both matrices was used as a measure of focused attention.
Digit span forward Block span forward[30]	Attention span From the revised German version of the Wechsler Memory Scale (WMS-R[29]). For the digit span the subject has to recall digit sequences of increasing length that are read out by the examiner. For the block span sequences of increasing lengths are tapped by the examiner and the participant has to tap the blocks in the same order. The number of correctly reproduced sequences is used as test score.
Stroop test[31,32]	Selective attention The test consists of three sub-tasks where the subject is required to (i) read names of colors written in black ink, (ii) name the color of squares printed in different colors, and (iii) name the color of color name words printed in both congruent colors (i.e. “green” printed in green ink) and incongruent colors (i.e. “green” printed in red ink). The difference between the (log-transformed) time for color naming of squares and the (log-transformed) time for color naming of the ink of a color word is taken as a measure of the Stroop effect.
Memory	
Digit span backward Block span backward[30]	Working memory The subject is confronted with sequences of increasing lengths and has to reproduce them in the backward order. The number of sequences correctly reproduced backward is used as the test score.
Munich Verbal Memory Test (MVG)[33] [*Münchner Verbaler Gedächtnistest*]	Learning and memory German version of the California Verbal Learning test (CVLT[33]). Sixteen items are presented once and according to a selective reminding procedure for 4 consecutive trials with immediate recall after each. Following the presentation and immediate recall of another 16-item interference list, free and category cued recall trials are presented after short and long delays (45 - 60 minutes). Scores used in the present analysis are immediate recall from trial 1, learning performance represented as recall from the last trial 5, short-term and long-term free recall.
Executive functioning	
Regensburg Verbal Fluency Test (RWT)[35] - Subtasks 1 and 2 [*Regensburger Wortflüssigkeitstest*]	Verbal fluency The test asks the subject to name as many words as possible from a category within 2 minutes. This category is phonemic (e.g. words that begin with the letter “p”) or semantic (e.g. “animals” or “fruits”).
Regensburg Verbal Fluency Test (RWT)[35] - Subtasks 3 and 4	Cognitive flexibility and set shifting Two further subtasks of the RWT ask the subject again to name as many words as possible, but this time words have to come from alternating categories (e.g. letters “a” and “t” or categories “sport” and “clothing”). For each task, the total number of correct words produced within 2 minutes was taken as a measure of cognitive flexibility.
Raven's Progressive Matrices (RPM)[36,37]	Abstract reasoning RPM are multiple-choice tests of abstract reasoning, originally developed by Raven in 1936[387]. The subject to whom five sets (A to E) of items are presented is asked to identify, among several choices, the missing segment required to complete a larger pattern. Difficulty increases from sets A to E. For the present study, all subjects had 45 minutes to complete all five sets, and the measured outcome was the number of correct choices per set and the total number of correct choices.

Neuropsychological Testing

All subjects were tested in the morning for approximately 2 hours between 08:00 and 13:00 hour and all RLS subjects were symptom-free at the time of testing. Cognitive tasks are described in Table 1 and were selected to assess a broad range of cognitive functions including short-term attention[27-32], memory[30,33,34], verbal fluency[35], and executive functions[35-38]. In addition, all subjects completed a standard vocabulary test (Mehrfachwahlwortschatztest, MWT-B[39]) to compare overall pre-morbid intelligence.

Performance Scores and Statistical Analysis

For the statistical analysis of neuropsychological performance, raw performance scores were transformed to z-scores relative to German age- and/or gender-specific normative data[27,28,30,31,33,35,36].

Neuropsychological testing yielded a total of 16 z-scores for each subject. To control for overall error level these were grouped into six domains (attention, working memory, memory, verbal fluency, cognitive flexibility, and abstract reasoning) and multivariate analysis of variance (mANOVA) was employed for each domain to explore overall differences between RLS subjects and controls. Only significant multivariate effects were followed up by univariate ANOVAs. Because controls were matched individually to the respective RLS subject, the group factor (RLS vs. control) was implemented as a repeated-measures factor.

Table 2. Description of RLS subjects.

	Mean ± SD (Range) n = 23
IRLS	23.52 ± 7.40 (10 - 37)
Age of onset, years (n = 22)	34.95 ± 15.90 (10 - 69)
Age of onset ≤ 45 years (n, %)	16 (70%)
Familial RLS (n, %)	6 (26%)
Possibly familial RLS (n, %)	4 (17%)
Sporadic RLS (n, %)	12 (52%)
Family history unknown (n, %)	1 (4%)
Medication status before study*	
De novo (n, %)	9 (39%)
De novo + L-dopa test (n, %)	7 (30%)
Dopaminergic medication (n, %)	6 (26%)
Other RLS medication (n, %)	1 (4%)
Ferritin (ng/ml) (n = 17)	116.47± 108.12 (8 - 457)
Ferritin < 20 ng/ml (n, %) (n = 17)	4 (23%)
Ferritin < 50 ng/ml (n, %) (n = 17)	7 (41%)

IRLS = International RLS severity scale
*All patients were medication-free for at least 14 days at the time of cognitive testing.

Data was missing from one control subject for Raven's Progressive Matrices and the respective patient-control pair was excluded from the analysis for this task. To standardize case-control differences across measures, Cohen's *d*, an effect size measure[40], was computed for all variables and is listed in Table 2 for descriptive purposes. Conventionally, for Cohen's *d* an effect size of 0.2 to 0.3 is considered a "small" effect, around 0.5 a "medium" effect and 0.8 or larger, a "large" effect[40]. Differences between RLS patients and controls regarding demographic characteristics, sleep, and mood-related information were explored with paired t-tests and Chi^2-tests as appropriate. Correlations and t-tests were used to assess age, RLS severity, subjective sleep duration, sleep quality, daytime sleepiness, and ferritin levels as potential determinants of cognitive dysfunction in RLS subjects.

Table 3. Description of study participants.

	RLS Mean ± SD (Range) **n = 23**	**Control** Mean ± SD (Range) **n = 23**	**Test statistics, p**
Age, years	54.91 ± 12.17 (20 - 73)	54.70 ± 12.94 (19 - 73)	$t_{(df=22)}$ = 0.69 p = 0.496
Male/female (n)	9/14	9/14	
Vocabulary (MWT-B)	30.61 ± 4.38 (20 - 36)	31.83 ± 4.36 (19 - 37)	$t_{(df=22)}$ = - 1.41 p = 0.172
Beck Depression Inventory	8.35 ± 6.23 (1 - 26)	2.61 ± 2.72 (0 - 9)	$t_{(df=22)}$ = 3.56 **p = 0.002**
STAI-State	39.48 ± 9.44 (25 - 67)	36.43 ± 10.21 (24 - 56)	$t_{(df=22)}$ = 0.99 p = 0.331
STAI-Trait	37.56 ± 8.66 (26 - 57)	33.13 ± 7.19 (24 - 46)	$t_{(df=22)}$ = 1.72 p = 0.099
SCL-90R Global severity index	0.46 ± 0.29 (0.01 - 1.12)	0.16 ± 0.10 (0.02 - 0.33)	$t_{(df=22)}$ = 4.46 **p < 0.001**
SCL-90R Positive symptom distress index	1.45 ± 0.23 (1.00 - 1.79)	1.11 ± 0.20 (1.00 - 1.67)	$t_{(df=22)}$ = 5.38 **p < 0.001**
SCL-90R Positive symptom total	27.30 ± 16.39 (1.00 - 60.00)	13.30 ± 8.42 (2.00 - 29.00)	$t_{(df=22)}$ = 3.58 **p = 0.002**
PSQI	10.91 ± 4.83 (2 - 20)	3.43 ± 1.93 (1 - 7)	$t_{(df=22)}$ = 6.52 **p < 0.001**
PSQI > 5 (n, %)	18 (78%)	4 (17%)	$Chi^2_{(df=1)}$ = 14.72 **p < 0.001**
Sleep duration, hours (PSQI)	5:30 ± 1:12 (3:30 - 7:30)	7:08 ± 0:47 (06:00 - 09:00)	$t_{(df=22)}$ = -5.48 **p < 0.001**
ESS	9.65 ± 5.38 (0 - 17)	5.83 ± 2.95 (1 - 11)	$t_{(df=22)}$ = 3.63 **p = 0.001**
ESS > 11 (n, %)	11 (48%)	0 (0%)	$Chi^2_{(df=1)}$ = 11.95 **p < 0.001**

MWT-B = Mehrfachwahl-Wortschatztest; ESS = Epworth Sleepiness Scale; PSQI = Pittsburgh Sleep Quality Index; SCL-90R = Symptom Check List 90, revised; STAI = State-Trait Anxiety Inventory

Results

A total of 27 RLS subjects participated in the study. From these four subjects were excluded: one subject because of a formerly undisclosed history of brain infarction, and three subjects (aged 70 to 79 years) because we were unable to locate matching control subjects. The final study group comprised 23 subjects with RLS (9 males, 14 females) aged 20 to 73 years (see Tables 2 and 3). The age of onset of RLS symptoms was between 10 and 69 years of age and was below the age of 45 in 16 of the patients (69%). More than half of the patients (n = 16, 69%) had never been treated for RLS, although seven of these had a single L-dopa test to which all responded positive. Six RLS subjects had taken dopaminergic medication in the past, one subject had used gabapentin for several weeks. All were medication-free for at least 14 days before the study. Ferritin levels were available for 17 of the RLS subjects (74%) and were below 50 ng/ml for seven subjects (41%), four of which had levels below 20 ng/ml (23%).

Although none of the RLS subjects had a psychiatric disorder, self-report questionnaires revealed elevated depression scores (BDI) compared to the control group (Table 3). Self-reported state anxiety did not differ between patients and controls, while there was a trend ($p < 0.10$) towards increased trait anxiety in RLS subjects. Also, overall psychological distress (SCL-90R), the number of self-reported symptoms and their intensity were increased in RLS subjects compared to controls.

Sleep quality was significantly worse in RLS subjects and more RLS subjects showed PSQI values above the threshold of five (78% vs. 17%, Table 3). Self-reported sleep duration was significantly shorter in patients, and daytime sleepiness (ESS) was increased in the RLS group, with half of them (48%) showing increased daytime sleepiness (ESS > 11) while none of the control group did (Table 3).

Multivariate analysis revealed that cognitive functioning differed between RLS subjects and controls in the area of attention and verbal fluency (Table 4). There was no overall difference between the groups in tasks assessing working memory, learning and memory, cognitive flexibility, and abstract reasoning. Within the attention domain, RLS subjects performed worse than controls in the d2-cancellation task and the Stroop task, which evaluate short-term selective visual attention and vulnerability to interference. In the verbal fluency domain, both letter and category fluency were reduced in RLS subjects. Effect sizes (ES) indicated that these differences ranged from medium (around 0.5, d2-cancellation, verbal and category fluency) to large (0.8, Stroop task, Table 4). Because RLS subjects differed from controls in depression scores, we repeated the analysis for the d2-cancellation task, the Stroop task and verbal fluency in a restricted sample of 15 RLS subjects with BDI scores within the normal range (0-9). A difference between patients and controls was no longer apparent for the d2-cancellation task (ES: 0.39) and category fluency (ES: 0.36) while deficits in the Stroop task (ES: 1.01; $F_{1,14}=8.076$, $p = 0.013$) and letter fluency (ES: 0.72, $F_{1,14}=9.467$, $p = 0.008$) became even more pronounced.

To explore potential determinants of these reduced task performances we correlated RLS subjects' z-scores in the four tasks with age, RLS severity, sleep quality, subjective sleep duration, daytime sleepiness, depression, and psychopathology scores. We also investigated whether task performance differed between patients with impaired sleep quality (PSQI > 10), increased daytime sleepiness (ESS > 11) or low ferritin levels (< 50 ng/ml) versus patients with normal values in the respective measurements. Task performance did not correlate with age, sleep quality, sleep duration, daytime sleepiness, or psychopathology scores ($rho < 0.360$, $p > 0.09$). IRLS scores were significantly associated with performance in the d2-cancellation task ($rho = -0.440$, $p = 0.036$). Furthermore, both depression scores ($rho = -0.531$, $p = 0.011$) and IRLS scores correlated with category fluency ($rho = -0.612$, $p = 0.002$).

There was, however, a strong association between depression scores and RLS severity (rho = 0.669, p < 0.001). Partial correlations revealed that the association between IRLS scores and category fluency was distinctly attenuated when controlling for depression scores (r = -0.350, p = 0.110) and *vice versa* (correlation of depression scores with category fluency controlling for IRLS scores: r = -0.295, p = 0.183).

Table 4. Cognitive functions in RLS subjects and matched controls (z-scores).

	RLS **Mean ± SD** **n = 23**	**Control** **Mean ± SD** **n = 23**	**Test statistics, p**	**Effect size**
Attention			$F_{(5,18)} = 3.22$ **p = 0.030**	
D2 Cancellation Test	-0.37 ± 1.05	0.09 ± 0.85	$F_{(1,22)} = 5.83$ **p= 0.025**	0.49
Trail-Making Test (ZVT)	**0.02 ± 1.28**	0.01 ± 0.77	$F_{(1,22)} = 0.01$ p= 0.963	-0.01
Digit span forward	-0.05 ± 0.75	0.26 ± 1.10	$F_{(1,22)} = 1.67$ p= 0.209	0.34
Block span forward	0.63 ± 0.84	0.69 ± 0.98	$F_{(1,22)} = 0.43$ p= 0.837	0.07
Stroop task	-0.27 ± 1.03	0.41 ± 0.71	$F_{(1,22)} = 8.14$ **p= 0.009**	0.78
Working memory			$F_{(2,21)} = 0.35$ p = 0.710	
Digit span backward	0.24 ± 0.97	0.19 ± 0.95		-0,05
Block span backward	0.52 ± 0.97	0.73 ± 0.91		0.23
Memory (MVG)			$F_{(4,19)} = 0.33$ p = 0.854	
Immediate recall	-0.11 ± 1.32	-0.02 ± 0.90		0.08
Learning	0.66 ± 0.87	0.69 ± 0.82		0.03
Delayed recall (short)	0.58 ± 0.93	0.49 ± 0.98		-0.10
Delayed recall (long)	0.69 ± 0.81	0.49 ± 0.98		-0.23
Verbal fluency (RWT)			$F_{(2,21)} = 3.88$ **p = 0.037**	
Letter fluency	-0.20 ± 0.99	0.29 ± 0.86	$F_{(1,22)} = 4.77$ **p = 0.040**	0.54
Category fluency	0.12 ± 1.02	0.56 ± 0.86	$F_{(1,22)} = 4.55$ **p = 0.047**	0.48
Cognitive flexibility (RWT)			$F_{(2,21)} = 2.25$ p = 0.130	
Alternating letters fluency	-0.16 ± 1.04	0.14 ± 1.04		0.29
Alternating categories fluency	0.11 ± 0.74	0.50 ± 0.82		0.51
Abstract reasoning				
Raven matrices (n = 22)	1.27 ± 0.62	1.29 ± 0.55	$F_{(1,21)} = 0.03$ p = 0.869	0.09

MVG = Münchner Verbaler Gedächtnistest; RWT = Regensburger Wortflüssigkeitstest; ZVT = Zahlenverbindungstest

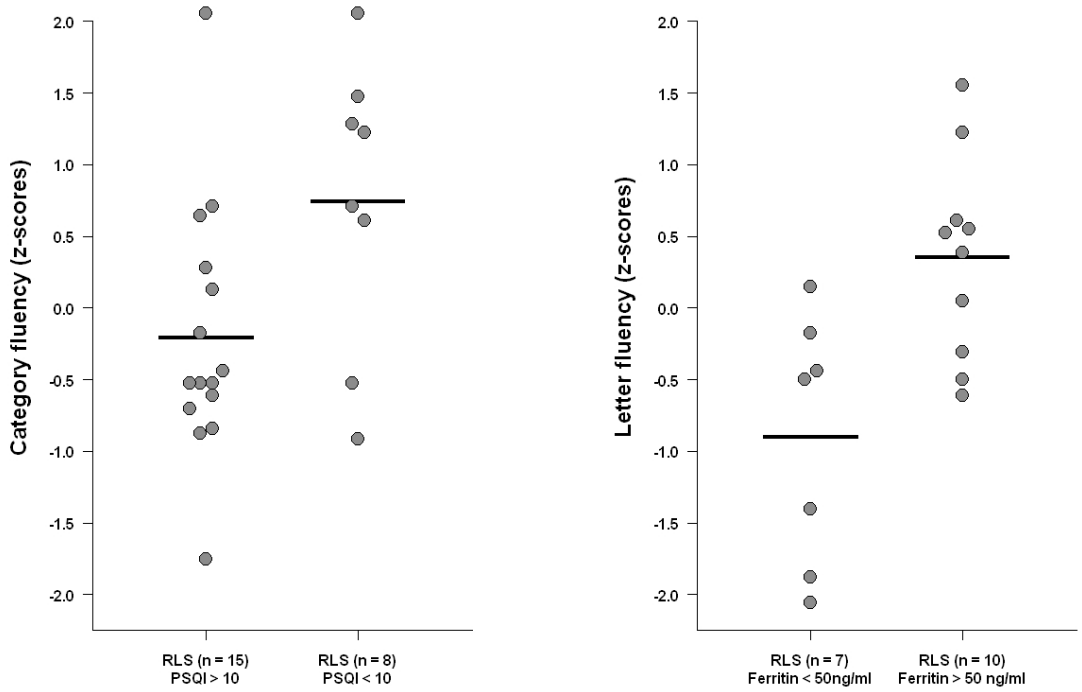

Fig. 1. Left panel: Significant differences in semantic verbal fluency (measured in z-scores) between RLS subjects with more impaired sleep quality (PSQI score > 10) and RLS subjects with less impaired sleep quality (PSQI score < 10). Right panel: Significant differences in phonemic verbal fluency (measured in z-scores) between RLS subjects with low ferritin levels (< 50 ng/ml) and those with normal ferritin levels. The z-scores represent in standard deviation units the extent to which a raw performance score deviates relative to German age- and/or gender-specific normative data and show a mean of 0 and standard deviation of 1.

Patients with more severely impaired sleep quality (PSQI > 10, n = 15) showed a significantly lower performance in the category fluency task than patients with better sleep quality (-0.21±0.89 vs. 0.74±1.01, $t_{(df=21)}$ = 2.335, p = 0.030, Figure 2). Finally, for 17 of the RLS subjects ferritin levels were available, and within this group those with low ferritin (< 50 ng/ml, n = 7) showed a worse performance in the letter fluency task (-0.90±0.87 vs. 0.35±0.71, $t_{(df=15)}$ = 3.260, p = 0.005, Figure 2).

Discussion

We compared cognitive functioning of RLS patients to that of individually matched controls and found a decreased verbal fluency and selective attention and increased vulnerability to So far, there have been only four previous studies exploring cognitive functioning in subjects with RLS. Saletu and co-workers[10] found that in 12 unmedicated patients with idiopathic RLS and matched controls fine motor activity of the left hand was decreased, reaction time was slower, and reaction time variability and errors of omission were increased in patients. The study of the group of Drs. Allen and Early[11] assessed attention, verbal fluency, and executive functioning in 16 unmedicated patients with idiopathic RLS and healthy controls. Patients performed worse on the Trail-Making Test B and the category verbal fluency task compared

to controls. In the largest study to date, Celle and co-workers[13] reported cognitive functioning in RLS subjects in an elderly French population (all 68±1 years). There was no difference between 77 subjects with RLS and 241 subjects without RLS in episodic memory, visual memory, digit span, trail making tests, and a test of reasoning. However, RLS subjects performed worse than controls in verbal fluency and the Stroop task. Recently, a retrospective study[12] found no differences in a wide range of cognitive performance measures between 26 elderly subjects with RLS and 208 control subjects (average age: 77 years). However, in this group RLS severity was very mild (average IRLS score: 11), RLS medications were not withdrawn for testing, concurrent depression was not excluded and antidepressant and benzodiazepine use was permitted, which limits the comparability with previous studies and impedes the interpretation of negative findings.

The overlap with the study of Saletu and co-workers[10] is small but nevertheless the numerical memory tasks of this study are similar to the digit span employed in the present study, and in both studies no difference between RLS subjects and controls was found. Our finding of reduced verbal fluency matches those reported by Pearson et al.[11] and Celle et al.[13]. In the present study and the study of Celle et al. both letter and category fluency were affected while in the study of Pearson et al. a significant difference was reported only for category fluency. Since in the latter work there was a trend ($p = 0.09$) also towards reduced letter fluency, the smaller sample size and reduced statistical power could explain this difference. Both studies[11,13] also employed a version of the Trail-Making task A, a measure of attention, and like the present study found no difference. Likewise, performance in Raven's Progressive Matrices, a measure of reasoning, did not differ between RLS subjects and controls in Pearson's and the present study. All three studies, however, also employed the Stroop task. Pearson et al. reported no differences in performance, Celle et al. found that RLS subjects were slower in word and color naming but not the color word naming, and we found a strong effect for this task. Potential explanations for these varying findings are differences in the samples employed or the specific scoring of the Stroop task. We have analyzed z-scores of performance, while the other two studies computed the raw difference between color naming and color word naming. However, in our sample also the raw performance scores for the Stroop task differed significantly between RLS subjects and controls (39.83±18.59 vs. 28.26±11.10; $t_{df=22} = 3.22$, $p = 0.004$; effect size $d = 0.77$). On average RLS subjects were 10 years older in Pearson's study and 14 years older in the French study. The difference in the average age of subjects could suggest that the Stroop task is more sensitive in a middle-aged as opposed to an elderly population, perhaps because the strong age-related decrease in performance obscures group differences[41].

In post-hoc analyzes we found that RLS severity was associated with selective attention and ferritin status with letter fluency which had not been documented before. Iron deficiency can affect cognitive performance also in adults[42-44], and the potential influence of iron status on cognitive performance in RLS subjects warrants further systematic investigation. For category fluency we observed a strong association with both RLS severity and depression scores, and RLS subjects with more severely impaired sleep quality showed decreased category fluency. All three factors, however, were not independent from each other suggesting that the influence of RLS severity on cognitive functioning was mediated by mood and sleep quality and vice versa. To disentangle the separate contribution of these factors a larger group with a broader range of symptoms would be needed. Our subgroup analysis in patients without elevated depression scores showed, however, that cognitive deficits in RLS patients are still evident in this group.

Potential limitations of the present study are sample selection and study design. We included consecutive RLS outpatients and thus the sample was representative of RLS sufferers, i.e. patients with severe and frequent RLS symptoms, at least severe enough to seek

professional help. However, RLS subjects had to be medication-free for at least 2 weeks, and this might have introduced a bias towards less severely affected patients. By including consecutive patients we also increased heterogeneity of the sample with regard to age, RLS severity, treatment history, and other factors that thereby might have obscured patient-control differences. We did not record objective data documenting sleep disturbances and periodic limb movements or the absence of sleep disorders in the control group. While both patients and controls were interviewed by a sleep specialist, sleep disorders cannot be ruled out with complete certainty without polysomnography. It also precluded us from associating objective indices of sleep disturbances with cognitive functioning in RLS, a topic that merits further investigation.

In summary, subjects with RLS showed specific and significant impairments in selective attention, vulnerability to interference, and verbal fluency, which are partly influenced by RLS severity, sleep disturbances, and iron status. Further research is needed to replicate these determining factors, which could then be used to identify RLS patients at risk for cognitive dysfunctions.

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Authors' roles

1. Research project: A. Conception, B. Organization, C. Execution;
2. Statistical Analysis: A. Design, B. Execution, C. Review and Critique
3. Manuscript: A. Writing of the first draft, B. Review and Critique

Fulda 1A, 1B, 1C, 2A, 2B, 3A; Beitinger 1B, 2C, 3A; Reppermund 1B, 2C, 3B; Wetter 1A, 2C, 3B; Winkelmann 1A, 1B, 1C, 2C, 3B.

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REFERENCES

1. Allen RP, Picchietti D, Hening WA, et al. 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;4:101-119.
2. Walters AS. Toward a better definition of the restless legs syndrome. The International Restless Legs Syndrome Study Group. Mov Disord 1995;10:634-642.
3. Garcia-Borreguero D, Egatz R, Winkelmann J, Berger K. Epidemiology of restless legs syndrome: the current status. Sleep Med Rev 2006;10:153-167.
4. Hening W, Walters AS, Allen RP, et al. Impact, diagnosis and treatment of restless legs syndrome (RLS) in a primary care population: the REST (RLS epidemiology, symptoms, and treatment) primary care study. Sleep Med 2004;5:237-246.
5. Allen RP, Walters AS, Montplaisir J, et al. Restless legs syndrome prevalence and impact: REST general population study. Arch. Intern. Med. 2005;165:1286-1292.
6. Abetz L, Allen R, Follet A, et al. Evaluating the quality of life of patients with restless legs syndrome. Clin Ther 2004;26:925-935.
7. Happe S, Reese JP, Stiasny-Kolster K, et al. Assessing health-related quality of life in patients with restless legs syndrome. Sleep Med 2009;10:295-305.
8. Kushida C, Martin M, Nikam P, et al. Burden of restless legs syndrome on health-related quality of life. Qual Life Res 2007;16:617-624.
9. Fulda S, Wetter TC. Is daytime sleepiness a neglected problem in patients with restless legs syndrome? Mov Disord 2007;22(Suppl 18):S409-413.
10. Saletu B, Anderer P, Saletu M, et al. EEG mapping, psychometric, and polysomnographic studies in restless legs syndrome (RLS) and periodic limb movement disorder (PLMD) patients as compared with normal controls. Sleep Med 2002;3(Suppl):S35-42.
11. Pearson VE, Allen RP, Dean T, et al. Cognitive deficits associated with restless legs syndrome (RLS). Sleep Med 2006;7:25-30.
12. Driver-Dunckley E, Connor D, Hentz J, et al. No evidence for cognitive dysfunction or depression in patients with mild restless legs syndrome. Mov Disord 2009;24(12):1843-1847.
13. Celle S, Roche F, Kerleroux J, et al. Prevalence and clinical correlates of restless legs syndrome in an elderly French population: the synapse study. J Gerontol A Biol Sci Med Sci 2010;65(2):167-173.
14. Fulda S, Plag J, Winkelmann J, Wetter TC. Quality of life, mood, and cognition in RLS patients. Sleep Med 2005;6(Suppl 2 (Abstract)):S32.
15. Weniger G, Trenkwalder C, Dörnte M, Irle E. Psychiatric comorbidity and neuropsychological changes in patients with restless-legs-syndrome. Pharmacopsychiatry 2005;38(Suppl (Abstract)):A249.
16. Tryggvadottir HB, Jonsdottir MK, Kristjansson K, Rye D, Sigmundsson T. Cognitive function in restless legs syndrome. J Sleep Res 2004;13(Suppl (Abstract)):732.
17. Ferini-Strambi L. Cognitive functions in RLS patients with PLM and in PLMD patients without RLS. Sleep Med 2005;6(Suppl 2 (Abstract)):S31.
18. Wittchen H, Pfister H. DIA-X-Interviews: Manual für Screening-Verfahren und Interview: (a) Interviewheft Längsschnittuntersuchung (DIA-X-Lifetime); (b) Ergänzungsheft (DIA-X-Lifetime); (c) Interviewheft Querschnittuntersuchung (DIA-X-12 Monate); (d) Ergänzungsheft (DIA-X-12 Monate); (e) PC-Programm zur Durchführung des Interviews (Längs- und Querschnittuntersuchung); (f) Auswertungsprogramm. (Manual). Frankfurt: Swets & Zeitlinger; 1997.
19. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28(2):193-213.
20. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540-545.
21. Derogatis LR. SCL-90-R, administration, acoring and procedures manual for the revised version of the SCL-90. (Manual). Baltimore: John Hopkins University Press; 1977.

22. Franke GH. SCL-90-R. Symptom-Checkliste von Derogatis – Deutsche Version – 2. Vollständig überarbeitete und neu normierte Auflage [Symptom checklist, 2nd revised version]. (Manual). Göttingen: Beltz Test GmbH; 2002.
23. Beck AT, Steer RA. Beck Depression Inventory. New York (NY): Harcourt Brace Jovanovich; 1987.
24. Spielberger CD, Gorsuch RL, Lushene RE. STAI, Manual for the State-Trait-Anxiety-Inventory. (Manual). Palo Alto, CA: Consulting Psychologist Press; 1970.
25. Walters A, LeBrocq C, Dhar A, et al. Validation of the International Restless Legs Syndrome Study Group rating scale for restless legs syndrome. Sleep Med 2003:121-132.
26. Hening WA, Allen RP. Restless legs syndrome (RLS): the continuing development of diagnostic standards and severity measures. Sleep Med 2003;4:95-97.
27. Brickenkamp R. Test d2. Aufmerksamkeits-Belastungstest (9.,überarbeitete und neu normierte Auflage) [d2 Letter Cancellation Test, 9th revised version].(Manual). Göttingen: Hogrefe; 2002.
28. Oswald WD, Roth E. Der Zahlen-Verbindungs-Test (2. überarbeitete und erweiterete Auflage)[Trail Making Test; 2nd revised version]. (Manual). Göttingen: Hogrefe; 1987.
29. Reitan RM, Wolfson D. The Halstead–Reitan Neuropsychological Battery. Theory and clinical interpretation. Tuscon, AZ: Neuropsychology Press; 1985.
30. Wechsler D. Wechsler-Gedächtnistest - Revidierte Fassung. Herausgegeben und bearbeitet von Ch. Härting, H. Markowitsch, H. Neufeld, P. Calabrese, K. Deisinger & J. Kessler. [German Adaptation of the revised version of the Wechsler Memory Scale]. Bern: Hans Huber; 2000.
31. Bäumler G. Farbe-Wort-Interferenztest (FWIT) nach J.R. Stroop. (Manual). Göttingen: Verlag für Psychologie, Dr. C.J. Hogrefe; 1985.
32. Stroop JR. Studies of interference in serial verbal reactions. J Exp Psychol 1935;18:643-662.
33. Ilmberger J. Münchner Verbaler Gedächtnistest (MVG). Deutsche Version des California Verbal Learning Test (CVLT). [German version of the California Verbal Learning Test] (Manual). Munich, Germany: Insitut für Medizinische Psychologie, Universität München; 1988.
34. Delis DC, Kramer JH, Kaplan E, Ober BA. California Verbal Learning Test: Adult Version Manual. (Manual). San Antonio, TX: The Psychological Corporation; 1987.
35. Aschenbrenner S, Tucha O, Lange KW. RWT Regensburger Wortflüssigkeits-Test. (Manual). Göttingen: Hogrefe; 2000.
36. Raven JC, Court JH, Raven J. A manual for Raven's Progressive Matrices. (Manual). London: H.K. Lewis; 1987.
37. Kratzmeier H, Horn R. SPM - Standard progressive Matrices. Manual (2. erweiterte und überarbeitete Auflage) [Standard progressive matrices, 2nd revised version]. (Manual). Weinheim: Beltz Test Gesellschaft; 1988.
38. Raven JC. Mental tests used in genetic studies: The performance of related individuals on tests mainly educative and mainly reproductive. (MSc Thesis). London: University of London; 1936.
39. Lehrl S. Mehrfachwahl-Wortschatz-Intelligenztest B (MWT-B). (Manual). Göttingen: Hogrefe Testzentrale; 2005.
40. Cohen J. Statistical Power Analysis for the Behavioral Sciences. second. Hilsdale, NJ: Lawrence Erlbaum Associates; 1988.
41. Ben-David BM, Schneider BA. A sensory origin for color-word Stroop effects in aging: a meta-analysis. Neuropsychol Dev Cogn B Aging Neuropsychol Cogn 2009;16(5):505-534.
42. Murray-Kolb LE, Beard JL. Iron treatment normalizes cognitive functioning in young women. Am J Clin Nutr 2007;85(3):778-787.
43. Khedr E, Hamed SA, Elbeih E, et al. Iron states and cognitive abilities in young adults: neuropsychological and neurophysiological assessment. Eur Arch Psychiatry Clin Neurosci 2008;258(8):489-496.
44. Youdim MBH. Brain iron deficiency and excess; cognitive impairment and neurodegeneration with involvement of striatum and hippocampus. Neurotox Res 2008;14(1):45-56.

III. Where dopamine meets opioids: a meta-analysis of the placebo effect in restless legs syndrome treatment studies*

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http://brain.oxfordjournals.org/cgi/reprint/awm244?ijkey=HHziocBtzVBsqWZ&keytype=ref

Abstract

The restless legs syndrome (RLS) is a common sensory-motor disorder of sleep/wake motor regulation with prevalence rates between 3% and 10%. In its more severe forms, RLS is a burdening disorder with disturbed sleep and significantly impaired quality of life. Restless legs symptoms are dramatically relieved with levodopa and dopamine agonists, which are first-line treatment for this disorder. In addition, opioids have been shown to provide a marked symptomatic relief. This unique responsiveness of RLS to both dopaminergic agents and opioids places it at the crossroad of the two systems implicated in the placebo response. Indeed, in recent large-scale studies a substantial placebo response was observed. We performed a meta-analysis to provide an evidence-based estimate of the magnitude of the placebo response in RLS.

Search strategies included the electronic databases PubMed and the Cochrane Clinical Trials Registry (from1966 to March 2007), the reference lists of retrieved articles, hand-searching abstract books of sleep, neurology and movement disorder congresses and visiting clinical trial register web sites. All randomized, double-blind, placebo-controlled studies exploring a pharmacological treatment in subjects with RLS were considered. Outcome measures from five domains were extracted: RLS severity, subjective sleep parameters, sleep parameters derived from nocturnal polysomnography, periodic leg movements during sleep (PLMS) and daytime functioning.

We identified 60 clinical trials and 36 of them were eligible for the meta-analysis. In 24 trials, the pooled placebo response rate was 40.09% (95% CI: 31.99 - 48.19). The placebo effect was large for the primary outcome measure in most studies, which is the International Restless Legs Severity Scale (21.48, CI: 21.81 to 21.14), notably smaller for other RLS severity scales, moderate for daytime functioning, small to moderate for subjective and objective sleep parameters, very small for PLMS and absent for sleep efficiency.

This meta-analysis yields several implications for the planning of both clinical RLS treatment studies and basic research programs.

Keywords: restless legs syndrome; placebo effect; meta-analysis

Abbreviations: CGI=Clinical Global Impression; CI=confidence interval; CO=cross-over trial; ES=effect size; IRLS=International Restless Legs Severity Scale; PD=Parkinson's disease; PG=parallel-group trial; PLMS=periodic leg movements during sleep; RLS=restless legs syndrome

* Fulda S, Wetter TC. Where dopamine meets opioids: a meta-analysis of the placebo effect in restless legs syndrome treatment studies. Brain 2008; 131: 902-917.

Introduction

The placebo response is an improvement of subjective and objective outcomes while taking an inert substance or undergoing a sham procedure. The precise mechanisms of the placebo response are not well understood (Hróbjartsson and Gøtzsche, 2001) and potential factors include regression to the mean, expectancies, non-specific effects of participating in research and physiological changes produced by placebos. The dopamine and opioid systems are thought to play a crucial role in the physiological response to a placebo (Colloca and Benedetti, 2005). The placebo effect in pain is powerful and has been known for long (Levine et al., 1978; Turner et al., 1994; Colloca and Benedetti, 2005); placebo analgesia can be blocked by naloxone, suggesting that placebos can induce the release of endogenous opioids (Levine et al., 1978; Amanzio and Benedetti, 1999; Benedetti et al., 1999). In a positron emission tomography (PET) study, analgesia induced by both a placebo and the opioid agonist remifentanil was associated with an increased activity in several pain-modulating brain regions including the rostral anterior cingulate cortex, the orbitotofrontal cortex and the anterior insula (Petrovic et al., 2002). Functional magnetic resonance imaging (fMRI) showed an increased activation in the same regions and in the dorsolateral prefrontal cortex during the anticipation phase of the placebo analgesic response, whereas placebo treatment was characterized by a decreased activation in the thalamus, anterior insula and the caudal rostral anterior cingulate cortex (Wager et al., 2004). A substantial placebo effect is also apparent in neurological disorders not directly involving pain such as Parkinson's disease (PD) (Shetty et al., 1999; Goetz et al., 2002). The dopaminergic system, particularly affected in PD, is involved in the regulation of several cognitive, behavioural and sensory-motor functions (Nieoullon, 2002), and notably in reward mechanisms (Ikemoto and Panksepp, 1999; Martin- Soelch et al., 2001). PET studies using the dopamine D2 receptor antagonist [11C]raclopride found that the placebo effect in PD is related to the release of dopamine in both the dorsal (de la Fuente-Fernández et al., 2001) and ventral striatum (de la Fuente-Fernández et al., 2002). In addition, the perception of the clinical placebo effect was related to the amount released in the dorsal striatum (de la Fuente- Fernández et al., 2001).

A disorder that is unique in the sense that it responds to both dopaminergic and opioidergic agents is the restless legs syndrome (RLS). RLS is a common sensory-motor disorder of sleep/wake motor regulation with prevalence rates estimated from population surveys between 3% and 10% (Phillips et al., 2000; Masood and Phillips, 2003). RLS is characterized by an imperative desire to move the extremities associated with paraesthesias, motor restlessness, worsening of symptoms at rest and in the evening or at night and, as a consequence, sleep disturbances (Allen et al., 2003). Additionally, most patients with RLS have periodic limb movements during sleep (PLMS) and relaxed wakefulness. In its more severe forms, RLS is a burdening disorder with significantly impaired quality of life (Hening et al., 2004). Restless legs symptoms are dramatically relieved with levodopa and dopamine agonists, which present first-line treatment (Fulda and Wetter, 2005), and this responsiveness is a supportive criterion for the diagnosis of RLS (Allen et al., 2003). In contrast to PD, RLS is not a degenerative disorder, and a dopaminergic deficit has not been proven (Paulus and Trenkwalder, 2006). So far, PET and single photon emission tomography (SPECT) studies are at best compatible with a subtle dysfunction of the dopaminergic system (Wetter et al., 2004). The sensory symptoms of RLS may be experienced as painful by a substantial number of patients (Winkelmann et al., 2000) and opioids have been shown to provide a marked symptomatic relief (Kaplan et al., 1993; Walters et al., 1993). Again, imaging results did not reveal major changes in opioid receptor binding. However, in a recent PET study using the non-selective opioid receptor ligand [^{11}C]diprenorphine, RLS severity correlated negatively with ligand binding in the medial pain

system including the thalamus, the amygdala and the anterior cingulate gyrus (von Spiczak et al., 2005).

The unique responsiveness of RLS to both dopaminergic agents and opioids places it at the crossroad of the two systems implicated in the placebo response. In addition, substantial placebo effects have also been reported in a broad spectrum of disorders of the central nervous system such as insomnia (Perlis et al., 2005) and depression (Walsh et al., 2002; McCall et al., 2003), both conditions that are also associated with RLS (Picchietti and Winkelman, 2005). And indeed, in recent treatment trials, a large placebo effect has been observed (Allen et al., 2004; Trenkwalder et al., 2004; Walters et al., 2004). The aim of the present meta-analysis was to quantify the magnitude of the placebo effect in RLS treatment studies by combining results from studies conducted during the past 25 years. We also explored whether the placebo effect differed between the various outcome modalities assessed in RLS treatment trials such as RLS severity, periodic leg movements, subjective and objective assessment of sleep and daytime functioning.

Methods

Location and selection of studies

We searched the electronic databases PubMed and the Cochrane Clinical Trials Registry (from 1966 to March 2007) using the following key words: ‘restless legs syndrome’ and ‘placebo’. In addition, reference lists of the retrieved articles were checked and we made an extensive effort to include unpublished material and trial information published only as abstracts by hand-searching abstract books of sleep, neurology and movement disorder congresses held in the last 4 years and visiting trial register web sites of companies known or suspected to conduct trials (Supplement 1 lists all resources used for the search). All randomized, double-blind, placebo-controlled studies exploring a pharmacological treatment in subjects with RLS were considered. Exclusion criteria were the use of concomitant pharmacological treatment for RLS during the trial and a withdrawal study design. We also considered non-English publications. A detailed list of the excluded studies with the reason for exclusion is available from the authors on request.

Outcome measures

For this meta-analysis, in addition to analysing response rates, we focused on five general domains that have been addressed in RLS treatment trials: RLS severity, subjective sleep parameters, sleep parameters derived from nocturnal polysomnography, PLMS and daytime functioning. Within each domain we selected those outcomes for which at least five effect sizes could be extracted. These were available for the International Restless Legs Severity Scale (IRLS) (Walters et al. 2003) and other RLS severity scores, subjective sleep duration and sleep quality, total sleep time and sleep efficiency derived from polysomnography, the PLMS index (number of periodic leg movements per hour of sleep), daytime sleepiness and quality of life.

Data extraction and computation of effect sizes

One reviewer extracted data and another reviewer verified the data extracted. Calculation of effect sizes and variances followed the general outline given by Morris and DeShon (2002) (for computational details see Appendix I). Effect sizes were computed for all outcome measures where means and standard deviations (SD, or standard errors) were given for baseline and endpoint of the placebo trial or for the differences between baseline and

endpoint. The effect size employed in this meta-analysis expressed the differences between baseline and endpoint in units of the standard deviation at baseline:

$$ES = \frac{\text{mean at baseline} - \text{mean at endpoint}}{\text{SD at baseline}}$$

All effect sizes were corrected for small sample bias following Hedges (1982). The sampling variance for the individual effect size was computed taking into account the correlation between baseline and endpoint (Morris and DeShon 2002) (see Appendix I). These correlations are rarely reported but can be computed by combining variances from baseline, endpoint and difference scores. The estimated correlations between baseline and placebo endpoint were 0.39 for the IRLS and other scales (n = 665, eight studies), 0.78 for subjective sleep parameters (n = 305, three studies), 0.91 for polysomnographic sleep parameters (n = 17, two studies), 0.71 for the PLMS index (n = 126, three studies) and 0.92 for daytime functioning (n = 195, two studies) (see Appendix II for specific references).

A random effects meta-analysis was conducted to yield a pooled estimate of the placebo effect and between-study heterogeneity assessed with the homogeneity index I^2. In case of significant between-study heterogeneity (I^2 > 25%), an attempt was made to find a homogeneous set of effect sizes by excluding studies based on study characteristics. A priori defined variables for the subgroup analysis were study design (parallel-group versus cross-over design), study duration (84 versus <84 days; 430 versus 535 days), number of treatment arms (one versus more than one), study population (idiopathic versus secondary RLS), study drug (dopaminergic versus non-dopaminergic drug) and outcome measures where applicable (e.g. periodic leg movements assessed with actigraphy versus polysomnography).

For descriptive purposes only, we also computed the corresponding effect sizes in the treatment groups for each outcome and explored whether treatment and placebo effect sizes were associated (Spearman correlation ρ). In trials employing multiple groups, treatment effects were taken from the group with the largest effect. Results are displayed as forest plots with the familiar diamond shape of the effect sizes replaced by circles to indicate that these are repeated-measures effect sizes instead of the standard independent group effect sizes. Analysis of response rates followed the general outline of Einarson (1997) but with confidence intervals of the point estimates computed according to Wilson (Agresti and Coull 1998). All analyses were performed with R (R Development Core Team, 2005) and the meta and nlme library (Pinheiro and Bates, 2000) in R.

Meta-regression

Meta-regression employing linear mixed models with known level-1 variance (Raudenbush and Bryk, 2002) and effect sizes nested within studies was employed to explore two basic questions. First, for response rates, the role of those study characteristics used for subgroup analysis (see above) was assessed. Initially, several other trial characteristics such as percentage of drug-naïve patients, mean age, gender composition, or severity of RLS were considered, but too little information was available in the first case and not enough variation was found in the latter variables (Table 1). Second, for the RLS severity measures, we explored whether differences between the IRLS and other scores persisted after controlling for differences in study characteristics. We chose not to perform meta-regression for the other outcome measures or for all available effect sizes due to several reasons: study design and study duration were not independent and both were associated with the year of publication and sample size, thus there was considerable confounding of the potential moderator variables. This was mostly due to the fact that all newer studies (after 2002) were long-term (12 weeks), parallel-group trials with large sample sizes. In addition, the use of

specific outcome measures was also associated with these trial characteristics. In particular, effect sizes for quality of life were only available for long-term, parallel-group studies and the IRLS and subjective sleep duration were mostly available from parallel-group trials.

Table 1 Study description including study design, placebo-drug allocation, study duration, age, gender, percentage of drug-naïve patients and number of idiopathic and secondary RLS patients

Drug	Study design	P:D	P:D n	Placebo group				
				Study duration (days)[a]	Age (mean)	Female (%)	Drug naïve (%)	Id:Sec n[b]
Dopaminergic drugs								
Levodopa								
Montplaisir *et al.,* 1996	SC CO	1:1	6	14:7	51	50	100	6:0
Beneš *et al.,* 1999	MC CO	1:1	32	28:0	56	59	50	28:4[c]
Levodopa + entacapone								
Polo *et al.,* 2005	CO	1:4	28	1:6	51	64		
Sr-levodopa / sr-valproic acid								
Eisensehr *et al.,* 2004	CO	1:2	20	21:0	59	60		20:0
Bromocriptine								
Walters *et al.,* 1988	SC CO	1:1	6	30:14	61	33		6:0
Pergolide								
Wetter *et al.,* 1999	MC CO	1:1	28	28:7	57	57	36	28:0
Ropinirole								
GlaxoSmithKline, 2005b; Trenkwalder *et al.,* 2004	MC PG	1:1	138:146	84	56	66		284:0
GlaxoSmithKline, 2005c; Walters *et al.,* 2004	MC PG	1:1	135:131	84	56	62	57	266:0
Allen *et al.,* 2004; GlaxoSmithKline, 2005d	MC PG	1:1	30:29	84	53	57	50	59:0
Kelly and Mistry, 2005	MC PG	1:2	17:37	42	56	76		54:0
Bogan *et al.,* 2006; GlaxoSmithKline, 2005a	MC PG	1:1	193:187	84	52	64		380:0
GlaxoSmithKline, 2006b; Kushida and Tolson, 2006	MC PG	1:1	184:175	84	51	62		359:0
GlaxoSmithKline, 2006a	MC PG	1:1	149:154	84	57	75		303:0
Adler *et al.,* 2004	CO	1:1	22	28:7	60	73	59	22:0
Pramipexole								
Montplaisir *et al.,* 1999	SC CO	1:1	10	28:14	49	50	60	10:0
Oertel *et al.,* 2006b	MC PG	1:1	114:224	42	56	68	68	338:0
Partinen *et al.,* 2006	PG	1:4	22:86	21	53	81	67	107:0
Winkelman *et al.,* 2006	MC PG	1:3	85:254	84	52	64	81	339:0
Inoue *et al.,* 2006	PG	1:1	19:19[d]	42				
Cabergoline								
Kohnen *et al.,* 2004; Stiasny-Kolster *et al.,* 2004a	MC PG	1:3	22:63	35	56	82	36	85:0
Oertel *et al.,* 2006a	MC PG	1:1	20:20	35	56	75	20	40:0

continued on next page

Table 1 continued

Lisuride patch								
Beneš *et al*., 2005	MC PG	1:3	52:156[d]	84	60[b]	70[b]		206:4[c]
Rotigotine patch								
Stiasny-Kolster *et al.,* 2004b	MC PG	1:3	14:49	7	60	50	14	63:0
Oertel *et al.,* 2005	MC PG	1:5	55:285	49	58[b]	70[b]		340:0
Sumanirole								
Garcia-Borreguero *et al.,* 2007	MC PG	1:4	52:218	56	53	58		270:0
Anticonvulsant drugs								
Carbamazepine								
Lundvall *et al.,* 1983	CO	1:1	6	28:0	53	33		
Telstad *et al.,* 1984	MC PG	1:1	90:84	35	52	72		
Gabapentin								
Garcia-Borreguero *et al.,* 2002	SC CO	1:1	22	42:7	55	73		22:2[e]
Thorp *et al.,* 2001	SC CO	1:1	16	42:7	64	6		0:16[c]
XP13512								
Kushida *et al.,* 2006	MC PG	1:2	33:62	14	50[b]	62[b]		
XenoPort 2006	MC CO	1:1	36	14:7	50	58		
Opioids								
Oxycodone								
Walters *et al.,* 1993	MC CO	1:1	11	14:0	55	45		11:0
Other drugs								
Clonazepam								
Boghen *et al.,* 1986	SC CO	1:1	6	28:0	46	50		
Montagna *et al.,* 1984	SC CO	1:2	6	7:3	54	50		
Clonidine								
Wagner *et al.,* 1996	MC CO	1:1	10	14:0	44	20		10:0
Hydroquinine								
van Dijk *et al.,* 1991	SC CO	1:1	59	14:14	55	47		

P : D: placebo : drug allocation, Id : Sec: idiopathic : secondary RLS, SC: single-centre, MC: multi-centre, CO: cross-over, PG: parallel-group,
a study duration : duration of wash-out phase
b complete group
c uremic RLS
d The exact number of participants in each group was not reported but estimated as being the appropriate fraction of the total numbers.
e iron deficiency

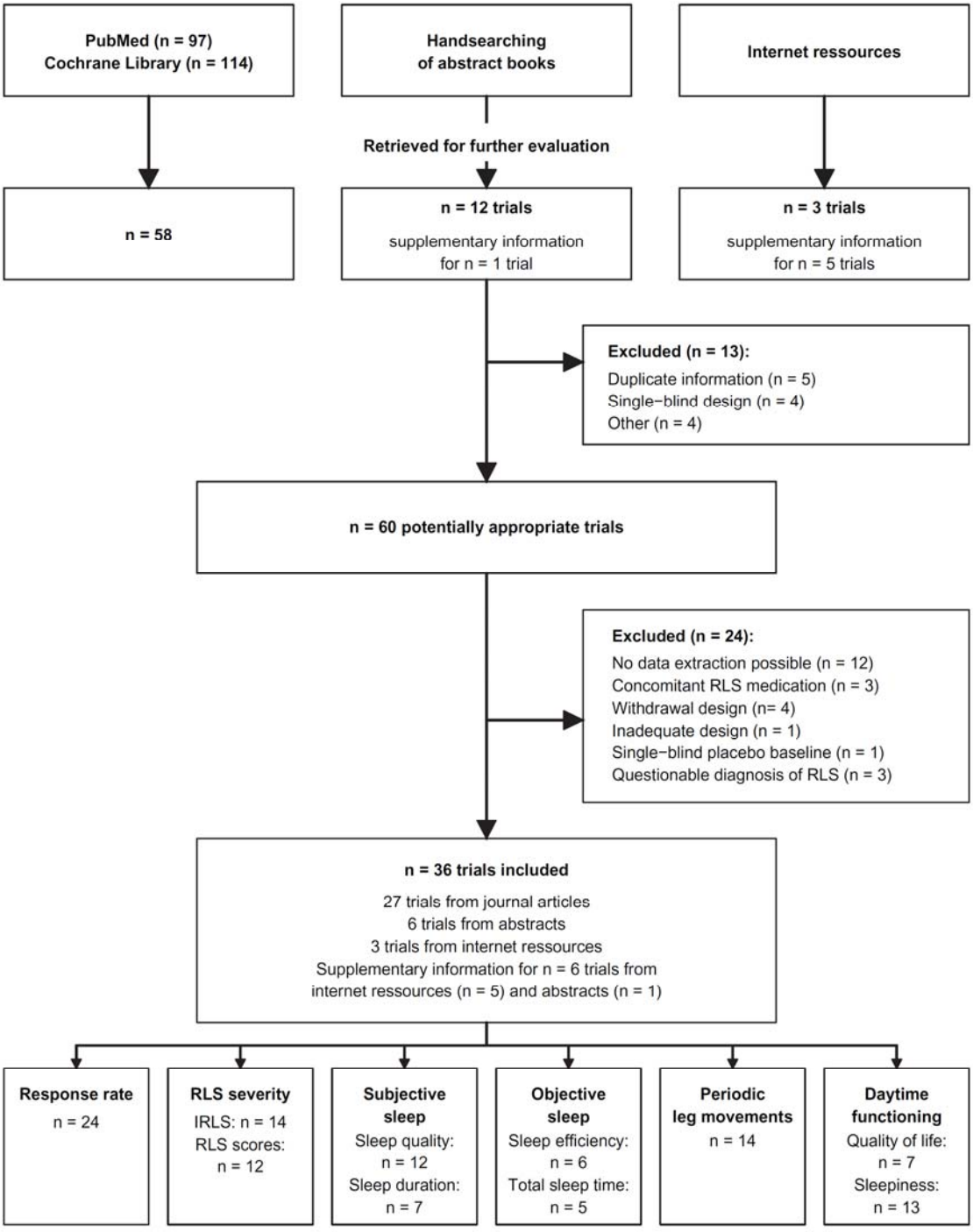

Fig. 1. Process of study selection of randomized controlled trials. (RLS: restless legs syndrome, IRLS: International Restless Legs Severity Scale).

Results

We identified 60 double-blind, randomized, placebo-controlled studies. Twenty-four studies were excluded for various reasons detailed in Fig. 1 and we included 36 studies published between 1983 and 2007. A detailed description of the included studies is given in Table 1. There were 17 crossover trials and 19 parallel-group trials with study durations between 1 day and 12 weeks (Table 1). The vast majority of trials included patients with idiopathic RLS, with three trials including also a low number of subjects with secondary RLS and one trial including only subjects with uraemic RLS. Summing over all trials, there were 1748 subjects in the placebo groups. The average age was 54 years (range: 44–64 years) and around 64% of the participants were female with the proportion ranging from 6% to 82%.

Response rates

Twenty-four studies reported response rates during placebo treatment (see Appendix 2.2 for specific references). In 17 studies, response rates were given as percentage of patients rated as 'much improved' or 'very much improved' on the clinical global impression (CGI) change of condition scale by the physician. One study defined response rates as an IRLS score indicative of none or mild symptoms; two studies relied on physician-rated improvement and in a single study each response was defined on a self-made RLS symptom scale, as no RLS 'attacks' during 1 week, or the wish to continue on placebo medication. Finally, for one study response rates pertaining to different scales were reported and averaged within study to yield a single estimate. The 24 studies included a total of 1527 patients in the placebo condition and 1665 patients in the treatment condition. Placebo response rates varied from 0% to 60% with substantial heterogeneity (I^2 = 91.6%, Fig. 2). We considered several subgroups of studies based on study characteristics (see Methods section and Supplement 2) but were not able to find a homogeneous set of effect sizes so that a random effects model was applied. The pooled weighted response rate during placebo treatment was 40.09% [95% confidence interval (CI): 31.99–48.19]. The corresponding mean weighted response rate in the treatment groups was 68.32% (CI: 63.36–73.29), again with significant between-study heterogeneity (I^2 = 77.3%). Placebo and treatment response rates did not correlate across studies (ρ 0.22, P = 0.30). We conducted a linear mixed model meta-regression with fixed level-1 variances of 41 available response rates from 24 studies including 17 additional effect sizes from eight studies that reported response rates for more than one time point during the trial and with the study characteristics as covariates. Of all the study characteristics only study duration was related to the placebo response which increased with study duration both within and across studies (Supplementary Fig. 1). Given an estimated placebo rate of 22.26% (±3.02, t = 7.36, P < 0.0001) at the end of the first week, the response rate during placebo treatment is expected to increase by almost 3% per week (2.75±0.19, t = 14.47, P < 0.0001).

IRLS and other RLS scores

A total of 22 studies reported placebo data regarding RLS symptom scales. The IRLS was the primary or secondary endpoint in 14 studies (see Appendix II for specific references); in four of these studies additional scales for rating restless legs symptoms have been employed as well. A further eight studies used other scores such as the RLS-6 scales, visual analogue scales of RLS severity, the CGI severity of RLS item, the number of RLS 'attacks' per week or various self-made RLS scores, with three studies reporting aggregated data for a 1-week diary of symptoms. Most studies that did not use the IRLS employed several different scores to assess RLS severity; these were averaged within study to yield a single effect size

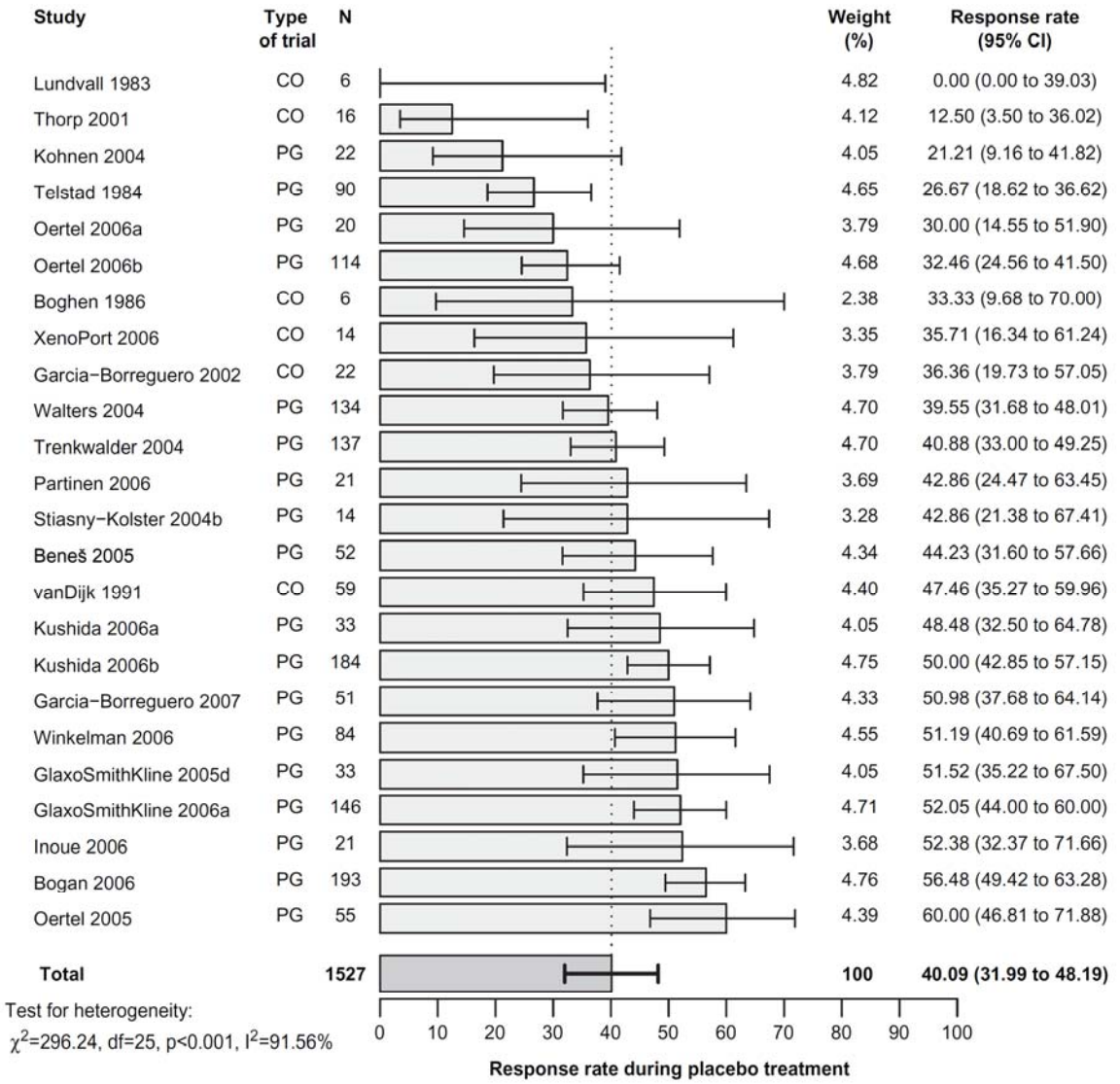

Fig. 2. Meta-analysis of response rates during placebo treatment in RLS therapy studies (CO: cross-over trial, PG: parallel-group trial, CI: confidence interval).

estimate. Standardized repeated-measures effect sizes for the IRLS ranged from -0.04 to -2.67 with significant between-study heterogeneity (I^2 = 89.0%, Fig. 3). Excluding the only crossover trial did not yield a homogeneous data set (I^2 = 87.5%) nor did restricting the analysis to trials with the longest study duration (I^2 = 90.5%, Supplement 2). The pooled random-effects estimator was -1.48 (CI: -1.81 to -1.14), which indicates a substantial decrease of RLS severity during placebo treatment. The corresponding pooled random-effect size for the treatment condition was -2.62 (CI: -2.97 to -2.27), again exhibiting significant between-study heterogeneity (I^2 = 84.2%). There was a positive correlation between the treatment and placebo effect sizes for the IRLS (ρ = 0.53, P = 0.05).

The standardized repeated-measures effect sizes for other RLS severity scores ranged from 0.04 to -1.21 with significant between-study heterogeneity (I^2 = 75.3%, Fig. 3). Between-study heterogeneity was no longer apparent when considering only the seven cross-over trials (I^2 = 0%), while it was still substantial for the parallel-group trials (I^2 = 87.0%). The pooled placebo effect sizes were -0.25 (CI: -0.49 to -0.01) for the cross-over trials and -0.78 (CI: -1.26 to -0.30) for the parallel-group trials. The corresponding pooled treatment effect

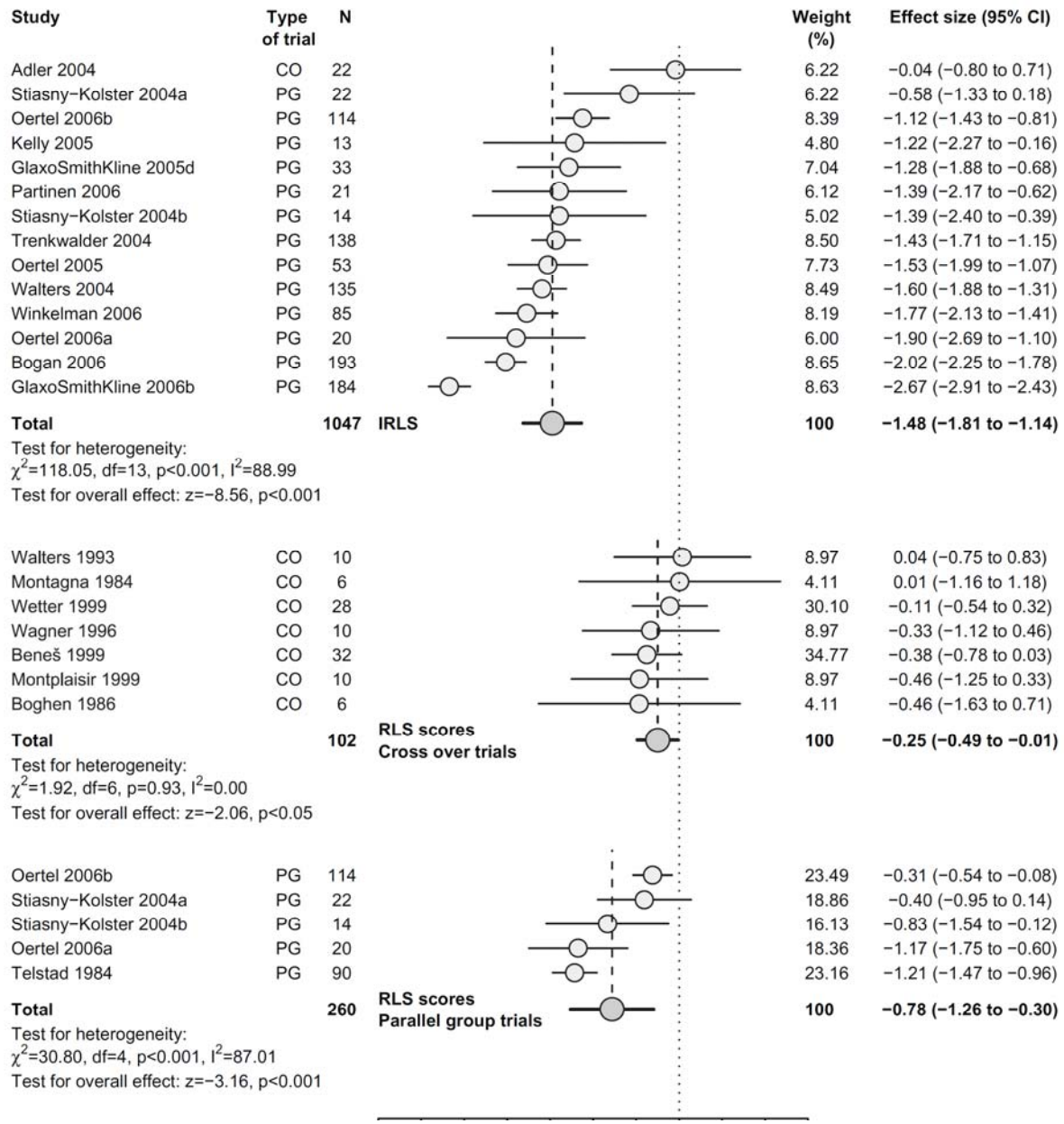

Fig. 3. Meta-analysis of standardized repeated-measures effect sizes for the placebo effect in the IRLS scale and other RLS severity scores (IRLS: International Restless Legs Severity Scale, RLS: restless legs syndrome, CO: cross-over trial, PG: parallel-group trial, CI: confidence interval).

sizes were -1.57 (CI: -2.21 to -0.94; I2 = 58.8%) for the cross-over trials and -1.48 (CI: -1.99 to -0.98; I^2 = 86.0%) for the parallel-group trials. There was no correlation between placebo and treatment effect sizes for the cross-over trials (ρ -0.04, P = 0.96), but a positive correlation for the parallel-group trials (ρ 0.70, P = 0.23).

We conducted a linear mixed model meta-regression with fixed level-1 variances of 31 available effect sizes from 22 studies including five additional effect sizes from four studies that reported RLS severity for more than one time point during the trial and with the type of scales (IRLS versus all other scores) and study characteristics as covariates. The pooled placebo effect was of greater magnitude for the IRLS than for other scales (-0.79±0.29, *t* = -2.72, *P* = 0.03) when considering only marginal effects. When controlling for study duration and type of trial, the differences in effect sizes between the different scales were still evident (-0.52±0.17, t =-3.01, P = 0.02) while there was only a trend for a larger placebo effects in

parallel-group trials versus crossover trials (-0.55±0.27, t =-2.01, P = 0.06). The placebo effect increased with study duration and each additional week was estimated to further reduce RLS severity by the standardized effect size of 0.09 (±0.01, t = 9.62, P < 0.0001).

Subjective sleep parameters: sleep quality and sleep duration

Twelve studies reported data on sleep quality during placebo treatment (see Appendix II for specific references). Outcomes included the Medical Outcome Study Sleep Scale item 'sleep adequacy' in six studies, the Schlaffragebogen A item 'sleep quality', the RLS-6 item 'sleep satisfaction', a visual analogue scale item 'satisfaction with sleep' and diary-derived sleep quality. Repeated-measures effect sizes ranged from 0.00 to 0.46 with significant between-study heterogeneity (I^2 = 70.7%) that remained after the exclusion of the three crossover trials (I^2 = 73.4%). The pooled placebo effect size estimate was 0.27 (CI: 0.18–0.36), indicative of a small increase in sleep quality during placebo treatment (Figure 4, upper panel). The corresponding pooled treatment effect size was 0.84 (CI: 0.63–1.04; I2 = 92.3%). The correlation between placebo and treatment effect sizes was negative but statistically insignificant (ρ -0.22, P = 0.50).

Subjective sleep duration was reported in seven trials (see Appendix II for specific references); six of them employed the Medical Outcome Study Sleep Scale item 'sleep quantity' and one study reported a diary-based estimate. Repeated-measures effect sizes ranged from -0.15 to 0.32 with significant between-study heterogeneity (I^2 = 78.2%) that remained after the exclusion of the only crossover study that was also the only study with a study duration less than 12 weeks (I^2 = 81.4%). The pooled random-effects estimate was 0.13 (CI: 0.03–0.24) pointing to a very small, albeit statistically significant increase in subjective sleep duration during placebo treatment (Fig. 4, upper middle panel). The corresponding pooled treatment effect size was 0.35 (CI: 0.23–0.47; I^2 = 76.6%) and there was a positive correlation between placebo and treatment effect sizes (ρ 0.54, P = 0.236).

Polysomnographic sleep parameters: sleep efficiency and total sleep time

Five studies reported data regarding the placebo effect on total sleep time with repeated-measures effect sizes ranging from 0.05 to 0.59 with moderate to substantial heterogeneity (I^2 = 68.2%) (see Appendix II for specific references). The pooled effect size of 0.24 (CI: 0.04–0.44) was indicative of a small increase in total sleep time during placebo treatment (Fig. 4, lower middle panel). The corresponding pooled treatment effect size was 0.37 (CI: 0.25–0.49; I^2 = 0%) and there was a positive but statistically insignificant correlation between placebo and treatment effect sizes (ρ 0.60, P = 0.35). Seven studies reported data regarding sleep efficiency with effect sizes ranging homogeneously (I^2 = 37.2%) from -0.21 to 0.25 around the pooled effects estimate of 0.07 (CI: -0.06–0.19) which did not significantly differ from zero (Fig. 4, lower panel) (see Appendix II for specific references). The corresponding pooled treatment effect size was 0.37 (CI: 0.26–0.49; I^2 = 0%) and there was a negative association between placebo and treatment effect sizes (ρ -0.60, P = 0.24).

PLMS

Fourteen studies measured the PLMS index during placebo treatment and individual effect sizes ranged from 0.44 (increase in PLMS index) to -0.28 (see Appendix II for specific references). As shown in Fig. 5, the pooled effect size estimate was -0.11 (CI: -0.20 to -0.03) and there was no indication of significant within-study heterogeneity (I^2 = 10.5%). The corresponding pooled treatment effect size was -0.88 (CI: -1.06 to -0.71; I^2 = 52.4%) with no apparent correlation between placebo and treatment effect sizes (ρ 0.27, P = 0.34).

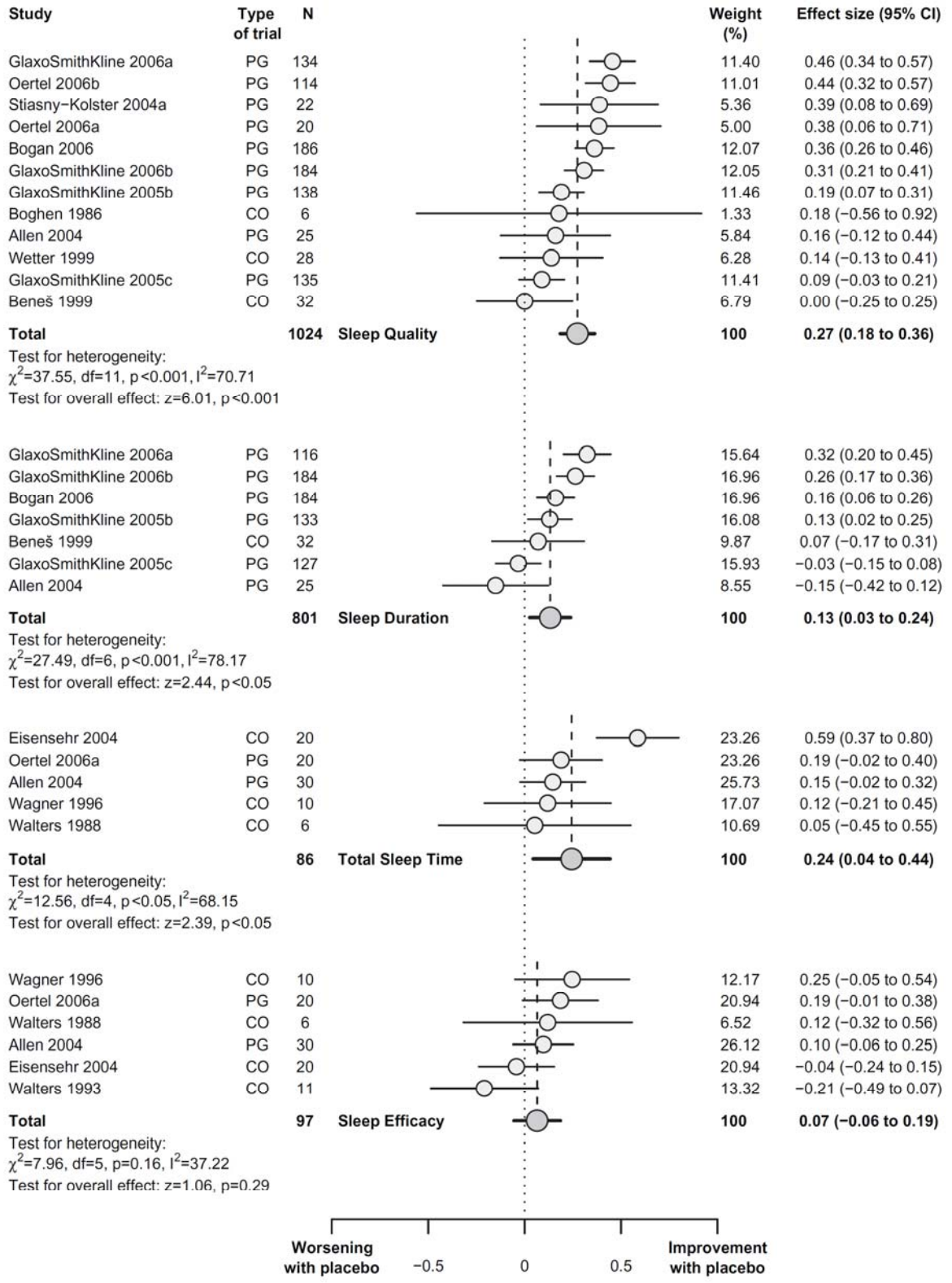

Fig. 4. Meta-analysis of standardized repeated-measures effect sizes for the placebo effect in sleep quality (upper panels), subjective sleep duration (upper middle panel), total sleep time derived from polysomnography (lower middle panel) and sleep efficiency derived from polysomnography (lower panel) (CO: cross-over trial, PG: parallel-group trial, CI: confidence interval).

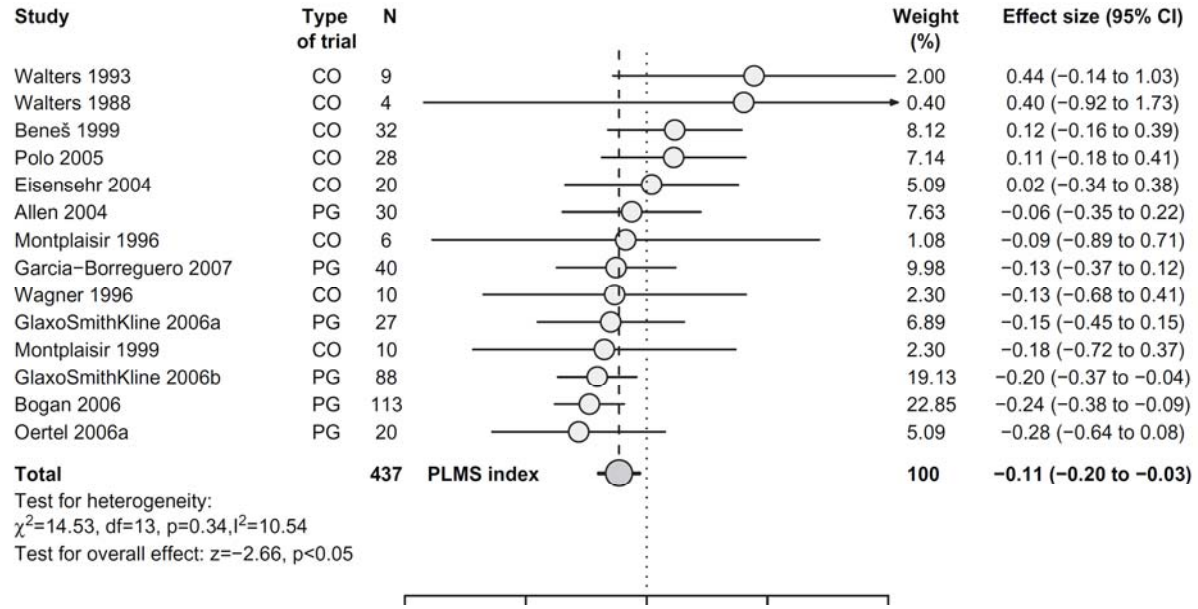

Fig. 5. Meta-analysis of standardized repeated-measures effect sizes for the placebo effect in the number of periodic leg movements per hour of sleep (PLMS index) (CO: cross-over trial, PG: parallel-group trial, CI: confidence interval).

Daytime functioning: sleepiness and quality of life

Thirteen studies contributed data concerning daytime sleepiness; six studies employed the MOS item daytime sleepiness, three studies used the Epworth Sleepiness Scale, two studies reported diary-derived measures of daytime fatigue or drowsiness and one study each used the RLS-6 item daytime tiredness or the IRLS item sleepiness (see Appendix II for specific references). A decrease in average daytime sleepiness was observed in all studies with effect sizes ranging from -0.07 to -0.96 with significant between-study heterogeneity (I^2 = 92.3%). The between-study heterogeneity remained elevated even when considering only the parallel-group trials (I^2 = 93.7%) or, within that group of studies, only those that used the MOS daytime sleepiness item (I^2 = 95.4%, Supplement 2). The pooled random effects estimate over all studies was -0.36 (CI: -0.48 to -0.25, Supplementary Fig. 2) indicating a moderate reduction in daytime sleepiness during placebo treatment. The corresponding pooled treatment effect size was -0.63 (CI: -0.80 to -0.46) with significant between-study heterogeneity (I^2 = 95.4%). Placebo and treatment effect sizes were significantly related across studies (ρ 0.87, P < 0.001).

Seven studies assessed quality of life in RLS subjects (see Appendix II for specific references). Six of the seven studies were parallel-group, 12-week studies that employed the RLS-Quality of Life Questionnaire (Abetz et al. 2004). One study used a German disease-specific RLS quality of life questionnaire (Kohnen et al. 2002). Effect sizes ranged from 0.30 to 1.08 with significant between-study heterogeneity (I^2 = 95.1%) that was not reduced after the exclusion of the only crossover study (I^2 = 94.9%, Supplement 2). The pooled effect size was 0.50 (CI: 0.32–0.68, Supplementary Fig. 3) indicating a moderate improvement in quality of life during placebo treatment. The corresponding pooled treatment effect size was 0.83 (CI: 0.58–1.09; I^2 = 95.7%). The rank order of treatment and placebo effect sizes across studies was in high concordance (ρ 0.96, P = 0.003).

Discussion

Our meta-analysis has revealed a substantial placebo response rate in RLS treatment studies. On average, more than one-third of RLS subjects experienced a major improvement of RLS symptoms while being treated with a placebo. This response rate was matched by a large placebo effect in overall RLS severity as measured by the IRLS. Compared to the IRLS, other scores of RLS severity had a lower—small to moderate—placebo effect even when accounting for differences in study design and study duration. There was a moderate effect for quality of life, and a smaller placebo effect was observed for daytime sleepiness, sleep quality and total sleep time. Even smaller placebo effects were apparent for subjectively estimated sleep duration and the PLMS index. A placebo effect was absent for sleep efficiency. Any discussion of differences in the magnitude of the placebo effect must, however, take the correlation between the placebo and treatment effect sizes into account. Therefore, in Fig. 6, the weighted mean placebo and treatment effect sizes are given for each outcome domain and in general the placebo effect size was proportional to the corresponding treatment effect size. Although linear mixed model analysis indicated that the placebo effect for the IRLS was larger than for other RLS scales even when taking differences in study features into account, it is apparent that this large placebo effect was also matched by a large treatment effect. Nevertheless, there were some notable exceptions to this proportionality of placebo and treatment effect sizes: the placebo effect for RLS scores other than the IRLS was considerably smaller in crossover trials than in parallel-group trials, while this difference was not observed for the treatment effects. For both sleep quality and in particular the PLMS index the treatment effects were disproportionally larger than the placebo effects.

Focusing on PLMS as a primary endpoint in RLS trials could appear promising but neglects the fact that only around 80% of subjects with RLS will exhibit PLMS to some degree (Montplaisir et al., 1997), thus making generalizations regarding the complete population of RLS sufferers questionable. In addition, the true clinical significance of PLMS still remains to be determined (Mahowald, 2003). Intriguingly from a theoretical point of view, PLMS are not consciously experienced due to their occurrence during sleep and it is tempting to speculate that therefore they might not be accessible to placebo-mediated expectations of the subject. In addition, apart from the distinction between PLMS and IRLS as subjective versus objective outcome measures, the IRLS is a multidimensional assessment instrument, whereas the PLMS index is one-dimensional. This multidimensionality of the IRLS may predispose the scale to be especially sensitive to placebo but also treatment effects. Future research, analysing individual items of the IRLS, should address the question whether this sensitivity can be traced back to a specific subset of items. Furthermore, as only a few studies (four) have employed another RLS severity score alongside with the IRLS, a direct comparison of the IRLS to other measures of severity was not feasible in the present analysis. In addition, the majority of response rates included in our analysis were based on the CGI and evaluated by an experienced investigator; thus, the placebo effect was not limited to the patients' self-report. Interestingly, for PD a recent study (Goetz et al., 2002) found a larger placebo effect for the objective part of the Unified Parkinson's Disease Rating Scale than for the subjective part, arguing against a purely subjective phenomenon.

A placebo effect has been observed in a broad spectrum of disorders of the central nervous system such as insomnia or depression and can be attributed to different mechanisms including expectation of clinical improvement and conditioning. For both insomnia and depression, comparable placebo effects have been reported (Walsh et al., 2002; McCall et al., 2003). In both disorders the placebo response increased with study duration (Walach and Maidhof, 1999; Perlis et al., 2005; Posternak and Zimmerman, 2005), a phenomenon we

observed also for response rates and RLS severity. Indeed, a recent review of the potential mechanisms of the placebo response in insomnia highlighted the importance of the episodic rather than chronic pattern of insomnia symptoms with regard to the placebo response in this disorder (Perlis et al., 2005). According to these authors, because insomnia symptoms will naturally vary across time, the cooccurrence of better sleep and placebo intake represents a strong reinforcement that could in part be responsible for the observation that placebo response rates increase over time. RLS is associated with insomnia and possibly mood disturbances (Picchietti and Winkelman, 2005) and its severity exhibits day-to-day variations. Although in our meta-analysis the placebo (and treatment) effect for sleep measures was considerably smaller than the effect for RLS severity, the association with sleep and mood disturbances may predispose subjects with RLS to the occurrence of a placebo response. Research on the placebo effect in depression, however, has also shown that response to both treatment and placebo during pharmacological (Benedetti et al., 2005) and cognitive-behavioural therapy (Mayberg et al., 2000) closely matches the active treatment response that the placebo was designed to stimulate (Lidstone and Stoessl, 2007). This would rather point to the involvement of the opioid or dopamine system in the placebo response in RLS.

Apart from the general limitations inherent to meta-analyses (Lyman and Kuderer, 2005) there are specific methodological issues to mention regarding our present study. First, our data basis is limited insofar as we identified 60 controlled trials but could only include 36. Even for these included trials, not all potentially available effect sizes could be extracted due to the data not being reported in an appropriate form. Given that a more detailed reporting would be more likely in the case of significant treatment effects, i.e. larger treatment-placebo differences, our present results could even be an underestimation of the magnitude of the placebo effect. A second issue pertains to the choice of the effect size measure. For the present analysis, we standardized the change from baseline to endpoint by the standard deviation at baseline. Thus, all other parameters being equal, the effect size will be larger if the standard deviation is smaller. If an outcome measure is also used as an inclusion criterion, a range and thus variance restriction is to be expected, effect sizes will be larger and regression to the mean is promoted. This pertains to the IRLS scale for which a minimum value of 10 or 15 was generally required. Finally, we included trials conducted within the last 25 years. While the aim was to guarantee the completeness of the data basis, there was also a notable change in methodology across this large period of time. Standards for treatment trials have changed in terms of design, duration and sample size. In addition, significant research progresses such as the development of the IRLS scale are reflected by the fact that this scale was the primary endpoint in almost all newer studies. For this reason, the use of meta-regression to explore the influence of potential moderators of the placebo effect was restricted to only two outcome domains and even their results must be treated with appropriate caution. Thus, the present analysis cannot claim to provide any insights into the role of possible moderators of the placebo effect.

This meta-analysis has several implications for the planning of both clinical RLS treatment studies and basic research programmes. RLS treatment studies have to reckon with a substantial placebo effect that increases with study duration. Thus, any long-term controlled trial should include a larger number of subjects into the trial. The present meta-analysis can only serve as a starting point for research into the nature of the placebo effect in RLS. To explore potential moderators and predictors of the placebo effect, more detailed data and preferably an individual patient data meta-analysis is needed, which, however, requires the cooperation of the respective drug companies to share their data. Potential moderator variables that could influence the placebo response are gender and age, the fact of whether subjects are drug-naïve or pre-treated or the presence of associated symptoms or comorbidities.

A further promising area of research would be the imaging of the opioid and dopamine systems in RLS in relation to the placebo response. So far, these research projects have been restricted to subjects with PD (de la Fuente-Fernández et al., 2001, 2002) and to subjects with pain disorders or experimentally induced pain (Colloca and Benedetti, 2005). Here, RLS offers the unique possibility to study both systems in the same group of subjects and may help to disentangle their respective contributions to the placebo effect. Indeed, the multitude of subjective and objective outcome measures used in assessing treatment efficacy in RLS, the association with sleep and mood disorders, the occurrence of symptoms both consciously experienced and not accessible for the subject, together with the responsiveness to dopaminergic as well as opioidergic agents, make RLS a model disease to study the placebo effect systematically. Finally, one necessary research project must be the inclusion of a no-treatment control group in a future trial. Without such data there is no way to differentiate between the natural course of the disease as opposed to the placebo effect. In summary, placebo treatment has a strong impact on outcome measures in RLS treatment studies, which may not only point to promising research avenues but may also challenge us to incorporate the therapeutic placebo effect into clinical practice.

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References

Abetz L, Allen R, Follet A, Washburn T, Earley C, Kirsch J, et al. Evaluating the quality of life of patients with restless legs syndrome. Clin Ther 2004; 26: 925–35.

Adler CH, Hauser RA, Sethi K, Caviness JN, Marlor L, Anderson WM, et al. Ropinirole for restless legs syndrome: a placebo-controlled crossover trial. Neurology 2004; 62: 1405–7.

Agresti A, Coull BA. Approximate is better than ‘‘exact’’ for interval estimation of binomial proportions. Am Stat 1998; 52: 119–26.

Allen R, Becker PM, Bogan R, Schmidt M, Kushida CA, Fry JM, et al. Ropinirole decreases periodic leg movements and improves sleep parameters in patients with restless legs syndrome. Sleep 2004; 27: 907–14.

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: 101–19.

Amanzio M, Benedetti F. Neuropharmacological dissection of placebo analgesia: expectation-activated opioid systems versus conditioningactivated specific subsystems. J Neurosci 1999; 19: 484–94.

Benedetti F, Arduino C, Amanzio M. Somatotopic activation of opioid systems by target-directed expectations of analgesia. J Neurosci 1999; 19: 3639–48.

Benedetti F, Mayberg HS, Wager TD, Stohler CS, Zubieta JK. Neurobiological mechanisms of the placebo effect. J Neurosci 2005; 25: 10390–402.

Benes› H, Kurella B, Kummer J, Kazenwadel J, Selzer R, Kohnen R. Rapid onset of action of levodopa in restless legs syndrome: a double-blind, randomized, multicenter, crossover trial. Sleep 1999; 22: 1073–81.

Beneš H. TULIR study group. Transdermal lisuride in patients with idiopathic restless legs syndrome: results from a placebo-controlled, double-blind, randomized, multicenter, 12-week dose-finding study. Sleep Med 2005; 6 (Suppl. 2): S156–7.

Bogan RK, Fry JM, Schmidt MH, Carson SW, Ritchie SY. Ropinirole in the treatment of patients with restless legs syndrome: a US-based randomized, double-blind, placebo-controlled clinical trial. Mayo Clin Proc 2006; 81: 17–27.

Boghen D, Lamothe L, Elie R, Godbout R, Montplaisir J. The treatment of the restless legs syndrome with clonazepam: a prospective controlled study. Can J Neurol Sci 1986; 13: 245–7.

Colloca L, Benedetti F. Placebo and painkillers: is mind as real as matter? Nat Rev Neurosci 2005; 6: 545–52.

de la Fuente-Fernández R, Phillips AG, Zamburlini M, Sossi V, Calne DB, Ruth TJ, et al. Dopamine release in human ventral striatum and expectation of reward. Behav Brain Res 2002; 136: 359–63.

de la Fuente-Fernández R, Ruth TJ, Sossi V, Schulzer M, Calne DB, Stoessl AJ. Expectation and dopamine release: mechanism of the placebo effect in Parkinson's disease. Science 2001; 293: 1164–6.

Einarson TR. Pharmacoeconomic applications of meta-analysis for single groups using antifungal onychomycosis lacquers as an example. Clin Ther 1997; 19: 559–69.

Eisensehr I, Ehrenberg BL, Rogge Solti S, Noachtar S. Treatment of idiopathic restless legs syndrome (RLS) with slow-release valproic acid compared with slow-release levodopa/benserazide. J Neurol 2004; 251: 579–83.

Fulda S, Wetter TC. Dopamine agonists for the treatment of restless legs syndrome. Expert Opin Pharmacother 2005; 6: 2655–66.

Garcia-Borreguero D, Larrosa O, de la Llave Y, Verger K, Masramon X, Hernandez G. Treatment of restless legs syndrome with gabapentin: a double-blind, cross-over study. Neurology 2002; 59: 1573–9.

Garcia-Borreguero D, Winkelman J, Adams A, Ellis A, Morris M, Lamb J, et al. Efficacy and tolerability of sumanirole in restless legs syndrome: a phase II, randomized, double-blind, placebo-controlled, dose-response study. Sleep Med 2007; 8: 119–27.

GlaxoSmithKline. Clinical Trial Register. Study ID: 101468/249. [updated 2005a February 28; cited 2006 October 30]. Available from: http://ctr.gsk.co.uk/Summary/ropinirole/studylist.asp

GlaxoSmithKline. Clinical Trial Register. Study ID: 101468/190. [updated 2005b March 1; cited 2006 October 30]. Available from: http://ctr.gsk.co.uk/Summary/ropinirole/studylist.asp

GlaxoSmithKline. Clinical Trial Register. Study ID: 101468/194. [updated 2005c March 1; cited 2006 October 30]. Available from: http://ctr.gsk.co.uk/Summary/ropinirole/studylist.asp

GlaxoSmithKline. Clinical Trial Register. Study ID: 101468/191. [updated 2005d March 1; cited 2006 October 30]. Available from: http://ctr.gsk.co.uk/Summary/ropinirole/studylist.asp

GlaxoSmithKline. Clinical Trial Register. Study ID: ROP101892. [updated 2006a January 16; cited 2006 October 30]. Available from: http://ctr.gsk.co.uk/Summary/ropinirole/studylist.asp

GlaxoSmithKline. Clinical Trial Register. Study ID: RRL100013. [updated 2006b March 15; cited 2006 October 30]. Available from: http://ctr.gsk.co.uk/Summary/ropinirole/studylist.asp

Goetz CG, Leurgans S, Raman R. Parkinson Study Group. Placebo-associated improvements in motor function: comparison of subjective and objective sections of the UPDRS in early Parkinson's disease. Mov Disord 2002; 17: 283–8.

Hedges LV. Estimation of effect size from a series of independent experiments. Psychol Bull 1982; 92: 490–9.

Hening W, Walters AS, Allen RP, Montplaisir J, Myers A, Ferini- Strambi L. Impact, diagnosis and treatment of restless legs syndrome (RLS) in a primary care population: the REST (RLS epidemiology, symptoms, and treatment) primary care study. Sleep Med 2004; 5: 237–46.

Hróbjartsson A, Gøtzsche PC. Is the placebo powerless? An analysis of clinical trials comparing placebo with no treatment. N Engl J Med 2001; 344: 1594–602.

Ikemoto S, Panksepp J. The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Brain Res Rev 1999; 31: 6–41.

Inoue Y, Fujita M, Shimizu T, Emura N, Kuroda K, Uchimuram N. Efficacy and safety of pramipexole in Japanese patients with restless legs syndrome. Mov Disord 2006; 21 (Suppl. 15): S442.

Kaplan PW, Allen RP, Buchholz DW, Walters JK. A double-blind, placebo-controlled study of the treatment of periodic limb movements in sleep using carbidopa/levodopa and propoxyphene. Sleep 1993; 16: 717–23.
Kelly M, Mistry P. Tolerability of a forced-dose escalating regimen of ropinirole in patients with RLS. Mov Disord 2005; 20 (Suppl. 10): S63.
Kohnen R, Beneš H, Heinrich CR, Kurella B. Development of the disease-specific Restless Legs Syndrome Quality of Life (RLS-QoL) questionnaire. Mov Disord 2002; 17 (Suppl 5): P743.
Kohnen R, Beneš H, Leroux M, Stiasny-Kolster K, Oertel WH. Defining clinical relevance of treatment outcome in studies with restless legs syndrome: example from the cabergoline dose-finding trial. Mov Disord 2004; 19 (Suppl. 9): S427–8.
Kushida CA, Becker PM, Ellenbogen AL, Walters AS, Canafax DM. XP13512 is well tolerated and effective in treating moderate to serve restless legs syndrome in a 2-week randomized, double-blind, placebo-controlled, exploratory trial. [updated 27 July 2006a; cited 1 March 2007]. Available from: http://www.xenoport.com/assets/pdf/PUB_XP045_Presentation032806.pdf.
Kushida CA, Tolson JM. Ropinirole is an effective treatment for patients with restless legs syndrome (RLS) needing extended treatment coverage. Neurology 2006b; 66 (Suppl. 5): A82.
Levine JD, Gordon NC, Fields HL. The mechanisms of placebo analgesia. Lancet 1978; 312: 654–7.
Lidstone SC, Stoessl AJ. Understanding the placebo effect: contributions from neuroimaging. Mol Imaging Biol 2007; 9: 176–85.
Lundvall O, Abom PE, Holm R. Carbamazepine in restless legs. A controlled pilot study. Eur J Clin Pharmacol 1983; 25: 323–4.
Lyman GH, Kuderer NM. The strengths and limitations of meta-analyses based on aggregate data. BMC Med Res Methodol 2005; 5: 14.
Mahowald MW. Restless leg syndrome and periodic limb movements of sleep. Curr Treat Options Neurol 2003; 5: 251–60.
Martin-Soelch C, Leenders KL, Chevalley A-F, Missimer J, Kunig G, Magyar S, et al. Reward mechanisms in the brain and their role in dependence: evidence from neurophysiological and neuroimaging studies. Brain Res Brain Res Rev 2001; 36: 139–49.
Masood A, Phillips B. Epidemiology of restless legs syndrome. In: Chokroverty S, Hening WA, Walters AS, editors. Sleep and movement disorders. Philadelphia: Butterworth Heinemann, 2003; p. 316–21.
Mayberg HS, Brannan SK, Tekell JL, Silva JA, Mahurin RK, McGinnis S, et al. Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response. Biol Psychiatry 2000; 48: 830–43.
McCall WV, D'Agostino R, Dunn A. A meta-analysis of sleep changes associated with placebo in hypnotic clinical trials. Sleep Med 2003; 4: 57–62.
Montagna P, Sassoli de Bianchi L, Zucconi M, Cirignotta F, Lugaresi E. Clonazepam and vibration in restless legs syndrome. Acta Neurol Scand 1984; 69: 428–30.
Montplaisir J, Boucher S, Gosselin A, Poirier G, Lavigne G. Persistence of repetitive EEG arousals (K-alpha complexes) in RLS patients treated with L-DOPA. Sleep 1996; 19: 196–9.
Montplaisir J, Boucher S, Poirier G, Lavigne G, Lapierre O, Lespérance P. Clinical, polysomnographic, and genetic characteristics of restless legs syndrome: a study of 133 patients diagnosed with new standard criteria. Mov Disord 1997; 12: 61–5.
Montplaisir J, Nicolas A, Denesle R, Gomez-Mancilla B. Restless legs syndrome improved by pramipexole: a double-blind randomized trial. Neurology 1999; 52: 938–43.
Morris SB, DeShon RP. Combining effect sizes in meta-analysis with repeated measures and independent-groups designs. Psychol Methods 2002; 7: 105–25.
Nieoullon A. Dopamine and the regulation of cognition and attention. Prog Neurobiol 2002; 67: 53–83.
Oertel WH, Beneš H, Geisler P, Eisensehr I, Garcia-Borreguero D, Clarenbach P, et al. Rotigotine patch efficacy and safety in the treatment of moderate to severe idiopathic restless legs syndrome - results from a multi-national double-blind placebo-controlled multi-center dose-finding study. Sleep Med 2005; 6 (Suppl. 2): S159.

Oertel WH, Beneš H, Bodenschatz R, Peglau I, Warmuth R, Happe S, et al. Efficacy of cabergoline in restless legs syndrome. A placebo-controlled study with polysomnography (CATOR). Neurology 2006a; 67: 1040–6.
Oertel WH, Stiasny-Kolster K, Bergtholdt B, Hallstrom Y, Albo J, Leissner L, et al. Efficacy of pramipexole in restless legs syndrome: a six-week, multicenter, randomized, double-blind study. Mov Disord 2006b; 22: 213–9.
Partinen M, Hirvonen K, Jama L, Alakuijala A, Hublin C, Tamminen I, et al. Efficacy and safety of pramipexole in idiopathic restless legs syndrome: a polysomnographic dose-finding study - the PRELUDE study. Sleep Med 2006; 7: 407–17.
Paulus W, Trenkwalder C. Less is more: pathophysiology of dopaminergictherapy- related augmentation in restless legs syndrome. Lancet Neurol 2006; 5: 878–86.
Perlis ML, McCall WV, Jungquist CR, Pigeon WR, Matteson SE. Placebo effects in primary insomnia. Sleep Med Rev 2005; 9: 381–9.
Petrovic P, Kalso E, Petersson KM, Ingvar M. Placebo and opioid analgesia - imaging a shared neuronal network. Science 2002; 295: 1737–40.
Phillips B, Young T, Finn L, Asher K, Hening WA, Purvis C. Epidemiology of restless legs symptoms in adults. Arch Int Med 2000; 160: 2137–41.
Picchietti D, Winkelman JW. Restless legs syndrome, periodic limb movements in sleep, and depression. Sleep 2005; 28: 891–8.
Pinheiro JC, Bates DM. Mixed effects models in S and S-PLUS. New York: Springer, 2000.
Polo O, Ylä-Sahra R, Hirvonen K, Karvinen J, Vahteristo M, Ellmén J. Levodopa optimized with entacapone decreases periodic limb movements in patients with restless legs syndrome. Mov Disord 2005; 20 (Suppl. 10): S102.
Posternak MA, Zimmerman M. Is there a delay in antidepressant effect? A meta-analysis. J Clin Psychiatry 2005; 66: 148–58.
R Development Core Team. R: A language and environment for statistical computing. 2005. R Foundation for statistical computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.
Raudenbush SW, Bryk AS. Hierachical linear models. Applications and data analysis. Thousand Oaks: Sage Publications, 2002.
Shetty N, Friedman JH, Kieburtz K, Marshall FJ, Oakes D. The placebo response in Parkinson's disease. Parkinson Study Group. Clin Neuropharmacol 1999; 22: 207–12.
Stiasny-Kolster K, Beneš H, Peglau I, Hornyak M, Holinka B, Wessel K, et al. Effective cabergoline treatment in idiopathic restless legs syndrome. Neurology 2004a; 63: 2272–9.
Stiasny-Kolster K, Kohnen R, Schollmayer E, Möller JC, Oertel WH. Patch application of the dopamine agonist rotigotine to patients with moderate to advanced stages of restless legs syndrome: a double-blind, placebo-controlled pilot study. Mov Disord 2004b; 19: 1432–510.
Telstad W, Sorensen O, Larsen S, Lillevold PE, Stensrud P, Nyberg-Hansen R. Treatment of the restless legs syndrome with carbamazepine: a double blind study. Br Med J 1984; 288: 444–6.
Thorp ML, Morris CD, Bagby SP. A crossover study of gabapentin in treatment of restless legs syndrome among hemodialysis patients. Am J Kidney Dis 2001; 38: 104–8.
Trenkwalder C, Garcia-Borreguero D, Montagna P, Lainey E, de Weerd AW, Tidswell P, et al. Ropinirole in the treatment of restless legs syndrome: results from the TREAT RLS 1 study, a 12 week, randomised, placebo controlled study in 10 European countries. J Neurol Neurosurg Psychiatry 2004; 75: 92–7.
Turner JA, Deyo RA, Loeser JD, Von Korff M, Fordyce WE. The importance of placebo effects in pain treatment and research. JAMA 1994; 271: 1609–14.
van Dijk JG, Bollen ELEM, Slootweg J, van der Meer CM, Durian FW, Zwinderman AH. Geen verschil in werkzaamheid tussen hydrokinine en placebo bij het 'restless legs'-syndroom. Ned Tijdschr Geneeskd 1991; 135: 759–63.
von Spiczak S, Whone AL, Hammers A, Asselin MC, Turkheimer F, Tings T, et al. The role of opioids in restless legs syndrome: an [^{11}C]diprenorphine PET study. Brain 2005; 128: 906–17.
Wager TD, Rilling JK, Smith EE, Sokolik A, Casey KL, Davidson RJ, et al. Placebo-induced changes in fMRI in the anticipation and experience of pain. Science 2004; 303: 1162–7.

Wagner ML, Walters AS, Coleman RG, Hening WA, Grasing K, Chokroverty S. Randomized, double-blind, placebo-controlled study of clonidine in restless legs syndrome. Sleep 1996; 19: 52–8.
Walach H, Maidhof C. Is the placebo effect dependent on time? A meta-analysis. In: Kirsch I, editor. How expectancies shape experience. Washington DC: American Psychological Association, 1999; p. 321–32.
Walsh BT, Seidman SN, Sysko R, Gould M. Placebo response in studies of major depression. Variable, substantial, and growing. JAMA 2002; 287: 1840–7.
Walters AS, Hening WA, Kavey N, Chokroverty S, Gidro-Frank S. A double-blind randomized crossover trial of bromocriptine and placebo in restless legs syndrome. Ann Neurol 1988; 24: 455–8.
Walters AS, LeBrocq C, Dhar A, Hening W, Rosen C, Allen RP, et al. Validation of the International Restless Legs Syndrome Study Group rating scale for restless legs syndrome. Sleep Med 2003; 4: 121–32.
Walters AS, Ondo WG, Dreykluft T, Grunstein R, Lee D, Sethi K. Ropinirole is effective in the treatment of restless legs syndrome. TREAT RLS 2: a 12-week, double-blind, randomized, parallel-group, placebo-controlled study. Mov Disord 2004; 19: 1414–23.
Walters AS, Wagner ML, Hening WA, Grasing K, Mills R, Chokroverty S, et al. Successful treatment of the idiopathic restless legs syndrome in a randomized double-blind trial of oxycodone versus placebo. Sleep 1993; 16: 327–32.
Wetter TC, Eisensehr I, Trenkwalder C. Functional neuroimaging studies in restless legs syndrome. Sleep Med 2004; 5: 401–6.
Wetter TC, Stiasny K, Winkelmann J, Buhlinger A, Brandenburg U, Penzel T, et al. A randomized controlled study of pergolide in patients with restless legs syndrome. Neurology 1999; 52: 944–50.
Winkelman JW, Sethi KD, Kushida CA, Becker PM, Koester J, Cappola JJ, et al. Efficacy and safety of pramipexole in restless legs syndrome. Neurology 2006; 67: 1034–9.
Winkelmann J, Wetter TC, Collado-Seidel V, Gasser T, Dichgans M, Yassouridis A, et al. Clinical characteristics and frequency of the hereditary restless legs syndrome in a population of 300 patients. Sleep 2000; 23: 597–602.
XenoPort. Data presented at the 20th Annual Meeting of the Associated Professional Sleep Societies (APSS). [updated 27 July 2006; cited 1 March 2007]. Available from: http://www.xenoport.com/assets/pdf/ PUB_APSS_Presentation_June2006.pdf.

Appendix

Calculation of effect sizes and variances followed the general outline given by Morris and DeShon (2002). Effect sizes (ES) were computed from means and standard deviations as $ES = (M_b - M_e)/(SD_b)$ with Mb the mean at baseline, Me the mean at endpoint and SD_b the standard deviation at baseline. In a minority of cases only the standard deviation of the difference between baseline and endpoint was available. In that case, the effect size in the change score metric $ES_{diff} = (M_b - M_e)/(SD_{diff})$ was transformed into the raw score metric $ES = ES_{diff}$ [2 (1 - r)]1/2 with SD_{diff} the standard deviation of difference scores and r the correlation between baseline and endpoint. All effect sizes were corrected for small sample bias following Hedges (1982). The sampling variance of the effect size was computed as $V_{es} = [2(1 - r)/n][(n - 1)/(n - 3)][1 + (n/2 (1 - r)) ES_g^2] - ES_g^2/c(df)^2$ with n the number of paired observations, ES_g the population effect sizes, and c(df) the bias function $c(df) = 1 - [3/(4 df - 1)]$ with $df = n - 1$. The correlation between baseline and endpoint was computed from standard deviations according to $r = (SD_b^2+SD_e^2+SD_{diff}^2)/(2 SD_b SD_e)$ with SD_e the standard deviation at the end of the trial.

Studies contributing to the meta-analysis

Studies used to compute correlations

RLS severity: Montagna et al. (1984), Boghen et al. (1986), Walters et al. (1993), Trenkwalder et al. (2004), Stiasny-Kolster et al. (2004b), Bogan et al. (2006), Oertel et al. (2006b), GlaxoSmithKline (2006b).

Subjective sleep parameters: Montagna et al. (1984), Bogan et al. (2006), Oertel et al. (2006b).

Polysomnographic sleep parameters: Walters et al. (1988), Walters et al. (1993).

PLMS index: Walters et al. (1988), Walters et al. (1993), Bogan et al. (2006).

Daytime functioning: Walters et al. (1988), Bogan et al. (2006).

Response rates

CGI: Beneš and TULIR study group (2005), Bogan et al. (2006), Garcia-Borreguero et al. (2007), GlaxoSmithKline (2005d), GlaxoSmithKline (2006a), Inoue et al. (2006), Kushida et al. (2006), Kushida and Tolson (2006), Oertel et al. (2005), Oertel et al. (2006a), Oertel et al. (2006b), Partinen et al. 2006), Stiasny-Kolster et al. 2004b), Trenkwalder et al. (2004), Walters et al. (2004), Winkelman et al. (2006), XenoPort (2006).

Other: IRLS: Garcia-Borreguero et al. (2002), Physician-rating: Boghen et al. (1986), Lundvall et al. (1983), Self-made RLS symptom scale: Thorp et al. (2001), No RLS 'attacks': Telstad et al. (1984), Wish to continue: van Dijk et al. 1991), Different scales: Kohnen et al. (2004).

Multiple time points: Telstad et al. (1984), Walters et al. (2004), GlaxoSmithKline (2005a), Winkelman et al. 2006), GlaxoSmithKline (2006a), GlaxoSmithKline (2006b), Oertel et al. (2006b), Bogan et al. (2006).

IRLS and other RLS scores

IRLS: Adler et al. (2004), Trenkwalder et al. (2004), Walters et al. (2004), Stiasny-Kolster et al. (2004a), Stiasny-Kolster et al. (2004b), Kelly and Mistry (2005), Oertel et al. (2005), GlaxoSmithKline (2006b), GlaxoSmithKline (2005d), Bogan et al. (2006), Partinen et al. (2006), Winkelman et al. (2006), Oertel et al. (2006a), Oertel et al. (2006b).

IRLS and other scales: Stiasny-Kolster et al. (2004a), Stiasny-Kolster et al. (2004b), Oertel et al. (2006a), Oertel et al. (2006b).

Other scales: RLS-6 scales: Stiasny-Kolster et al. (2004a), Stiasny-Kolster et al. (2004b), Oertel et al. (2006a), Visual analogue scales: Oertel et al. (2006b), CGI severity item: Wetter et al. (1999), Number of RLS 'attacks': Telstad et al. (1984), Diaries: Montagna et al. (1984), Walters et al. (1993), Montplaisir et al. (1999), Self-made RLS scores: Boghen et al. (1986), Wagner et al. (1996), Beneš et al. (1999), Wetter et al. (1999).

Multiple time points: Trenkwalder et al. (2004), Walters et al. (2004), Bogan et al. (2006), GlaxoSmithKline (2006b).

Subjective sleep parameters

Sleep quality: Medical Outcome Study Sleep Scale: Allen et al. (2004; GlaxoSmithKline (2005b), GlaxoSmithKline (2005c), Bogan et al. (2006), GlaxoSmithKline (2006a), GlaxoSmithKline (2006b), Schlaffragebogen A: Beneš et al. (1999), Wetter et al. (1999), Oertel et al. (2006a; RLS-6 item: Stiasny-Kolster et al. (2004a), Visual analogue scale: Oertel et al. (2006b), Diary: Boghen et al. (1986).

Subjective sleep duration: Medical Outcome Study Sleep Scale: Allen et al. (2004), GlaxoSmithKline (2005b), GlaxoSmithKline (2005c), Bogan et al. (2006), GlaxoSmithKline (2006a), GlaxoSmithKline (2006b), Diary: Benes› et al. (1999).

Polysomnographic sleep parameters

Total sleep time: Walters et al. (s1988), Wagner et al. (1996), Allen et al. (2004), Eisensehr et al. (2004), Oertel et al. (2006a).

Sleep efficiency: Walters et al. (1988), Walters et al. (1993), Wagner et al. (1996), Allen et al. (2004), Eisensehr et al. (2004), Oertel et al. (2006a).

PLMS

Walters et al. (1988), Montplaisir et al. (1996), Walters et al. (1993), Wagner et al. (1996), Benes› et al. (1999), Montplaisir et al. (1999), Allen et al. (2004), Eisensehr et al. (2004), Polo et al. (2005), Bogan et al. (2006), GlaxoSmithKline (2006a), Oertel et al. (2006a), GlaxoSmithKline (2006b), Garcia-Borreguero et al. (2007).

Daytime functioning

Sleepiness: Medical Outcome Study Sleep Scale: Allen et al. (2004), GlaxoSmithKline (2005b), GlaxoSmithKline (2005c), Bogan et al. (2006), GlaxoSmithKline (2006a), GlaxoSmithKline (2006b), Epworth Sleepiness Scale: Adler et al. (2004), Stiasny-Kolster et al. (2004b), Winkelman et al. (2006), Diary: Walters et al. (1993), Wagner et al. (1996), RLS-6: Oertel et al. (2006a), IRLS: Oertel et al. (2006b).

Quality of life: RLS-Quality of Life Questionnaire: Walters et al. (2004), GlaxoSmithKline (2005b), GlaxoSmithKline (2005d), Bogan et al. (2006), Winkelman et al. (2006), GlaxoSmithKline (2006a), German RLS quality of life: Oertel et al. (2006a).

Supplementary material

Supplement 1. Resources used for search

Ressource **Electronic databases**	**URL**	**Retrieved**
PubMed	http://www.ncbi.nlm.nih.gov/entrez/query.fcgi	53
Cochrane Library	http://www3.interscience.wiley.com/cgi-bin/mrwhome/106568753/HOME	5
Handsearching of abstract books	**Years**	
Sleep	2002-2006	3
Journal of Sleep Research	2002, 2004, 2006	0
Sleep Medicine	2005, 2007	3
Neurology	2002-2006	1
Annals of Neurology	2002-2006	1
European Journal of Neurology	2003-2006	1
Journal of Neurology	2002-2006	0
Movement Disorder	2002-2006	4
Internet ressources	**URL**	
PhRMA Clinical Study Results	www.clinicalstudyresults.org	0
IFPMA Clinical Trial Results	http://www.ifpma.org/clinicaltrials.html	0
GlaxoSmithKline Clinical Trial Register	http://ctr.gsk.co.uk/Summary/ropinirole/studylist.asp	6
Boehringer Ingelheim Trial Results	http://trials.boehringer-ingelheim.com/Trial_Results/index.jsp	0
Lilly clinical trial results	http://www.lillytrials.com/results/results_by_ta.html	0
Roche	http://www.roche-trials.com/results.html	0
XenoPort	http://www.xenoport.com/assets/pdf/	2

Supplement 2

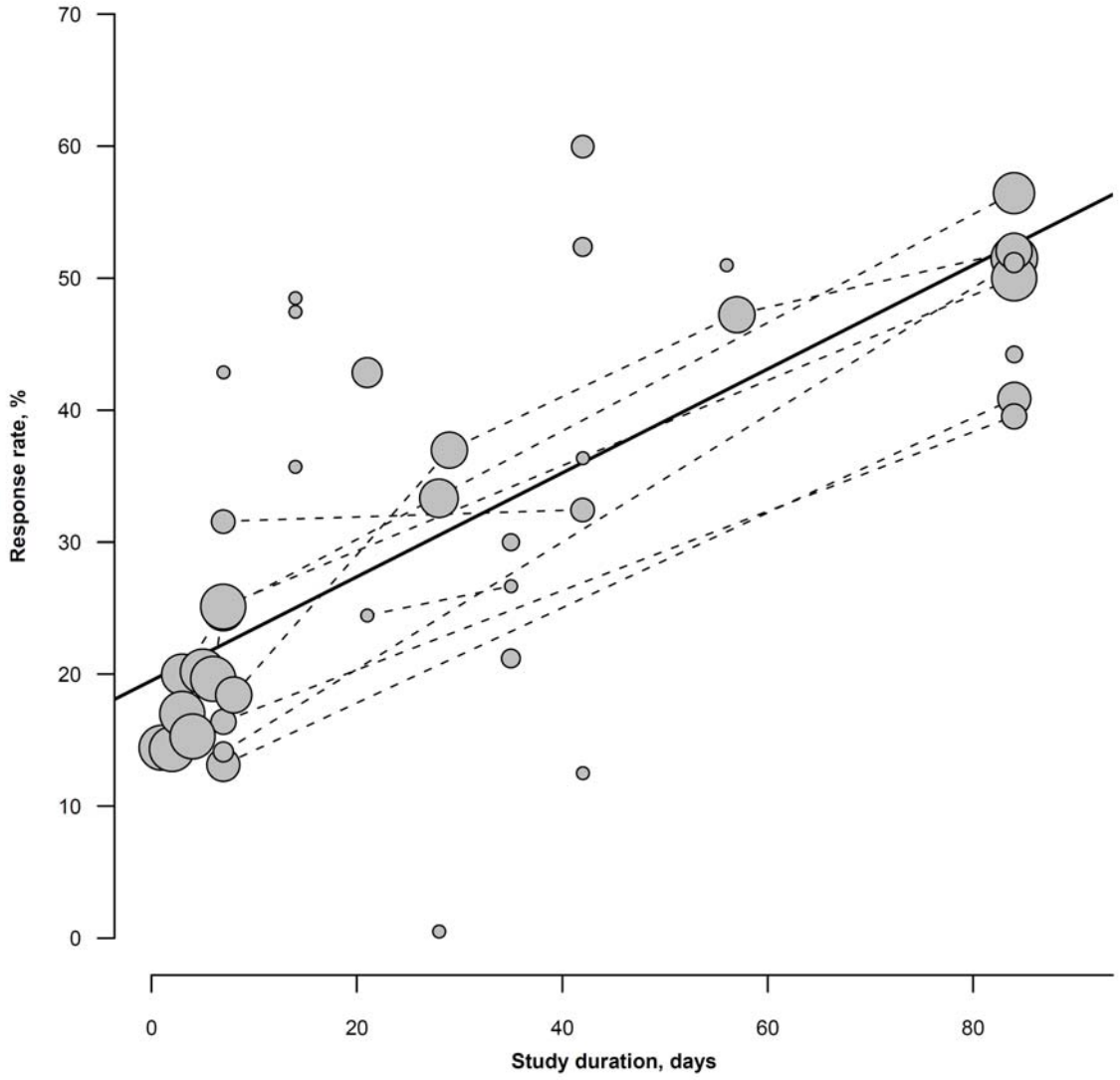
Investigating significant between-study heterogeneity with a priori planned subgroup analysis. Only subgroups with five or more trials are listed.

Outcome/Subgroup	n	Q	df	p	I^2 [CI]
Response rates	**24**	**269.46**	**23**	**< 0.0001**	**91.6% [89% – 94%]**
Parallel-group trials	18	59.45	17	< 0.0001	71.4% [54% – 82%]
Cross-over trials	6	55.94	5	< 0.0001	91.1% [83% – 95%]
Study duration = 84 days	8	14.42	7	0.0441	51.5% [0% – 78%]
Study duration < 84 days	16	152.09	15	< 0.0001	90.1% [86% – 93%]
Study duration ≤ 30 days	7	79.71	6	< 0.0001	92.5% [87% – 96%]
Study duration ≥ 35 days	17	74.8	16	< 0.0001	78.6% [66% – 86%]
Group allocation 1:1	16	237.48	15	< 0.0001	93.7% [91% – 95%]
Group allocation 1: >1	8	13.93	7	0.0525	49.7% [0% – 78%]
Idiopathic RLS	16	43.19	15	0.0001	65.3% [41% – 80%]
Dopaminergic drug	16	42.53	15	0.0002	64.7% [40% – 79%]
Non-dopaminergic drug	8	78.82	7	< 0.0001	91.1% [85% – 95%]
Only CGI response rate	17	34.71	16	0.0044	53.9% [20% – 73%]
Other response rates	7	62.3	6	< 0.0001	90.4% [83% – 95%]
IRLS	**14**	**118.05**	**13**	**< 0.0001**	**89.0% [83% – 93%]**
Parallel-group trials	13	95.78	12	< 0.0001	87.5% [80% – 92%]
Study duration = 84 days	6	52.53	5	< 0.0001	90.5% [82% – 95%]
Study duration < 84 days	8	25.04	7	0.0007	72.0% [43% – 86%]
Study duration ≥ 35 days	11	93.46	10	< 0.0001	89.3% [83% – 93%]
Group allocation 1:1	8	100.75	7	< 0.0001	93.1% [89% – 96%]
Group allocation 1: >1	6	9.87	5	0.0757	49.4% [0% – 80%]
RLS scores	**12**	**44.48**	**11**	**< 0.0001**	**75.3% [57% – 86%]**
Parallel-group trials	5	30.80	4	< 0.0001	87.0% [72% – 94%]
Cross-over trials	7	1.92	6	0.9270	0% [0% – 9%]
Study duration ≤ 30 days	8	4.64	7	0.7035	0% [0% – 51%]
Group allocation 1:1	9	42.34	8	< 0.0001	81.1% [65% – 90%]
Dopaminergic drug	7	12.17	6	0.0583	50.7% [0% – 79%]
Non-dopaminergic drug	5	11.10	4	0.0255	64.0% [5% – 86%]
Sleep quality	**12**	**37.55**	**11**	**< 0.0001**	**70.7% [47% – 84%]**
Parallel-group trials	9	30.12	8	0.0002	73.4% [48% – 86%]
Study duration = 84 days	6	24.74	5	0.0002	79.8% [56% – 91%]
Study duration < 84 days	6	12.00	5	0.0348	58.3% [0% – 83%]
Study duration ≥ 35 days	9	30.12	8	0.0002	73.4% [48% – 86%]
Group allocation 1:1	10	37.12	9	< 0.0001	75.8% [55% – 87%]
Dopaminergic drug	11	37.44	10	< 0.0001	73.3% [51% – 85%]
Subjective Sleep Duration	**7**	**27.49**	**6**	**< 0.0001**	**78.2% [55% – 90%]**
Parallel-group trials	6	26.89	5	< 0.0001	81.4% [60% – 91%]
Total Sleep Time	**5**	**12.56**	**4**	**0.0136**	**68.2% [18% – 88%]**
Sleep Efficiency	**6**	**7.96**	**5**	**0.1582**	**37.2% [0% - 75%]**

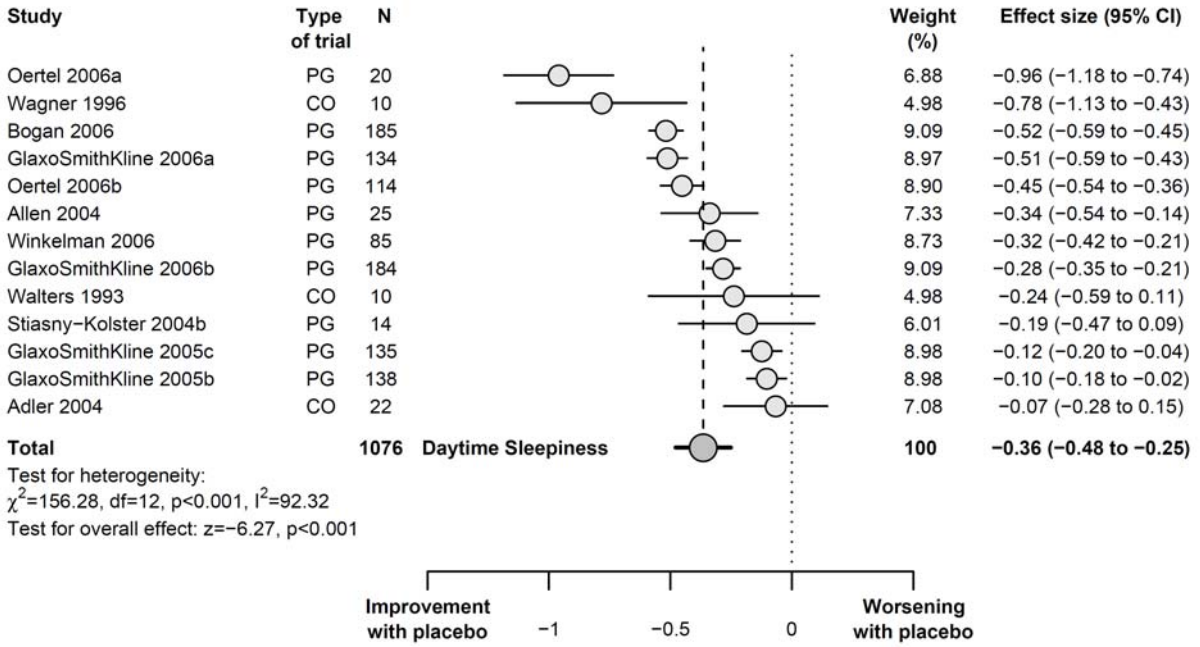
Table continued

Periodic Leg Movements	**14**	**14.53**	**13**	**0.3375**	**10.5% [0% – 49%]**
Quality of Life	**7**	**121.24**	**6**	**< 0.0001**	**95.1% [92% – 97%]**
Parallel-group trials	6	97.88	5	< 0.0001	94.9% [91% – 97%]
Group allocation 1:1	6	120.41	5	< 0.0001	95.8% [93% – 98%]
Sleepiness	**13**	**156.28**	**12**	**< 0.0001**	**92.3% [89% – 95%]**
Parallel-group trials	10	142.79	9	< 0.0001	93.7% [90% – 96%]
Study duration = 84 days	7	108.86	6	< 0.0001	94.5% [91% – 97%]
Study duration < 84 days	6	33.59	5	< 0.0001	85.1% [70% – 93%]
Study duration ≥ 35 days	9	141.08	8	< 0.0001	94.3% [91% – 96%]
Group allocation 1:1	11	153.69	10	< 0.0001	93.5% [90% – 96%]
Dopaminergic drug	11	150.53	10	< 0.0001	93.4% [90% – 96%]
MOS somnolence items	6	108.79	5	< 0.0001	95.4% [92% – 97%]
Other sleepiness scales	7	40.08	6	< 0.0001	85.0% [71% – 92%]

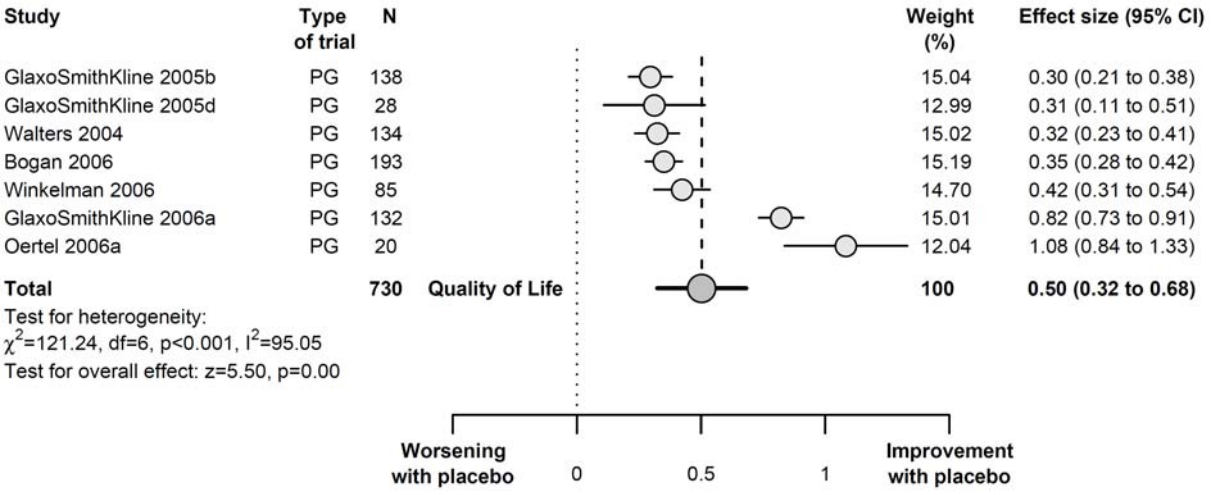
CI: confidence interval; RLS: restless legs syndrome; CGI: Clinical Global Impression scale; MOS: Medical Outcome Study scale



Supplementary Fig 1. [Response rates across all studies in relation to study duration. The size of circles is proportional to weight of study.]



Supplementary Fig 2. [Meta-analysis of standardized repeated-measures effect sizes for the placebo effect in measures of daytime sleepiness (CO: cross-over trial, PG: parallel-group trial, CI: confidence interval)].



Supplementary Fig 3. [Meta-analysis of standardized repeated-measures effect sizes for the placebo effect in quality of life (CO: cross-over trial, PG: parallel-group trial, CI: confidence interval)].

Material available upon request

Excluded studies:

Study	Reason for Exclusion
Saletu *et al.* 2000a	Single-blind study
Saletu *et al.* 2001	Single-blind study
Saletu *et al.* 2002	Single-blind study
Zucconi *et al.* 2003	Single-blind study
Tagaya *et al.* 2002	Same study as Wetter *et al.* 1999
Brodeur *et al.* 1988	Same study as Montplaisir *et al.* 1996
Larsen *et al.* 1985	Same study as Telstad *et al.* 1984
Brenning 1971	Sames study as Brenning 1969
Saletu *et al.* 2000b	Same study as Saletu *et al.* 2000a
Ausserwinkler & Schmidt 1989	Non-randomised study
Kaplan *et al.* 1993	Not all subjects had RLS
Reuter *et al.* 1999	Only a single subject
Hening *et al.* 1986	Naloxone placebo, concomitant medication
von Scheele 1986	Inadequate study design
Earley *et al.* 1998	No data extraction possible
Pieta *et al.* 1998	No data extraction possible
Akpinar 1987	No data extraction possible
Saletu *et al.* 2003	No data extraction possible
Walker *et al.* 1996	No data extraction possible
Trenkwalder *et al.* 1995	No data extraction possible
Winkelman & Johnston 2006	No data extraction possible
Leissner *et al.* 2004	No data extraction possible
Wang *et al.* 2006	No data extraction possible
Quinn and Biber 2006	No data extraction possible
Ulfberg *et al.* 2007	No data extraction possible
Hornyak *et al.* 2006	No data extraction possible
Sloand *et al.* 2004	Concomitant RLS medication
Davis *et al.* 2000	Concomitant RLS medication
Collado-Seidel *et al.* 1999	Concomitant RLS medication
Trenkwalder *et al.* 2004	Single-blind placebo baseline
Bliwise *et al.* 2005	Withdrawal design
Montplaisir *et al.* 2006	Withdrawal design
Trenkwalder *et al.* 2006	Withdrawal design
Beneš 2006	Withdrawal design
Brenning 1969	Questionable diagnosis of RLS
Hürlimann 1974	Questionable diagnosis of RLS
Christiansen 1970	Questionable diagnosis of RLS

References of excluded studies

Akpinar, S. Restless legs syndrome treatment with dopaminergic drugs. *Clin. Neuropharmacol.*, 1987, 10: 69-79.

Ausserwinkler, M. and Schmidt, P. Erfolgreiche Behandlung des "restless legs"-Syndroms bei chronischer Niereninsuffizienz mit Clonidin. *Schweiz. Med. Wochenschr.*, 1989, 119: 184-186.

Beneš, H. Transdermal lisuride: short-term efficacy and tolerability study in patients with severe restless legs syndrome. *Sleep Med.*, 2006, 7: 31-35.

Bliwise, D. L., Freeman, A., Ingram, C. D., Rye, D. B., Chakravorty, S. and Watts, R. L. Randomized, double-blind, placebo-controlled, short-term trial of ropinirole in restless legs syndrome. *Sleep Med.*, 2005, 6: 141-147.

Brenning, R. Enant- och furaldehyd vid molimina crurum nocturna inklusive "restless legs". *Nord. Med.*, 1969, 81: 528-534.

Brenning, R. Motor manifestations in molimina crurum nocturna (including "restless legs"). *J. Am. Geriatr. Soc.*, 1971, 19: 700-708.

Brodeur, C., Montplaisir, J., Godbout, R. and Marinier, R. Treatment of restless legs syndrome and periodic movements during sleep with L-dopa: a double-blind, controlled study. *Neurology*, 1988, 38: 1845-1848.

Christiansen, I. Mesoinositolhexanikotinat (hexanicit) og pentaerytritoltetranikotinat (bufon) ved restless legs. *Ugeskr Laeger*, 1970, 132: 1475-1476.

Collado-Seidel, V., Kazenwadel, J., Wetter, T. C., Kohnen, R., Winkelmann, J., Selzer, R., Oertel, W. H. and Trenkwalder, C. A controlled study of additional sr-L-dopa in L-dopa-responsive restless legs syndrome with late-night symptoms. *Neurology*, 1999, 52: 285-290.

Davis, B. J., Raiput, A., Raiput, M. L., Aul, E. A. and Eichhorn, G. R. A randomized, double-blind placebo-controlled trial of iron in restless legs syndrome. *Eur. Neurol.*, 2000, 43: 70-75.

Earley, C. J., Yaffee, J. B. and Allen, R. P. Randomized, double-blind, placebo-controlled trial of pergolide in restless legs syndrome. *Neurology*, 1998, 51: 1599-1602.

Hening, W. A., Walters, A., Kavey, N., Gidro-Frank, S., Cote, L. and Fahn, S. Dyskinesias while awake and periodic movements in sleep in restless legs syndrome: treatment with opioids. *Neurology*, 1986, 36: 1363-1366.

Hornyak, M., Riemann, D. and Voderholzer, U. Magnesium therapy in patients with idiopathic restless legs syndrome: a placebo-controlled, randomized, double-blind study. *Sleep*, 2006, 29: A289.

Hürlimann, F. Doppelblindstudie mit Circonyl bei peripheren venösen und arteriellen Durchblutungsstörungen unter Berücksichtigung der Symptome: "restless legs" und "nächtliche Crampi". *Schweiz Rundsch Med Prax*, 1974, 63: 194-195.

Kaplan, P. W., Allen, R. P., Buchholz, D. W. and Walters, J. K. A double-blind, placebo-controlled study of the treatment of periodic limb movements in sleep using carbidopa/levodopa and propoxyphene. *Sleep*, 1993, 16: 717-723.

Larsen, S., Telstad, W., Sorensen, O., Thom, E., Stensrud, P. and Nyberg-Hansen, R. Carbamazepine therapy in restless legs. Discrimination between responders and non-responders. *Acta Med. Scand.*, 1985, 218: 223-227.

Leissner, L. C., Grote, L. B. and Ulfberg, J. G. Cabergoline in restless legs syndrome - a double-blind, placebo controlled multicenter study. European Journal of Neurology 11[Suppl. 2], 302. 2004.

Montplaisir, J., Boucher, S., Gosselin, A., Poirier, G. and Lavigne, G. Persistence of repetitive EEG arousals (K-alpha complexes) in RLS patients treated with L-DOPA. *Sleep*, 1996, 19: 196-199.

Montplaisir, J., Karrasch, J., Haan, J. and Volc, D. Ropinirole is effective in the long-term management of restless legs syndrome: A randomized controlled trial. *Mov Disord.*, 2006, 21: 1627-1635.

Pieta, J., Millar, T., Zacharias, J., Fine, A. and Kryger, M. Effect of pergolide on restless legs and leg movements in sleep in uremic patients. *Sleep*, 1998, 21: 617-622.

Quinn, B. and Biber, M. P. Efficacy and safety of topiramate in restless legs syndrome. *Ann. Neurol.*, 2006, 60: S73.

Reuter, I., Ellis, C. M. and Chaudhuri, K. R. Nocturnal subcutaneous apomorphine infusion in Parkinson's disease and restless legs syndrome. *Acta Neurol. Scand.*, 1999, 100: 163-167.

Saletu, B., Gruber, G., Saletu, M., Brandstätter, N., Hauer, C., Prause, W., Ritter, K. and Saletu-Zyhlarz, G. Sleep laboratory studies in restless legs syndrome patients as compared with normals and acute effects of ropinirole. 1. Findings on objective and subjective sleep and awakening quality. *Neuropsychobiology*, 2000a, 41: 181-189.

Saletu, M., Anderer, P., Högl, B., Saletu-Zyhlarz, G., Kunz, A., Poewe, W. and Saletu, B. Acute double-blind, placebo-controlled sleep laboratory and clinical follow-up studies with a combination treatment of rr-L-dopa and sr-L-dopa in restless legs syndrome. *J. Neural Transm.*, 2003, 110: 611-626.

Saletu, M., Anderer, P., Saletu, B., Hauer, C., Mandl, M., Oberndorfer, S., Zoghlami, A. and Saletu-Zyhlarz, G. Sleep laboratory studies in restless legs syndrome patients as compared with normals and acute effects of ropinirole. 2. Findings on periodic leg movements, arousals and respiratory variables. *Neuropsychobiology*, 2000b, 41: 190-199.

Saletu, M., Anderer, P., Saletu-Zyhlarz, G., Hauer, C. and Saletu, B. Acute placebo-controlled sleep laboratory studies and clinical follow-up with pramipexole in restless legs syndrome. *Eur. Arch. Psychiatry Clin. Neurosci.*, 2002, 252: 185-194.

Saletu, M., Anderer, P., Saletu-Zyhlarz, G., Prause, W., Semler, B., Zoghlami, A., Gruber, G., Hauer, C. and Saletu, B. Restless legs syndrome (RLS) and periodic limb movement disorder (PLMD). Acute placebo-controlled sleep laboratory studies with clonazepam. *Eur. Neuropsychopharmacol.*, 2001, 11: 153-161.

Sloand, J. A., Shelly, M. A., Feigin, A., Bernstein, P. and Monk, R. D. A double-blind, placebo-controlled trial of intravenous iron dextran therapy in patients with ESRD and restless legs syndrome. *Am. J. Kidney Dis.*, 2004, 43: 663-670.

Tagaya, H., Wetter, T. C., Winkelmann, J., Rubin, M., Hundemer, H. P., Trenkwalder, C. and Friess, E. Pergolide restores sleep maintenance but impairs sleep EEG synchronization in patients with restless legs syndrome. *Sleep Med.*, 2002, 3: 49-54.

Telstad, W., Sorensen, O., Larsen, S., Lillevold, P. E., Stensrud, P. and Nyberg-Hansen, R. Treatment of the restless legs syndrome with carbamazepine: a double blind study. *Br. Med. J.*, 1984, 288: 444-446.

Trenkwalder, C., Stiasny, K., Pollmächer, T., Wetter, T., Schwarz, J., Kohnen, R., Kazenwadel, J., Kruger, H. P., Ramm, S. and Kunzel, M. L-dopa therapy of uremic and idiopathic restless legs syndrome: a double-blind, crossover trial. *Sleep*, 1995, 18: 681-688.

Trenkwalder, C., Hundemer, H. P., Lledo, A., Swieca, J., Polo, O., Wetter, T. C., Ferini-Strambi, L., de Groen, H., Quail, D., Brandenburg, U. and PEARLS Study Group Efficacy of pergolide in treatment of restless legs syndrome: the PEARLS Study. *Neurology*, 2004, 62: 1391-1397.

Trenkwalder, C., Stiasny-Kolster, K., Kupsch, A., Oertel, W. H., Koester, J. and Reess, J. Controlled withdrawal of pramipexole after 6 months of open-label treatment in patients with restless legs syndrome. *Mov Disord.*, 2006, 21: 1404-1410.

Ulfberg, J., Leissner, L., Grote, L. and Hedner, J. Beneficial effects of iron-sucrose in restless legs syndrome: a randomised, double-blind, placebo-controlled comparison. One year follow-up. *Sleep Med.*, 2007, 8: S19.

von Scheele, C. Levodopa in restless legs. *Lancet*, 1986, 2: 426-427.

Walker, S. L., Fine, A. and Kryger, M. H. L-DOPA/carbidopa for nocturnal movement disorders in uremia. *Sleep*, 1996, 19: 214-218.

Wang, J. Y., Mysliwiec, V. and Mysliwiec, A. Efficacy of iron in patients with restless legs syndrome and low-normal ferritin: a randomized, double-blind, placebo controlled study. *Sleep*, 2006, 29: A273.

Wetter, T. C., Stiasny, K., Winkelmann, J., Buhlinger, A., Brandenburg, U., Penzel, T., Medori, R., Rubin, M., Oertel, W. H. and Trenkwalder, C. A randomized controlled study of pergolide in patients with restless legs syndrome. *Neurology*, 1999, 52: 944-950.

Winkelman, J. W. and Johnston, L. Efficacy and tolerability of levetiracetam in the treatment of restless legs syndrome: a double-blind placebo controlled crossover trial. Sleep 29[Abstract Suppl.], A274. 2006.

Zucconi, M., Oldani, A., Castronovo, C. and Ferini-Strambi, L. Cabergoline is an effective single-drug treatment for restless legs syndrome: clinical and actigraphic evaluation. *Sleep*, 2003, 26: 815-818.

IV. Prevalence of the restless legs syndrome in transsexual patients: the hormonal hypothesis revisited*

This is a pre-copy-editing, author-produced PDF of an article accepted for publication in Journal of Neurology *following peer review. The original publication [Prevalence of the restless legs syndrome in transsexual patients: the hormonal hypothesis revisited. Stephany Fulda, Günther K. Stalla, Thomas C. Wetter. Journal of Neurology 2007; DOI 10.1007/100415-007-0624-6*] *is available at: www.springerlink.com*

Sirs: Epidemiological studies that have employed established diagnostic criteria[1] show a markedly higher prevalence of the restless legs syndrome (RLS) in women[2,3]. The increased risk for RLS in females is related to the number of pregnancies[3]. In addition, around 25% of females will experience RLS symptoms during pregnancy with the highest prevalence during the last trimester[8,9,11]. Iron requirements during pregnancy are increased and may play a role in the etiology of RLS[8]. However, a hormonal hypothesis regarding the incidence of RLS during pregnancy has already been postulated by Ekbom[5], and a recent study[6] has shown that pregnant women with RLS had significantly higher estradiol levels only during late-term pregnancy compared to those without RLS.

We therefore explored the prevalence of RLS in a group of transsexual patients treated with either testosterone or estrogens to elucidate the role of gender and steroid hormones as a risk factor for RLS. The study was approved by the local ethic committee. Questionnaires were mailed to 288 transsexual patients registered at the outpatient clinic for endocrinology at the Max Planck Institute of Psychiatry. Accompanying the questionnaires was a letter

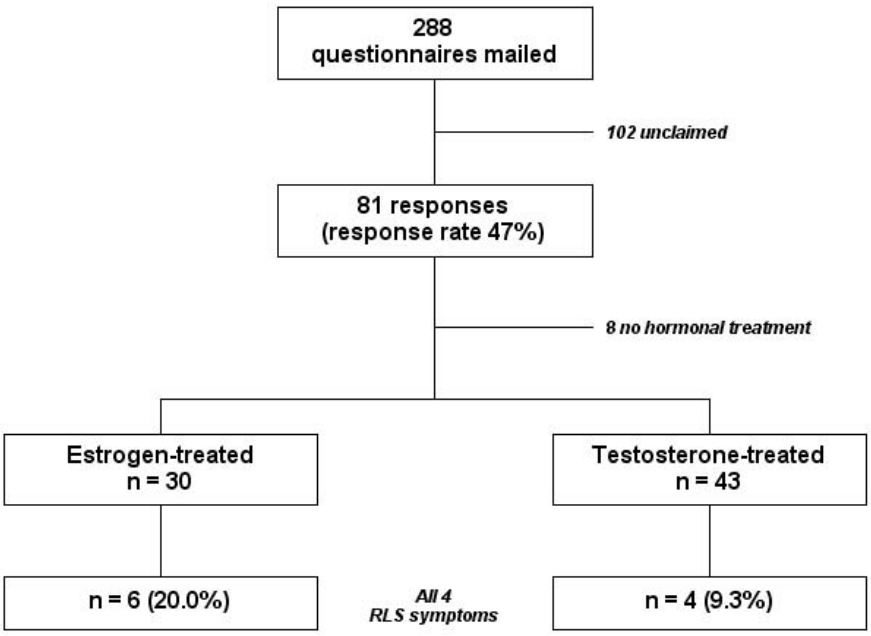


Fig. 1. Study design and response rates. (RLS = restless legs syndrome)

* Fulda S, Stalla GK, Wetter TC. Prevalence of the restless legs syndrome in transsexual patients: the hormonal hypothesis revisited. Journal of Neurology 2007; 254: 1748-1749.

Table 1. Characteristics of participants (mean ± standard deviation)

	Estrogen-treated subjects N = 30	Testosterone-treated subjects N = 43	Test statistic[a], p
Age, years	46.36 ± 7.10	31.93 ± 7.98	t = 7.95, p < 0.001
Age range, years	31 - 61	21-54	
BMI	24.61 ± 3.35	23.58 ± 3.47	F[b] = 3.16, p = 0.080
Duration of hormone treatment, months	74.93 ± 75.58	75.88 ± 56.51	U[c] = 597, p = 0.594
PSQI	5.16 ± 2.39 (n = 25)	5.62 ± 4.29 (n = 35)	F[b] = 0.79, p = 0.378
ESS	7.44 ± 4.16 (n = 29)	7.62 ± 4.07 (n = 42)	F[b] = 0.01, p = 0.936
Sleep onset latency, min[e]	15.13 ± 12.92 (n = 30)	18.53 ± 16.76 (n = 43)	F[b] = 0.27, p = 0.871
Sleep duration, hours	7.42 ± 2.09 (n = 29)	7.48 ± 2.11 (n = 41)	F[b] = 0.21, p = 0.886
All 4 RLS symptoms (n, %)	6 (20.0 %)	4 (9.3%)	p[d] = 0.300

PSQI = Pittsburgh Sleep Quality Index; ESS = Epworth Sleepiness Scale

[a] Because of missing data for some of the parameters a multivariate approach to control the overall type-I error rate was not feasible.

[b] Analysis of variance F-tests included age as a covariate

[c] Mann-Whitney U test

[d] Fisher's Exact Test

[e] due to substantial skewness, sleep onset latency was log-transformed before being entered into the analysis

explaining the purpose of the study and assuring anonymity to all participants. The returning of the questionnaires was regarded to signify informed consent. Basic information was ascertained concerning age, weight, height, co-morbidities, intake of medication and hormonal treatment. Sleep quality (Pittsburgh Sleep Quality index[4]) and daytime sleepiness (Epworth Sleepiness Scale[7] (ESS)) was assessed. Subjects were asked for the four cardinal symptoms of RLS[1], a rating of symptom severity and their first occurrence (lifetime). Differences between the two groups were explored with univariate analysis of variance with age as a covariate due to the significant age difference between the two groups (t-test). Non-parametric tests (Mann-Whitney U-Test, Fisher's Exact Test) were applied for non-normally distributed variables and frequencies.

Eigthy-one questionnaires were returned and 73 subjects were currently treated with testosterone or estrogens (Figure 1). All 43 testosterone-treated subjects were female-to-male transsexual patients while the estrogen-treated group (n = 30) included one hermaphrodite beside the female-to-male transsexual patients. The main results are given in Table 1. Twenty percent of the estrogen-treated subjects affirmed all four RLS criteria compared to 9.3% of the testosterone-treated subjects (p = 0.30). Because the testosterone-treated subjects were younger on average than the estrogen-treated subjects we repeated the analysis considering only the 22 testosterone-treated participants within the age range of the estrogen-treated participants. The difference in prevalence (20.0% vs. 4.5%) became more pronounced but still did not reach statistical significance (p = 0.22). In half of the ten

subjects with RLS, symptoms started after hormonal treatment began. None of them was treated for RLS. Two participants took medication that could have aggravated RLS (venlafaxine, 75 mg (one subject) and flupentixol, 10 mg (one subject)), however, these two subjects did not report restless legs symptoms.

Overall, we found a higher, although statistically not significant, prevalence in estrogen-treated transsexuals as compared to testosterone-treated subjects. It is most likely that the study was underpowered regarding the number of subjects. Although we contacted nearly 300 persons, ultimately a considerable lower number replied. For one, it is known that there is a limited accessibility for this target population[10], and this might account for the 35% of subjects we were unable reach by mail. Secondly, we assured subjects' anonymity, and thus we were not able to re-contact subjects to achieve a higher response rate and to gather more detailed data and follow-up of the affected individuals. Finally, we explored the presence of restless legs symptoms at the time of investigation and may thus have missed those subjects in whom hormonal treatment may have induced a transient RLS. Despite these constraints, this pilot study suggests that estrogens may play a role in the development of RLS. Further studies, however, are needed to replicated and extend this findings and to assess the prevalence of RLS in a larger number of transsexual patients.

ACKNOWLEDGMENT

The authors like to thank Dr. Markus Riedel for inspiring this study.

REFERENCES

1. Allen RP, Picchietti D, Hening WA, Trenkwalder C, Walters AS, Montplaisir J, Restless Legs Syndrome and Epidemiology workshop at the National Institutes of Health, International Restless Legs Syndrome Study Group (2003) 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 4:101-119
2. Allen RP, Walters AS, Montplaisir J, Hening W, Myers A, Bell TJ, Ferini-Strambi L (2005) Restless legs syndrome prevalence and impact. REST general population study. Arch Int Med 165:1286-1292
3. Berger K, Luedemann J, Trenkwalder C, John U, Kessler C (2004) Sex and the risk of restless legs syndrome in the general population. Arch Int Med 164:196-202
4. Buysse DJ, Reynolds CF, III, Monk TH, Hoch CC, Yeager AL, Kupfer DJ (1991) Quantification of subjective sleep quality in healthy elderly men and women using the Pittsburgh Sleep Quality Index (PSQI). Sleep 14:331-338
5. Ekbom KA (1945) Restless legs: clinical study of hitherto overlooked disease in legs characterized by peculiar paresthesia ('anxietas tibiarum'), pain and weakness and occuring in two main forms, asthenia crurum paresthetica and asthenia crurum dolorosa; short review of paresthesias in general. Acta Med Scand 158:1-123
6. Fulda S, Dzaja A, Lancel M, Pollmächer T (2004) Sleep and periodic leg movements in late term pregnancy and postpartum in women with and without RLS. J Sleep Res 13 (Suppl. 1):252

7. Johns MW (1994) Sleepiness in different situations measured by the Epworth sleepiness scale. Sleep 17:703-710
8. Lee KA, Zaffke ME, Baratte-Beebe K (2001) Restless legs syndrome and sleep disturbance during pregnancy: the role of folate and iron. J Womens Health Gend Based Med 10:335-341
9. Manconi M, Govoni V, De Vito A, Economou NT, Cesnik E, Casetta I, Mollica G, Ferini-Strambi L, Granirei E (2004) Restless legs syndrome and pregnancy. Neurology 63:1065-1069
10. Michel A, Ansseau M, Legros JJ, Pitchot W, Mormont C (2002) The transsexual: what about the future? Eur Psychiatry 17:353-362
11. Suzuki K, Ohida T, Sone T, Takemura S, Yokoyama E, Miyake T, Harano S, Motojima S, Suga M, Ibuka E (2003) The prevalence of restless legs syndrome among pregnant women in Japan and the relationship between restless legs syndrome and sleep problems. Sleep 26:673-677

V. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions*

This is a pre-copy-editing, author-produced PDF of an article accepted for publication in Nature Genetics *following peer review. The definitive publisher-authenticated version [Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Juliane Winkelmann, Barbara Schormair, Peter Lichtner, Stephan Ripke, Lan Xiong, Shapour Jalilzadeh, Stephany Fulda, Benno Pütz, Gertrud Eckstein, Stephanie Hauk, Claudia Trenkwalder, Alexander Zimprich, Karian Stiasny-Kolster, Wolfgang Oertel, Cornelius G. Bachmann, Walter Paulus, Ines Peglau, Ilonka Eisensehr, Jacques Montplaisir, Gustavo Turecki, Guy Rouleau, Christian Gieger, Thomas Illig, H. Erich Wichmann, Florian Holsboer, Bertram Müller-Myhsok, Thomas Meitinger. Nature Genetics 2007; DOI 10.1038/ng2099] is available at:*
www.nature.com/ng/journal/v39/n9/abs/ng2099.html

Abstract

Restless legs syndrome (RLS) is a frequent neurological disorder characterized by an imperative urge to move the legs during night, unpleasant sensation in the lower limbs, disturbed sleep and increased cardiovascular morbidity. In a genome-wide association study we found highly significant associations between RLS and intronic variants in the homeobox gene MEIS1, the BTBD9 gene encoding a BTB(POZ) domain as well as variants in a third locus containing the genes encoding mitogen-activated protein kinase MAP2K5 and the transcription factor LBXCOR1 on chromosomes 2p, 6p and 15q, respectively. Two independent replications confirmed these association signals. Each genetic variant was associated with a more than 50% increase in risk for RLS, with the combined allelic variants conferring more than half of the risk. MEIS1 has been implicated in limb development, raising the possibility that RLS has components of a developmental disorder.

Nightwalkers, as individuals with RLS call themselves, are forced to move their legs during periods of rest especially in the evening and night to relieve uncomfortable or painful sensations in the deep calf[1]. This diurnal variation leads to impaired sleep onset, and the periodic leg movements during sleep in the majority of patients contribute to sleep disruption and a reduced quality of life as a major consequence[2]. There are recognized secondary forms of RLS such as in iron deficiency, pregnancy and end-stage renal disease and associated morbidity such as increased cardiovascular risk[2,3]. RLS is one of prevalence of up to 10% in the elderly in North America and Europe2. Dopaminergic agents originally developed for Parkinson's disease have been used to treat RLS, with an unknown mode of action[2]. Neurophysiological, pharmacological and neuroimaging studies suggest that the characteristic symptoms originate in the central nervous system, yet the underlying

* Winkelmann J, Schormaier B, Lichtner P, Ripke S, Xiong L, Jalilzadeh S, Fulda S, Pütz B, Eckstein G, Hauk S, Trenkwalder C, Zimprich A, Stiasny-Kolster K, Oertel W, Bachmann CG, Paulus W, Peglau I, Eisensehr I, Montplaisir J, Turecki G, Rouleau G, Gieger C, Illig T, Wichmann HE, Holsboer F, Müller-Myhsok B, Meitinger T. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Nat Genet 2007; 39(8): 1000-1006.

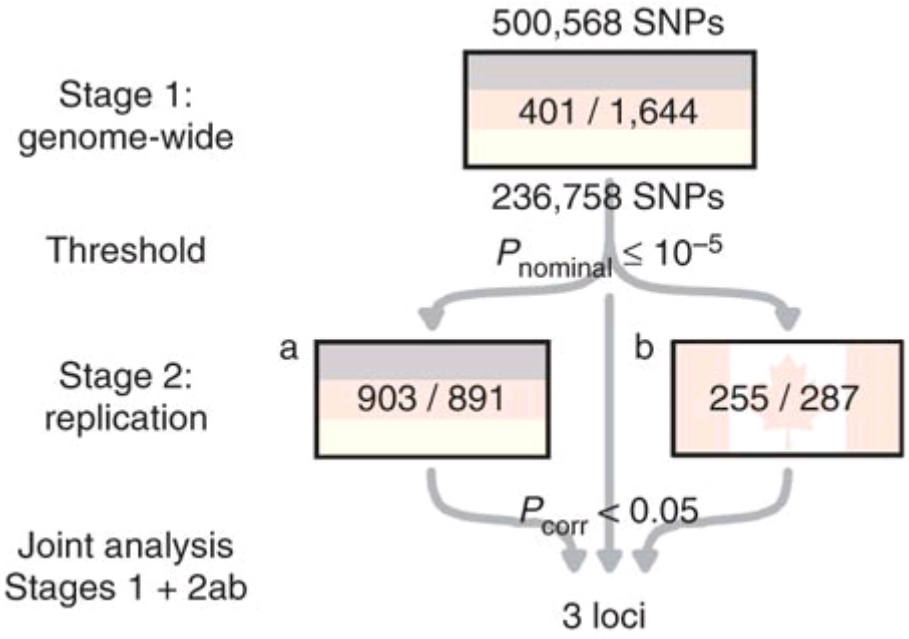


Fig. 1. Numbers refer to cases and controls and SNPs genotyped and analyzed. The 13 most significant SNPs together with neighboring SNPs were replicated in a German ('a') and a Canadian ('b') case/control sample. Three loci were confirmed in both stage 2 samples of the study.

neurobiology remains obscure[4]. A family history of RLS is present in more than 50% of affected individuals, and similar figures have been reported for heritability in twin studies[5,6]. Linkage analysis uncovered five loci based on recessive (RLS1) or dominant inheritance (RLS2–RLS5), but so far the most common neurological disorders, with an age-dependent no causally related sequence variants have been identified[5–7]. With SNP arrays becoming a mature technology, we conducted a genomewide association study (GWAS), typing 500,568 SNPs in individuals with RLS and in a large control cohort from the general population.

Genome-wide association

The study design involved an exploratory stage (stage 1) followed by replication in two further case-control samples (stages 2a and 2b) (Fig. 1). In stage 1, we performed a GWAS, typing cases and controls on a single platform with the Affymetrix 500K Array Set. To enrich for risk alleles and minimize phenotypic heterogeneity, we selected subjects with familial RLS (n = 401). Controls were selected randomly from a population-based cohort (*n* = 1,644, from the KORA-S3/F3 survey, described previously)[8]. For statistical analysis, we selected SNPs by including only high-quality genotypes to reduce the number of false-positive signals (Supplementary Table 1). A total of 236,758 SNPs passed all quality control filters (mean call rate = 99.48%). The effect of population stratification was negligible (inflation factor λ = 1.09 via genomic control)[9] (Fig. 2). Eigenvalue-based analysis showed only minimal population substructure (Fig. 2). An Armitage trend test uncovered four SNPs with *P* values < 10^{-6} (Fig. 3 and Supplementary Table 2). After correcting for multiple testing, we identified a single SNP within MEIS1 that reached genome-wide significance (rs2300478, $P_{corrected} < 0.0002$).

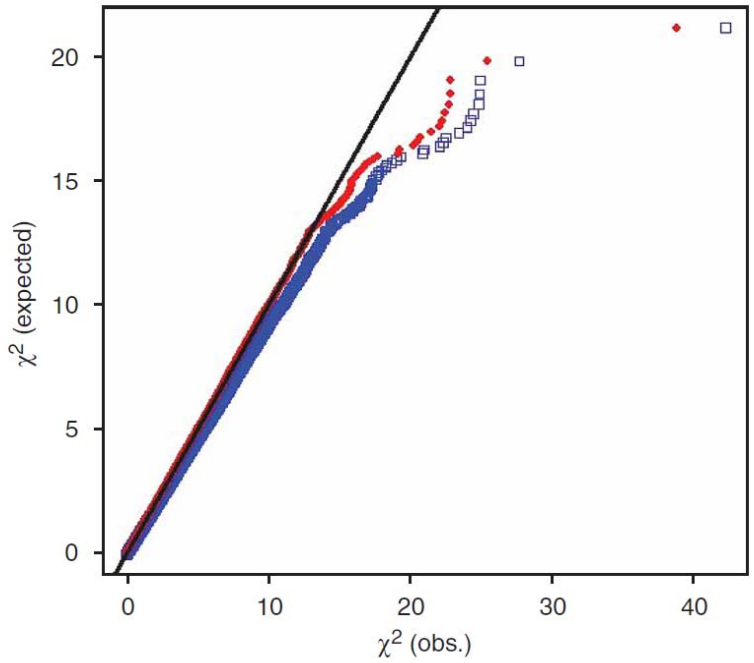


Fig. 2. Extent of population stratification. The distribution of expected (under the null hypothesis) versus observed χ^2 values (all *P* values obtained in the analysis of sample 1, using Armitage trend test with age and sex as covariates) before (blue) and after (red) correction by division with λ. Adherence to the diagonal indicates lack of inflation of the statistic. As can be seen in the uncorrected plot, there is evidence for a systematic deviation toward higher-than-expected values. After the correction, there is nearperfect adherence to the diagonal for most of the values obtained, indicating that the correction performed well.

Replication of genome-wide findings

For stage 2 replication, 13 SNPs passed our inclusion criteria based on *P* value, location within a linkage peak and visual inspection of clustering data. We selected these and 15 neighboring SNPs for replication. They mapped to six discernible regions. Of these 28 SNPs, 25 were successfully genotyped in stage 2a and 24 in stage 2b (Supplementary Table 3). Individuals in 2a (*n* = 903, familial or sporadic RLS) had been recruited separately using the sampling design of stage 1. Control subjects were selected from KORA-S4 (*n* = 891).

In stage 2a, we found nominally significant evidence for association in five regions, of which three withstood correction for multiple testing (Fig. 4 and Supplementary Table 4). The first region was on 2p, located in a 32-kb linkage disequilibrium (LD) block containing exon 9 of MEIS1. Here, two of three SNPs showed significant association ($P < 10^{-11}$). MEIS1 is a member of a family of highly conserved TALE homeobox genes. Heterodimers of MEIS1 with PBX and HOX proteins augment the affinity and specificity of DNA binding by HOX proteins[10]. MEIS1 has been found to be overexpressed in acute myeloid leukemia[10], and studies in *Xenopus laevis* have shown involvement in neural crest development[11]. In addition, there are several potential links to RLS: during embryonic development, MEIS1 is essential for proximo-distal limb formation[12], and children with restless legs syndrome are often described as having growing pains[13]. MEIS1 is part of a Hox transcriptional regulatory network that specifies spinal motor neuron pool identity and connectivity[14]. Notably, spinal hyperexcitability is an established component in the genesis of periodic leg movements found in individuals with RLS[15]. Specific functions of MEIS1 in postembryonic tissues still remain to be established. The protein is known to be expressed in the adult mouse brain in

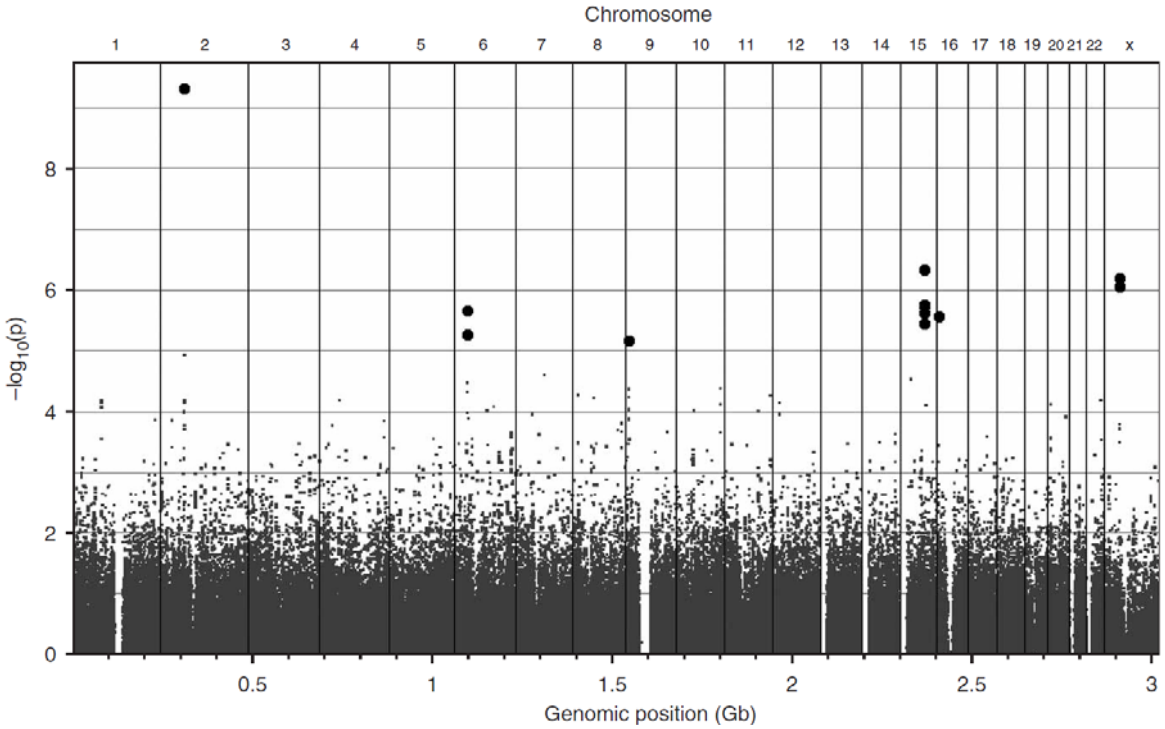


Fig. 3. Genome-wide association study for RLS susceptibility loci. The analysis compared 393 successfully genotyped RLS cases with 1,602 population-based KORA controls. The x-axis represents genomic position, and the y-axis shows $-\log_{10}(P)$. Thirteen SNPs that passed inclusion criteria for the replication study of stage 2 are highlighted in bold. Note that the P values of three SNPs on chromosome 15 are very similar, and these SNPs appear as one single dot.

cerebellar granule cells, the forebrain and, notably, in dopaminergic neurons of the substantia nigra[16].

The second region with significant association was on chromosome 6p, within a 113-kb LD block in intron 5 of the BTBD9 gene. All five SNPs tested were significant, four of these with P values < 10^{-5}. Little is known about BTBD9 other than that it belongs to the BTB(POZ) proteins. BTB stands for *broad complex*, *tramtrack* and *bric a` brac*, genes that in *Drosophila melanogaster* are required for embryonic development, cell fate determination in the eye, metamorphosis and pattern formation in the limbs[17,18]. Functions of BTB(POZ) proteins include transcription repression, cytoskeleton regulation, tetramerization and gating of ion channels, as well as ubiquitindependent protein degradation[17]. The modular nature of this protein and the universal occurrence of the particular domains of BTBD9 make assignment of a specific function difficult at present.

The third region, defined by seven SNPs tested on 15q, showed significant evidence for association, with P < 10^{-4}. This region contains a 48-kb LD block overlapping the 3' end of MAP2K5, a member of the mitogen-activated protein kinase family, and the adjacent LBXCOR1 gene. MAPK pathways are conserved from yeast to human and are activated by a signaling cascade that mediates the transduction of extracellular signals to cytoplasmic nuclear effectors[19]. MAP2K5 is a specific upstream activator of ERK5, and this pathway is activated by oxidative stress, hyperosmolarity and growth factors. In addition, MAP2K5 and ERK5 are abundantly expressed in heart and skeletal muscles, and the MAP2K5/ERK5 MAP kinase cascade is critical at early stages of muscle cell differentiation[19]. The possible link between RLS risk alleles and known biological functions of the MAP2K5-ERK5 pathway is of particular interest, as this pathway is important in neuroprotection of dopaminergic neurons[20]. LBXCOR1 is annotated as being downstream of MAP2K5 and acting as a transcriptional corepressor of LBX1. This homeobox gene is critical in the development of sensory the

Table 1. Confirmed association results

dbSNP ID	Chr	Genome position	Gene	MAF (cases)	Risk allele	MAF (controls)
rs2300478	2p	66634957	*MEIS1*	0.367 (G)	G	0.241 (G)
rs9296249	6p	38473819	*BTBD9*	0.162 (C)	T	0.235 (C)
rs9357271	6p	38473851	*BTBD9*	0.165 (C)	T	0.238 (C)
rs12593813	15q	65823906	*MAP2K5*	0.258 (A)	G	0.330 (A)
rs11635424	15q	65824632	*MAP2K5*	0.257 (A)	G	0.330 (A)
rs4489954	15q	65859129	*MAP2K5*	0.239 (T)	G	0.311 (T)
rs3784709	15q	65859329	*MAP2K5*	0.251 (T)	C	0.321 (T)
rs1026732	15q	65882139	*MAP2K5*	0.252 (A)	G	0.327 (A)
rs6494696	15q	65890260	*[MAP2K5/LBXCOR1]*	0.253 (C)	G	0.326 (C)

dbSNP ID	OR (95% c.i.)	Stage 1 P_{nom}	Stage 2a P_{nom}	Stage 2b P_{nom}	Stage 1+2a+2b P_{nom}	Stage 1+2a+2b $P^{a}_{corrected}$
rs2300478	1.74 (1.57–1.92)	4.89E–10	5.93E–12	2.19E–03	3.41E–28	8.08E–23
rs9296249	1.67 (1.49–1.89)	2.19E–06	1.61E–06	4.14E–03	3.99E–18	9.44E–13
rs9357271	1.66 (1.48–1.87)	5.48E–06	1.85E–06	2.48E–03	6.31E–18	1.50E–12
rs12593813	1.50 (1.36–1.66)	1.85E–06	4.95E–05	1.57E–02	1.06E–15	2.51E–10
rs11635424	1.51 (1.37–1.67)	1.77E–06	2.54E–05	6.60E–03	3.65E–16	8.64E–11
rs4489954	1.51 (1.36–1.67)	2.44E–06	2.60E–05	1.66E–02	2.68E–15	6.35E–10
rs3784709	1.52 (1.37–1.68)	3.56E–06	7.46E–05	1.79E–03	4.06E–16	9.61E–11
rs1026732	1.53 (1.39–1.70)	4.67E–07	2.78E–05	5.22E–03	6.09E–17	1.44E–11
rs6494696	1.52 (1.38–1.69)	1.79E–06	5.20E–05	5.22E–03	2.00E–16	4.74E–11

SNPs with significant association that were successfully genotyped in all three case-control samples, located in three different genomic regions. Genome positions refer to the human March 2006 (hg18) assembly. [MAP2K5/LBXCOR1] denotes an intergenic position of the SNP. MAF, minor allele frequency; OR, odds ratio; c.i., confidence interval; P_{nom} = nominal P value. MAF refers to stage 2a data only; OR was calculated using combined data from all stages. *P* values for stage 1, 2a and combined analysis were calculated using logistic regression implementing an Armitage trend test and taking sex and age as covariates into account. *P* values in stage 1 and 2a resulting from this regression were further corrected for population stratification by dividing the resulting χ^2 by the inflation factor 1. $^{a}P_{corrected}$ = *P* value corrected for multiple testing using Bonferroni's method, correcting for 236,758 SNPs.

pathways in the dorsal horn of the spinal cord that relay pain and touch[21]. Three SNPs within PTPRD gene in the chromosome 9 linkage region (RLS3) and one SNP on chromosome 16 in the A2BP1 gene were nominally significant.

In stage 2b, we genotyped the same SNPs in affected individuals (*n* = 255) and controls (*n* = 287) from a French-Canadian population. Here, we found nominally significant evidence for association in four regions (two SNPs on chromosome 2p, five SNPs on 6p, seven SNPs on 15q and one SNP on 16p, Supplementary Table 5). The same three regions as in stages 1 and 2a remained significant after correction for multiple testing. Odds ratios (ORs) and risk alleles were very similar to those for stage 2a. Table 1 shows those nine SNPs in the three loci confirmed in all three sample sets and in joint analysis withstanding genome-wide correction for multiple testing.

Fine mapping, haplotype and risk analysis

We genotyped tagging SNPs and all known coding and splice-site SNPs for fine mapping in the stage 2a samples. This confirmed the candidate regions defined by the explorative phase of the study (Fig. 4). Haplotype analysis for MEIS1 delineated a haplotype block (rs3890755 to rs12469063). A haplotype completely described by allele A (rs6710341) and allele G (rs12469063) was more strongly associated than each single SNP in this block ($P = 5.87 \times 10^{-20}$, OR = 2.75 [95% confidence interval, 2.23–3.41]). This haplotype was also maximally associated in the Canadian sample ($P = 8.51 \times 10^{-7}$, OR = 2.36 [1.40–3.97], Fig. 5). For

BTBD9 and the MAP2K5 and LBXCOR1 region haplotype analysis confirmed the results of single-SNP analysis.

In exploratory analysis, we compared the ORs obtained under the allele dosage model to those obtained under the unrestricted model. For MEIS1 and BTBD9, we did not find any significant difference between the models tested (MEIS1: $P = 0.714$; BTBD9: $P = 0.913$), but the allele dosage model was more parsimonious. For the MAP2K5 and LBXCOR1 region, the allele dosage model was significantly less likely than the unrestricted model ($P = 0.006$). Estimates pointed to a recessive model. This model was significantly better than the allele dosage model ($P = 0.009$) and not worse than the unrestricted model ($P = 0.395$). There was no difference in effect estimates between samples (Supplementary Table 6).

In the combined German samples, lower limits of the sequential attributable fraction (SAFs)[22,23] were estimated at 0.092, 0.303 and 0.079 for MEIS1, BTBD9 and the MAP2K5 and LBXCOR1 region respectively. Corresponding upper limits (equal to the population attributable risk fraction (ARF)) were 0.227, 0.492 and 0.201. In the Canadian sample, the lower limits of the SAFs were 0.075, 0.316 and 0.090, respectively, and we estimated the upper limits at 0.226, 0.550 and 0.258, respectively.We could not identify any statistical interaction between these loci, either in the individual samples or in the combined German or combined German-Canadian samples. Overall, although the single ARF and SAF estimates may be slightly overestimated, they clearly indicate that the three loci account for a large part of the phenotype in the populations studied. We estimated the ARF jointly attributable to the three loci at 68.6% in the German population and 74.2% for the Canadian population.

A comparison of familial versus sporadic cases in the combined stage 1 and 2a data set demonstrated virtually indistinguishable ORs for the regions on 6p and 15q. For the region on 2p, the risk was higher in familial (rs2300478: OR = 1.82 [1.55–2.14]) than in sporadic cases (OR = 1.59 [1.34–1.90]). However, confidence intervals were overlapping with no significant difference in allele distributions ($P = 0.22$, Supplementary Table 7). The familial relative risk figures estimated by the risk to siblings λ_s were 1.13 for MEIS1, 1.02 for BTBD9 and 1.03 for MAP2K5/LBXCOR1 in the German data set, with almost identical estimates in the Canadian data.

The increasing medical attention to RLS in recent years is matched by our ignorance about its underlying molecular basis. The genetic heterogeneity of RLS has made linkage studies notoriously difficult and favors association approaches. In agreement with power calculations, an initial genome-wide screen for common variants in 400 cases and 1,600 controls enabled us to detect risk alleles with odds ratios > 1.5. Sample size in the replicate was twice as high as in the initial GWAS and provided unequivocal evidence for the signals. The effects were strong enough that a second replication in a small independent sample from Canada also yielded significant signals for all three regions. A particular feature of our study design is the use of a control group from the general population. This provided us with very accurate estimates of the genotype frequencies and it avoided any bias to which a disease-negative population is prone.

The identification of significant signals in genes that have not been considered candidates from previous biological knowledge is a recurring theme in GWASs[24]. The current knowledge about MEIS1, BTBD9, MAP2K5 and LBXCOR1 opens new avenues of RLS research, and the involvement of developmental genes challenges us to rethink our basic concept of this widespread disease.

A major proportion of the risk for RLS is explained by variants in the loci identified. We could not derive any different contributions from any of these loci to familial versus sporadic RLS. The associated variants all convey very low familial relative risk ($\lambda_s < 1.15$ in all cases). The lack of positive results within the known linkage regions does not argue against the validity of

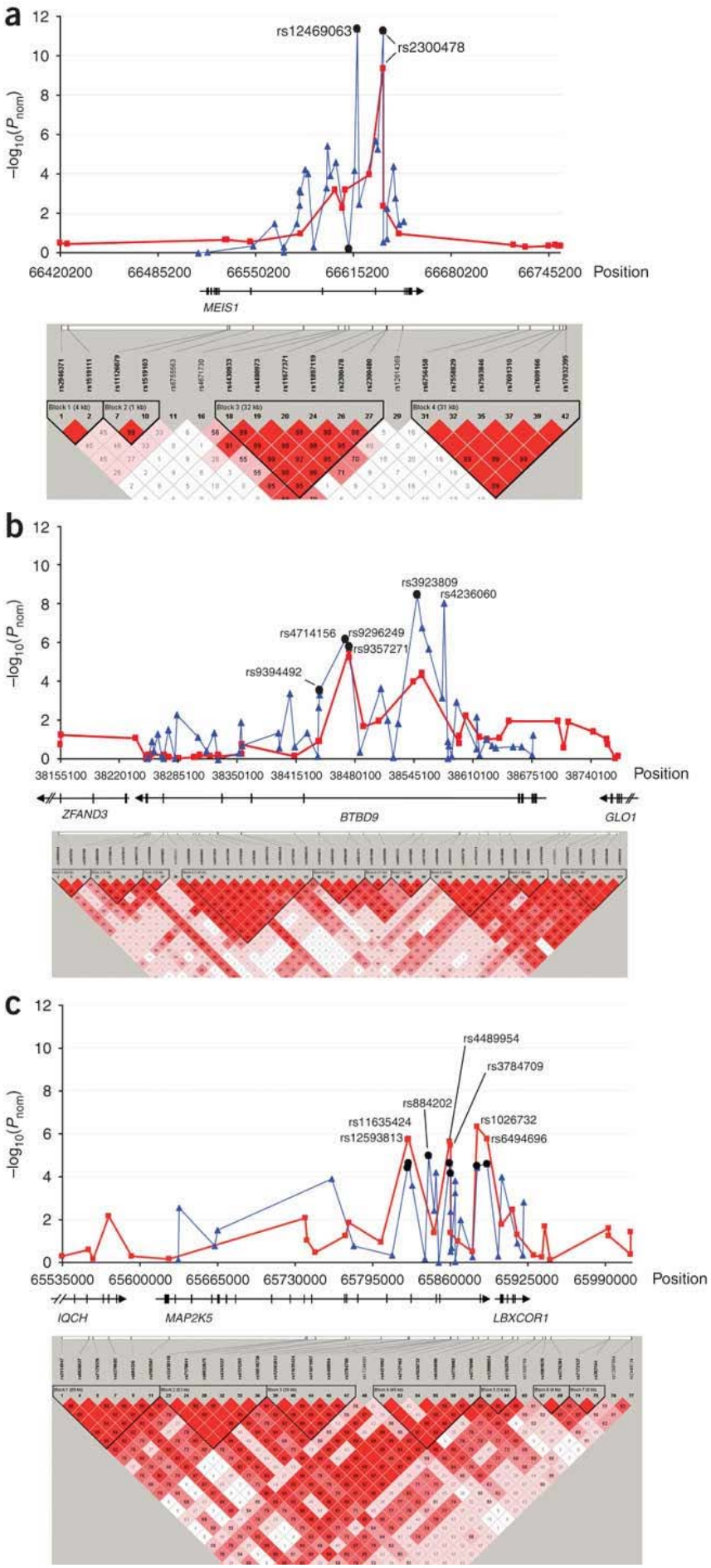


Fig. 4. Pairwise linkage disequilibrium diagrams for three RLS-associated loci. (a) MEIS1. (b) BTBD9. (c) Region of MAP2K5 and LBXCOR1. The P values based on the stage 1 Affymetrix data clearly delineate the regions of interest within a single LD block in the limits of the transcribed genomic unit for MEIS1 and three joint LD blocks in BTBD9. For the region of MAP2K5 and LBXCOR1, the region of interest is limited to a single LD block beginning in the transcribed unit of MAP2K5 and ending in the transcribed unit of LBXCOR1. Pairwise LD, measured as D', was calculated from the stage 1 control data set using the methods of Gabriel as implemented in Haploview. Shading represents the magnitude and significance of pairwise LD, with a white-to-red gradient reflecting lower to higher LD values. Stage 1 Affymetrix SNPs are indicated by red squares, replication SNPs (Stage 2a) by black circles and fine mapping SNPs (Stage 2a) by blue triangles. *x*-axis shows genomic position, and *y*-axis shows $-\log_{10}(P)$. Transcriptional units are indicated by black arrows, with exons depicted as black bars.

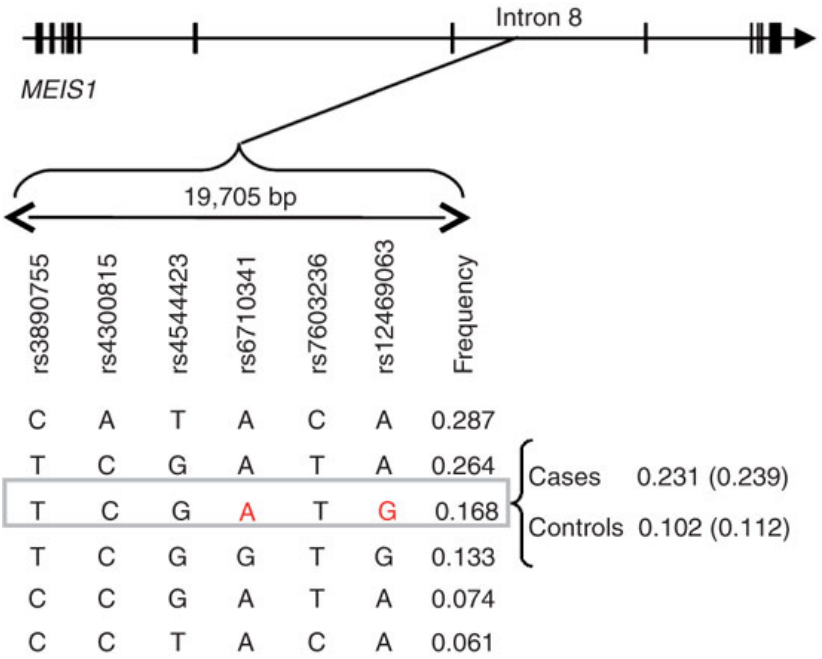


Fig. 5. A haplotype consisting of six SNPs (of which rs6710341 and rs12469063 fully tagged the risk haplotype) is associated with RLS with odds ratios of 2.75 and 2.36 in the stage 2a and 2b samples, respectively. Haplotype frequencies for all haplotypes occurring with these six SNPs are based on cases and controls jointly and are given for cases and controls separately for the risk haplotype. For the Canadian sample, the frequencies are given in brackets and are based on the two tagging SNPs.

the linkage results. The nominally significant signals detected in the RLS3 linkage region might indicate an allelic series of variants conferring weak and strong effects within the same gene.

This study is not exhaustive in identifying genetic factors contributing to RLS, and further investigations will provide a better picture of what constitutes the genetic architecture of the complex phenotype of restless legs syndrome. Future studies should investigate endophenotypes or secondary RLS cases, which might show alternative signal patterns. An interesting question is also whether the loci identified have a role in other dopaminergicdisorders such as Parkinson's disease or in other associated disorders such as attention deficit hyperactivity disorder or sleep disorders. Further experimental advances might include features such as higher sample numbers in the exploratory stage, higher SNP density, modification of clustering algorithms[25], inclusion of lower frequency polymorphisms, investigation of copy number changes and use of lower statistical thresholds using *a priori* information.

METHODS

Study population and phenotype assessment.

Cases of stages 1 and 2a were of European descent and were diagnosed according to standard criteria[2] in a personal interview. Familial RLS was defined by at least one affected first-degree relative. We excluded subjects with secondary RLS due to uremia, dialysis and iron deficiency.

Controls of stage 1 and 2a were of European descent and from the KORA S3/ F3 and S4 surveys, representative of the general population. KORA procedures have been described8. For stage 1, we included 1,644 subjects from S3/F3, ages 35–84 years, and for stage 2a,

891 age- and sex-matched subjects from S4. In 2a, 102 affected individuals were outside the age range of KORA and were matched to the next age group.

Affected individuals and controls of stage 2b were of French-Canadian ancestry. Affected individuals (n = 255) were diagnosed according to standard criteria[2], and polysomnography was performed in 156 subjects; of those, 82.1% (n = 128) showed significant periodic leg movements during sleep. Controls were recruited from the general population (n = 287). Secondary cases were excluded.

Studies were performed according to the declaration of Helsinki and approved by institutional review boards in Germany, Austria, and Canada. Written informed consent was obtained from participants. For demographic data of successfully genotyped samples, see Supplementary Table 8.

Genome-wide assays, SNP genotyping and quality control.

Stage 1 genotyping was performed using the Affymetrix 500K Array Set. Genotypes were determined using the BRLMM algorithm with cases and controls undergoing a joint cluster analysis. From 500,568 SNPs, a total of 236,758 were selected for subsequent analyses based on stringent quality control criteria. Exclusion criteria were call rate < 98% (n = 146,297), minor allele frequency (MAF) < 10% (n = 151,583), deviations from HWE (P < 0.00001, n = 22,536) and low number of heterozygotes (<10, n = 33,122). 14,069 SNPs were monomorphic. For a detailed breakdown, see Supplementary Table 1.

For the 13 SNPs passing the inclusion criteria for genotyping in stages 2, visual inspection of clustering was performed using the Affymetrix SNP Signaling Tool 1.0.0.12. All clusters passed this test. To validate the stage 1 experiment, we genotyped 15 SNPs in 400 samples on another platform (Sequenom MassArray system) with a genotype discordance rate of 0.2%.

Stage 2 and fine-scale mapping were performed using MALDI-TOF mass spectrometry on a Sequenom system (Autoflex HT and SpectroTYPER RT 3.4 analysis software). Assays were designed using AssayDesign 3.1.2.2 with iPLEX Gold chemistry default parameters. Supplementary Table 9 lists oligonucleotide sequences of replication and fine mapping.

SNP quality control criteria leading to exclusion were call rate < 97%, MAF < 10% and P < 0.001 for deviations from HWE in controls. This resulted in an exclusion of one SNP (rs2110974) in stage 2a, two SNPs (rs2110974, rs7881785) in stage 2b and 51 SNPs in fine mapping. All coding SNPs were monomorphic. A total of 28 affected individuals and 55 controls in stage 2a and 44 affected individuals and 46 controls in stage 2b were excluded owing to low call rate (< 90%) of all SNPs within a single DNA sample.

SNP selection for stage 2.

We used the following inclusion criteria: (i) $P < 10^{-6}$ in stage 1 analysis (four SNPs); (ii) $P \leq 10^{-5}$ with two neighboring SNPs (± 100 kb) with $P \leq 10^{-3}$ (eight SNPs); (iii) $P \leq 10^{-4}$ for SNPs within described linkage peaks (one SNP in RLS3). For these 13 SNPs, we chose 15 additional neighboring SNPs based on LD structure for genotyping in the replication samples 2a and 2b (Supplementary Table 3).

SNP selection for fine mapping.

SNPs in the coding regions and 10 kb of flanking sequences were selected using the Tagger algorithm (r^2 = 0.8) implemented in HAPLOVIEW 3.3.2 (ref. [26]). In addition, all coding-region SNPs and splice-site SNPs were included. This led to 41 SNPs on chromosome 2p (38 tagging, 1 synonymous and 2 nonsynonymous), 77 SNPs on chromosome 6p (tagging only)

and 46 SNPs on chromosome 15q (37 tagging, 1 synonymous, 4 nonsynonymous, 2 splice site, 2 frameshift coding). In total, 164 SNPs were selected, of which 163 were converted into genotyping assays, and 103 with a MAF >10% were analyzed.

Analysis of genetic effects.

To test and correct for possible population stratification, we performed an EIGENSOFT[27,28] analysis. We used a random sample of 16,000 SNPs passing the quality criteria for the stage 1 sample and allowed for ten rounds of outlier removal. In the first six rounds, a total of 50 outliers (8 cases and 42 controls) were removed, with none removed in the remaining rounds. To assess stratification, we compared the expected distribution of *P* values for association versus the expected χ^2 distribution with one degree of freedom[29]. We compared the empirically observed mean of the lower 90% of the distribution of the statistics observed and divided it by its expectation[9]. This led to an inflation factor (λ) of 1.09 (Fig. 2).

We performed logistic regression analysis coding the number of minor alleles as the dependent variables, thus implementing Armitage's trend test, including age and sex as covariates and allowing for interactions between age, sex and the number of alleles. Odds ratios and confidence interval limits were obtained through logistic regression analysis. The χ^2 values resulting from these analyses (stage 1, 2a and fine mapping) were divided by λ, assuming similar conditions for both German samples.

Haplotype analysis was performed using HAPLOVIEW 3.3.2 (refs. [26,29]), with the fraction of strong LD informative comparisons set at 0.9, and using UNPHASED 3.0.8, which allows the incorporation of age and sex as covariates[30]. Haplotype blocks were delineated using the method of Gabriel implemented in HAPLOVIEW[26]. ORs were obtained using logistic regression with age and sex as covariates in the stage 2 samples. χ^2 and *P* values in 2a were λ corrected. Differences between familial and sporadic cases were tested using Fisher's exact test. Familial attributable risks were calculated using the power calculator described in ref. [22].

Multiple testing.

Using WG-PERMER, a program for rapid permutation of genome-wide data, preliminary analysis showed that *P* values after Westfall- Young and Bonferroni correction, with the number of tests set at the number of SNPs tested (*n* = 236,758), were in good agreement. This may reflect stringent criteria for SNPs to enter the analysis, resulting in low average r^2 values between SNPs. To maintain comparability across results, we show Bonferroni-corrected P values for stage 1 and the combined analysis. For stages 2a and 2b, we give Westfall-Young–corrected *P* values based on 10,000 permutations, as only a few candidate regions were tested with high LD between them, and thus Bonferroni would be conservative.

Power analysis.

Power analysis for the combined German sample was performed using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/ ~purcell/gpc/). The power of any SNP tested with MAF ≥ 0.2 and OR ≥ 1.5 (or 1/1.5 or lower) was beyond 90%. The *P* value used was the *P* value required for a significant result after Bonferroni correction ($P = 0.05/236{,}758 = 2.112 \times 10^{-7}$).

Testing the mode of inheritance.

OR values and likelihoods were obtained using logistic regression analysis with age, sex and samples as covariates in the combined stage 1, 2a and 2b samples. Significance testing

between models was done using the likelihood ratio test.

Attributable risk fraction.

To quantify the contribution of these loci to RLS, we estimated the population attributable risk fraction (ARF)[22] and the sequential attributable fraction (SAF)[23]. We used the allele dosage model for MEIS1 and BTBD9 and the recessive model for MAP2K5 and LBXCOR1 and calculated upper and lower limits of SAFs[23]. For each locus, we used the SNP with the lowest *P* value, aware of the fact that this may lead to slight overestimation of the ARF. The ARF for the three loci combined was calculated by allowing for the possibility of simultaneous exposure to several of the risk genotypes.

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AUTHOR CONTRIBUTIONS

Study design: J.W., P.L., G.R., F.H., B.M.-M., T.M.; recruitment and biobanking of individuals with RLS: J.W., S.H., C.T., A.Z., K.S.-K., W.O., C.B., W.P., I.P., I.E., T.M.; recruitment and biobanking of KORA controls: C.G., T.I., H.-E.W.; recruitment and biobanking of Canadian affected individuals and controls: L.X., J.M., G.T., G.R.; Affymetrix genotyping: B.S., P.L., G.E.; Sequenom genotyping: B.S., P.L., S.J.; supervision of typing of all markers: J.W., P.L.; software development and data processing: S.R.,B.P.; statistical analysis: S.R., B.P., B.M.-M.; clustering of Affymetrix genotypes: S.R., B.M.-M.; manuscript writing: J.W., B.S., S.F., L.X., F.H., B.M.-M., T.M.

COMPETING INTERESTS STATEMENT

Declaration: J.W., B.S., P.L., B.M.-M., F.H. and T.M. have filed a patent application.

References

1. Walters, A.S. et al. A questionnaire study of 138 patients with restless legs sydrome: the 'night-walkers' survey. Neurology 46, 92–95 (1996).
2. Allen, R.P. et al. 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. 4, 101–119 (2003).
3. Winkelman, J.W., Finn, L. & Young, T. Prevalence and correlates of restless legs syndrome symptoms in the Wisconsin Sleep Cohort. Sleep Med. 7, 545–552 (2006).
4. Barrie`re, G., Cazalets, J.R., Bioulac, B., Tison, F. & Ghorayeb, I. The restless legs syndrome. Prog. Neurobiol. 77, 139–165 (2005).
5. Winkelmann, J. et al. Genetics of restless legs syndrome (RLS): state-of-the-art and future directions. Mov. Disord., published online 7 June 2007 (doi:10.1002/ mds.21587).
6. Rao, S., Winkelmann, J. & Wang, Q.K. in Restless Legs Syndrome. Diagnosis and Treatment (ed. Ondo,W.G.) 111–123 (Informa Healthcare, New York, 2007).
7. Kemlink, D. et al. Family-based association study of the restless legs syndrome loci 2 and 3 in a European population. Mov. Disord. 22, 207–212 (2007).
8. Wichmann, H.E., Gieger, C., Illig, T. & MONICA/KORA Study Group KORA-genresource for population genetics, controls and a broad spectrum of disease phenotypes. Gesundheitswesen 67, S26–S30 (2005).
9. Clayton, D.G. et al. Population structure, differential bias and genomic control in a large-scale, case-control association study. Nat. Genet. 37, 1243–1246 (2005).
10. Azcoitia, V., Aracil, M., Martinez, A.C. & Torres, M. The homeodomain protein Meis1 is essential for definitive hematopoiesis and vascular patterning in the mouse embryo. Dev. Biol. 280, 307–320 (2005).
11. Maeda, R. et al. Xmeis1, a protooncogene involved in specifying neural crest cell fate in Xenopus embryos. Oncogene 20, 1329–1342 (2001).
12. Mercader, N. et al. Conserved regulation of proximodistal limb axis development by Meis/Hth. Nature 402, 425–429 (1999).
13. Rajaram, S.S., Walters, A.S., England, S.J., Mehta, D. & Nizam, F. Some children with growing pains may actually have restless legs syndrome. Sleep 27, 767–773 (2004).
14. Dasen, J.S., Tice, B.C., Brenner-Morton, S. & Jessel, T.M. A Hox regulatory network establishes motor neuron pool identity and target-muscle connectivity. Cell 123, 477–491 (2005).
15. Bara-Jimenez, W., Aksu, M., Graham, B., Sato, S. & Hallett, M. Periodic limb movements in sleep. State-dependent excitability of the spinal flexor reflex. Neurology 54, 1609–1615 (2000).
16. Allen Institute for Brain Science. Allen Brain Atlas. http://www.brain-map.org (2004).
17. Stogios, P.J., Downs, G.S., Jauhal, J.J., Nandra, S.K. & Prive, G.G. Sequence and structural analysis of BTB domain proteins. Genome Biol. 6, R82 (2005).
18. Godt, D., Couderc, J.L., Cramton, S.E. & Laski, F.A. Pattern formation in the limbs of Drosophila: bric a` brac is expressed in both a gradient and a wave-like pattern and is required for specification and proper segmentation of the tarsus. Development 119, 799–812 (1993).
19. Dinev, D. et al. Extracellular signal regulated kinase 5 (ERK5) is required for the differentiation of muscle cells. EMBO Rep. 2, 829–834 (2001).
20. Cavanaugh, J.E., Jaumotte, J.D., Lakoski, J.M. & Zigmond, M.J. Neuroprotective role of ERK1/2 and ERK5 in a dopaminergic cell line under basal conditions and in response to oxidative stress. J. Neurosci. Res. 84, 1367–1375 (2006).
21. Gross, M.K., Dottori, M. & Goulding, M. Lbx1 specifies somatosensory association interneurons in the dorsal spinal cord. Neuron 34, 535–549 (2002).
22. Skol, A.D., Scott, L.J., Abecasis, G.R. & Boehnke, M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association. Nat. Genet. 38, 209–213 (2006).
23. Rowe, A.K., Powell, K.E. & Flanders, W.D. Why population attributable fractions can sum to more than one. Am. J. Prev. Med. 26, 243–249 (2004).
24. WTCCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447, 661–678 (2007).
25. Plagnol, V., Cooper, A.D., Todd, J.A. & Clayton, D.G. A method to address differential

bias in genotyping in large–scale association studies. Plos Genetics 3, e74 10.1371/ journal.pgen.0030074 (2007).
26. Barrett, J.C., Fry, B., Maller, J. & Daly, M.J. Haploview: analysis and visualisation of LD and haplotype maps. Bioinformatics 21, 263–265 (2005).
27. Patterson, N., Price, A.L. & Reich, D. Population structure and Eigenanalysis. Plos Genetics 2, e190 (2006).
28. Price, A.L. et al. Principal components analysis corrects for stratification in genomewide association studies. Nat. Genet. 38, 904–909 (2006).
29. Devlin, B. & Roeder, K. Genomic control for association studies. Biometrics 55, 997–1004 (1999).
30. Dudbridge, F. UNPHASED user guide. Technical Report 2006/5. MRC Biostatistics Unit, Cambridge, UK, (2006).

Supplementary material

Supplementary Table 1. Stage 1 SNP exclusion. Detailed breakdown of the SNPs that did not pass the quality control or were monomorphic and therefore not entered subsequent analysis.

Reason for exclusion	Number of SNPs excluded
Monomorphic >10%	14,069
HWE (deviation from HWE (P < 0.00001 in controls))	854
MAF (minor allele frequency < 0.10)	72,099
HET (< 10 heterozygotes for this SNP)	0
CR (call rate < 98%)	79,851
MAF + HWE	552
MAF + HET	29,889
CR + HWE	17,453
CR + MAF	42,194
HET + MAF + HWE	50
CR + MAF + HWE	3,616
CR + HET + MAF	3,172
CR + HET + MAF + HWE	11
Total	263,810

Supplementary Table 2. Stage 1 association results

SNP ID	Chr	Genome pos	Gene	HWE cases	HWE controls	MAF cases	MAF controls	MAF HapMap	OR (95% CI)	P_{nom}	$P_{corrected}$ (B)
rs2300478	2p	66,634,957	MEIS1	0.669	0.948	0.368 (G)	0.253 (G)	0.242 (G)	1.77 (1.49-2.10)	4.89E-10	<0.0002
rs9296249	6p	38,473,819	BTBD9	0.432	0.587	0.150 (C)	0.239 (C)	0.200 (C)	0.59 (0.48-0.74)	2.19E-06	0.519
rs9357271	6p	38,473,851	BTBD9	0.336	0.736	0.154 (C)	0.240 (C)	0.198 (C)	0.61 (0.49-0.75)	5.48E-06	1
rs4626664	9p	9,251,737	PTPRD	0.527	0.162	0.199 (A)	0.133 (A)	0.133 (A)	1.68 (1.36-2.08)	6.81E-06	1
rs12593813	15q	65,823,906	MAP2K5	0.016	0.296	0.249 (A)	0.340 (A)	0.317 (A)	0.64 (0.53-0.76)	1.85E-06	0.437
rs11635424	15q	65,824,632	MAP2K5	0.016	0.272	0.249 (A)	0.340 (A)	0.317 (A)	0.64 (0.53-0.76)	1.77E-06	0.419
rs4489954	15q	65,859,129	MAP2K5	0.007	0.211	0.230 (T)	0.318 (T)	0.288 (T)	0.63 (0.53-0.76)	2.44E-06	0.578
rs3784709	15q	65,859,329	MAP2K5	0.007	0.345	0.244 (T)	0.334 (T)	0.325 (T)	0.65 (0.54-0.77)	3.56E-06	0.844
rs1026732	15q	65,882,139	MAP2K5	0.019	0.245	0.241 (A)	0.337 (A)	0.317 (A)	0.62 (0.51-0.74)	4.67E-07	0.111
rs6494696	15q	65,890,260	[MAP2K5/ LBXCOR1]	0.007	0.245	0.246 (C)	0.338 (C)	0.317 (C)	0.64 (0.53-0.76)	1.79E-06	0.423
rs6500963	16p	7,407,490	A2BP1	0.288	0.408	0.253 (T)	0.343 (T)	0.246 (T)	1.4 (1.11-1.78)	2.69E-06	0.638
rs1983167	Xp	42,733,328		0.478	0.107	0.263 (A)	0.367 (A)	0.356 (A)	0.66 (0.56-0.77)	6.48E-07	0.153
rs7881785	Xp	42,739,550		0.573	0.045	0.265 (A)	0.367 (A)	0.356 (A)	0.66 (0.56-0.78)	9.01E-07	0.213

These 13 SNPs showed nominally significant association and were selected for replication. Chr, Chromosome; HWE, P value for the deviation from Hardy-Weinberg-Equilibrium; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; P_{nom}, nominal P value. Genome positions (Genome pos) refer to the Human March 2006 (hg18) assembly (http://genome.ucsc.edu/index.html). [gene] denotes intergenic position of SNP. HapMap refers to HapMap rel21a_NCBI_Build35 (http://www.hapmap.org). Minor allele annotation refers to HapMap data. Nominal P values were calculated using logistic regression implementing Armitage trend test and taking sex and age as covariates into account. Odds ratios and confidence limits were calculated from logistic regression. P values resulting from this regression were further corrected for population stratification by dividing the resulting χ^2 by the inflation factor λ, i.e. by dividing with 1.09. $P_{corrected}$, P value corrected for multiple testing using Bonferroni (B).

Supplementary Table 3. Stage 2a and 2b SNP selection.

Region	Chr	Genome position	dbSNP ID	Distance (bp) to * SNP	r^2	Genotyped in stage 1	stage 2a	stage 2b
1	2p	66,670,073	rs6710341	23,031	0.576	-	+	+
		66,675,959	rs12469063	17,145	0.953	-	+	+
		66,693,104	rs2300478	*	*	+	+	+
		66,700,242	rs2110974	-7,138	0.517	-	-	-
2	6p	38,440,588	rs9394492	33,231	0.482	-	+	+
		38,469,090	rs4714156	4,729	0.948	-	+	+
		38,473,819	rs9296249	*	*	+	+	+
		38,473,851	rs9357271	-32	1	+	+	+
		38,548,948	rs3923809	-75,129	0.436	-	+	+
3	9p	9,206,980	rs10816064	44,757	0.722	-	+	+
		9,243,587	rs7872553	8,150	0.858	-	+	+
		9,251,737	rs4626664	*	*	+	+	+
		9,262,841	rs4302899	-11,104	0.769	-	+	+
4	15q	65,823,906	rs12593813	35,223	0.779	+	+	+
		65,824,632	rs11635424	34,497	0.779	+	+	+
		65,841,442	rs884202	17,687	0.809	-	+	+
		65,859,129	rs4489954	*	*	+	+	+
		65,859,329	rs3784709	-200	0.820	+	+	+
		65,882,139	rs1026732	-23,010	0.852	+	+	+
		65,890,260	rs6494696	-31,131	0.852	+	+	+
5	16p	7,406,554	rs6500961	936	0.714	-	+	+
		7,407,490	rs6500963**	*	*	+	-	-
		7,407,527	rs6500964**	-37	0.832	-	-	-
		7,408,353	rs7194617	-863	0.742	-	+	+
6	Xp	42,591,364	rs6520824	13,274	0.790	-	+	+
		42,604,638	rs1983167	*	*	+	+	+
		42,610,860	rs7881785	-6,222	1	+	+	-
		42,621,074	rs6610746	-16,436	0.335	-	+	+

28 SNPs that were chosen for genotyping in stage 2a and 2b (13 SNPs from stage 1 and 15 additional SNPs). The additional SNPs were selected based on LD structure: We choose one of the original SNPs for each of the six regions and sought one SNP with an r^2-value of ≥ 0.9 as a technical replicate, two SNPs with an $r^2 \approx 0.8$ and two SNPs with an $r^2 \approx 0.7$ upstream and downstream, respectively, of the original SNP. In regions where these criteria could not be met, neighbouring SNPs with maximum r^2 obtainable in this region were chosen.

+ indicates successfully genotyped. Genome positions (Genome pos) refer to the Human March 2006 (hg18) assembly (http://genome.ucsc.edu/index.html). * SNP, reference SNP from which the distance within the region was computed. r^2, r^2-values between the reference *SNP and its respective neighboring SNPs as downloaded from HapMap rel21a_NCBI_Build35 (http://www.hapmap.org). ** not genotyped in stage 2 because no PCR-primer could be designed.

Supplementary Table 4. Stage 2a association results.

dbSNP ID	Chr	Genome pos	Gene	HWE cases	HWE controls	MAF cases	MAF controls	MAF HapMap	OR (95% CI)	P_{nom}	$P_{corrected}$ (WY)
rs6710341	2p	66,611,925	MEIS1	1	1	0.141 (G)	0.142 (G)	0.158 (G)	0.96 (0.77-1.17)	6.53E-01	1
rs12469063	2p	66,617,811	MEIS1	0.661	0.923	0.363 (G)	0.236 (G)	0.241 (G)	1.78 (1.52-2.10)	6.52E-12	0.001
rs2300478	2p	66,634,957	MEIS1	0.383	1	0.367 (G)	0.241 (G)	0.242 (G)	1.78 (1.52-2.09)	5.93E-12	0.001
rs9394492	6p	38,440,588	BTBD9	0.027	0.820	0.292 (T)	0.352 (T)	0.342 (T)	0.76 (0.64-0.87)	4.07E-04	0.008
rs4714156	6p	38,469,089	BTBD9	0.262	0.924	0.163 (T)	0.240 (T)	0.214 (T)	0.61 (0.51-0.74)	6.50E-07	0.001
rs9296249	6p	38,473,819	BTBD9	0.262	0.848	0.162 (C)	0.235 (C)	0.200 (C)	0.62 (0.52-0.75)	1.61E-06	0.001
rs9357271	6p	38,473,851	BTBD9	0.178	0.849	0.165 (C)	0.238 (C)	0.198 (C)	0.63 (0.52-0.75)	1.85E-06	0.001
rs3923809	6p	38,548,947	BTBD9	0.003	0.465	0.207 (G)	0.307 (G)	0.259 (G)	0.57 (0.48-0.68)	1.75E-09	0.001
rs10816064	9p	9,206,979	PTPRD	0.895	1	0.152 (A)	0.134 (A)	0.100 (A)	1.21 (0.98-1.48)	9.04E-02	0.917
rs7872553	9p	9,243,586	PTPRD	0.306	0.547	0.156 (C)	0.133 (C)	0.117 (C)	1.27 (1.03-1.56)	3.22E-02	0.616
rs4626664	9p	9,251,737	PTPRD	0.232	0.213	0.170 (A)	0.147 (A)	0.133 (A)	1.26 (1.03-1.55)	3.01E-02	0.716
rs4302899	9p	9,262,840	PTPRD	0.407	0.107	0.172 (A)	0.151 (A)	0.167 (A)	1.24 (1.01-1.52)	4.39E-02	0.853
rs12593813	15q	65,823,906	MAP2K5	0.479	0.814	0.258 (A)	0.330 (A)	0.317 (A)	0.71 (0.60-0.83)	4.95E-05	0.001
rs11635424	15q	65,824,632	MAP2K5	0.595	0.584	0.257 (A)	0.330 (A)	0.317 (A)	0.70 (0.59-0.82)	2.54E-05	0.001
rs884202	15q	65,841,441	MAP2K5	0.653	0.481	0.250 (G)	0.328 (G)	0.319 (G)	0.69 (0.58-0.81)	1.17E-05	0.001
rs4489954	15q	65,859,129	MAP2K5	0.779	0.872	0.239 (T)	0.311 (T)	0.288 (T)	0.69 (0.59-0.82)	2.60E-05	0.001
rs3784709	15q	65,859,329	MAP2K5	0.928	0.579	0.251 (T)	0.321 (T)	0.325 (T)	0.71 (0.60-0.83)	7.46E-05	0.001
rs1026732	15q	65,882,139	MAP2K5	0.530	0.582	0.252 (A)	0.327 (A)	0.317 (A)	0.70 (0.59-0.82)	2.78E-05	0.001
rs6494696	15q	65,890,260	[MAP2K5/ LBXCOR1]	0.476	0.431	0.253 (C)	0.326 (C)	0.317 (C)	0.71 (0.60-0.83)	5.20E-05	0.001
rs6500961	16p	7,406,553	A2BP1	0.489	0.600	0.326 (A)	0.363 (A)	0.283 (A)	0.84 (0.73-0.98)	3.67E-02	0.342
rs7194617	16p	7,408,352	A2BP1	0.888	0.724	0.399 (T)	0.429 (T)	0.317 (T)	0.89 (0.77-1.03)	1.28E-01	0.726
rs6520824	Xp	42,720,053		0,254	0,450	0.393 (C)	0.411 (C)	0.411 (C)	0.96 (0.83-1.09)	5.21E-01	0.596
rs1983167	Xp	42,733,328		0,491	0,215	0.339 (A)	0.358 (A)	0.356 (A)	0.95 (0.83-1.08)	4.43E-01	0.530
rs7881785	Xp	42,739,550		0,488	0,285	0.334 (A)	0.356 (A)	0.356 (A)	0.95 (0.83-1.09)	4.57E-01	0.453
rs6610746	Xp	42,749,763		0,348	0,430	0.367 (A)	0.346 (A)	0.378 (A)	1.06 (0.93-1.21)	4.17E-01	0.478

Chr, Chromosome; HWE, P value for the deviation from Hardy-Weinberg-Equilibrium; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; P_{nom}, nominal P value. Genome positions (Genome pos) refer to the Human March 2006 (hg18) assembly (http://genome.ucsc.edu/index.html). HapMap refers to HapMap rel21a_NCBI_Build35 (http://www.hapmap.org). Minor allele annotation refers to HapMap data. Nominal P values were calculated using logistic regression implementing Armitage trend test and correcting for population stratification. $P_{corrected}$, P value corrected for multiple testing using Westfall-Young method (WY).

Supplementary Table 5. Stage 2b association results.

dbSNP ID	Chr	Genome pos	Gene	HWE cases	HWE controls	MAF cases	MAF controls	MAF HapMap	OR (95% CI)	P_{nom}	$P_{corrected}$ (WY)
rs6710341	2p	66,611,925	MEIS1	1	0.277	0.133 (G)	0.141 (G)	0.158 (G)	0.87 (0.59-1.29)	4.93E-01	1
rs12469063	2p	66,617,811	MEIS1	0.882	0.607	0.355 (G)	0.248 (G)	0.241 (G)	1.68 (1.24-2.27)	6.54E-04	0.018
rs2300478	2p	66,634,957	MEIS1	0.653	0.505	0.358 (G)	0.261 (G)	0.242 (G)	1.59 (1.18-2.15)	2.19E-03	0.059
rs9394492	6p	38,440,588	BTBD9	1	0.888	0.280 (T)	0.355 (T)	0.342 (T)	0.71 (0.53-0.95)	1.99E-02	0.429
rs4714156	6p	38,469,089	BTBD9	1	0.177	0.140 (T)	0.213 (T)	0.214 (T)	0.60 (0.41-0.87)	6.79E-03	0.173
rs9296249	6p	38,473,819	BTBD9	1	0.451	0.140 (C)	0.218 (C)	0.200 (C)	0.59 (0.41-0.85)	4.14E-03	0.109
rs9357271	6p	38,473,851	BTBD9	1	0.578	0.140 (C)	0.222 (C)	0.198 (C)	0.57 (0.40-0.83)	2.48E-03	0.067
rs3923809	6p	38,548,947	BTBD9	0.139	1	0.207 (G)	0.313 (G)	0.259 (G)	0.58 (0.42-0.8)	8.68E-04	0.024
rs10816064	9p	9,206,979	PTPRD	0.015	1	0.133 (A)	0.106 (A)	0.100 (A)	1.40 (0.93-2.09)	1.05E-01	0.954
rs7872553	9p	9,243,586	PTPRD	0.055	1	0.128 (C)	0.104 (C)	0.117 (C)	1.35 (0.90-2.04)	1.46E-01	0.988
rs4626664	9p	9,251,737	PTPRD	0.084	0.746	0.138 (A)	0.108 (A)	0.133 (A)	1.39 (0.93-2.07)	1.02E-01	0.951
rs4302899	9p	9,262,840	PTPRD	0.144	0.513	0.140 (A)	0.112 (A)	0.167 (A)	1.34 (0.91-1.98)	1.43E-01	0.986
rs12593813	15q	65,823,906	MAP2K5	0.168	0.442	0.225 (A)	0.303 (A)	0.317 (A)	0.69 (0.51-0.94)	1.57E-02	0.357
rs11635424	15q	65,824,632	MAP2K5	0.240	0.372	0.227 (A)	0.315 (A)	0.317 (A)	0.67 (0.49-0.90)	6.60E-03	0.169
rs884202	15q	65,841,441	MAP2K5	0.158	0.452	0.220 (G)	0.311 (G)	0.319 (G)	0.65 (0.48-0.88)	4.56E-03	0.120
rs4489954	15q	65,859,129	MAP2K5	0.093	0.157	0.206 (T)	0.285 (T)	0.288 (T)	0.69 (0.51-0.94)	1.66E-02	0.374
rs3784709	15q	65,859,329	MAP2K5	0.147	0.368	0.211 (T)	0.309 (T)	0.325 (T)	0.62 (0.46-0.84)	1.79E-03	0.049
rs1026732	15q	65,882,139	MAP2K5	0.158	0.548	0.220 (A)	0.309 (A)	0.317 (A)	0.66 (0.49-0.88)	5.22E-03	0.136
rs6494696	15q	65,890,260	[MAP2K5/ LBXCOR1]	0.158	0.548	0.220 (C)	0.309 (C)	0.317 (C)	0.66 (0.49-0.88)	5.22E-03	0.136
rs6500961	16p	7,406,553	A2BP1	0.111	0.772	0.386 (A)	0.330 (A)	0.283 (A)	1.27 (0.96-1.67)	8.72E-02	0.922
rs7194617	16p	7,408,352	A2BP1	0.217	1	0.476 (T)	0.383 (T)	0.317 (T)	1.46 (1.11-1.91)	5.60E-03	0.145
rs6520824	Xp	42,720,053		0.107	0.391	0.405 (C)	0.444 (C)	0.411 (C)	0.90 (0.72-1.13)	3.63E-01	1
rs1983167	Xp	42,733,328		0.096	0.479	0.357 (A)	0.384 (A)	0.356 (A)	0.92 (0.73-1.16)	4.73E-01	1
rs6610746	Xp	42,749,763		0.849	0.838	0.372 (A)	0.306 (A)	0.378 (A)	1.25 (0.99-1.59)	6.45E-02	0.844

Chr, Chromosome; HWE, P value for the deviation from Hardy-Weinberg-Equilibrium; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; P_{nom}, nominal P value. Genome positions (Genome pos) refer to the Human March 2006 (hg18) assembly (http://genome.ucsc.edu/index.html). HapMap refers to HapMap rel21a_NCBI_Build35 (http://www.hapmap.org). Minor allele annotation refers to HapMap data. Nominal P values were calculated using logistic regression implementing Armitage trend test and correcting for population stratification. $P_{corrected}$, P value corrected for multiple testing using Westfall-Young method (WY).

Supplementary Table 6. Delineation of genetic model.

dbSNP ID	Chr	Genome position	Region	Risk allele	Unrestricted model OR (95 % CI) Number of risk alleles = 1	Unrestricted model OR (95 % CI) Number of risk alleles = 2	Allele dosage model OR (95 % CI) Number of risk alleles = 1	Allele dosage model OR (95 % CI) Number of risk alleles = 2
rs2300478	2p	66,634,957	MEIS1	G	1.80 (1.43-2.27)	3.07 (2.45-3.86)	1.74 (1.57-1.92)	3.01 (2.47-3.67)
rs9296249	6p	38,473,819	BTBD9	T	1.67 (1.45-1.92)	2.85 (1.93-4.20)	1.67 (1.49-1.89)	2.80 (2.21-3.56)
rs1026732	15q	65,882,139	MAP2K5/ LBXCOR1	G	1.11 (0.87-1.42)	1.94 (1.51-2.48)	1.53 (1.39-1.70)	2.36 (1.92-2.89)

Chr, Chromosome; OR, odds ratio; CI, confidence interval. Odds ratios were obtained using logistic regression with age, sex and samples as covariates in the combined stage 1 and 2a and b samples.

Supplementary Table 7. Analysis of familial versus sporadic cases in combined 1 and stage 2a data set.

			OR (95% CI)	
SNP	Chr	$P_{nominal}$	Familial	Sporadic
rs2300478	2p	2.23E-01	1.82 (1.55-2.14)	1.59 (1.34-1.9)
rs9296249	6p	7.31E-01	1.55 (1.27-1.89)	1.63 (1.31-2.02)
rs9357271	6p	6.81E-01	1.53 (1.25-1.86)	1.63 (1.31-2.02)
rs4626664	9p	1.02E-01	1.2 (0.97-1.49)	1.5 (1.21-1.86)
rs12593813	15q	1	1.5 (1.26-1.78)	1.5 (1.25-1.8)
rs11635424	15q	1	1.5 (1.27-1.79)	1.5 (1.25-1.8)
rs4489954	15q	7.19E-01	1.52 (1.28-1.82)	1.45 (1.21-1.75)
rs3784709	15q	8.60E-01	1.53 (1.29-1.83)	1.5 (1.25-1.81)
rs1026732	15q	9.53E-01	1.53 (1.28-1.81)	1.51 (1.26-1.82)
rs6494696	15q	1	1.52 (1.28-1.81)	1.51 (1.26-1.82)
rs1983167	Xp	1.97E-02	1.05 (0.89-1.23)	1.35 (1.13-1.61)
rs7881785	Xp	2.90E-02	1.07 (0.91-1.26)	1.37 (1.15-1.63)

Chr, Chromosome, OR, odds ratio; CI, confidence interval. Odds ratios were obtained using logistic regression with age and sex as covariates in the combined stage 1 and 2a samples. Nominal P values for differences between familial and sporadic cases were tested using Fisher`s exact test on the allele counts in familial and sporadic cases, respectively.

Supplementary Table 8. Description of study subjects.

	Stage 1		Stage 2a		Stage 2b	
	Cases GER1	Controls KORAS3/F3	Cases GER2	Controls KORAS4	Cases CAN	Controls CAN
N individuals	393	1602	875	836	211	241
N females	287	815	645	618	133	136
N males	106	787	230	218	78	105
Mean age (SD)	60.7 (8.0)	62.6 (9.9)	60.6 (12.1)	69.9 (11.3)	53.0 (12.4)	42.2 (16.0)
Mean age at onset (SD)	33.2 (13.7)*	-	38.8 (16.9)*	-	27.7 (12.4)*	-
AaO (SD) females	32.6 (13.6)*	-	38.1 (16.5)*	-	27.0 (16.1)*	-
AaO (SD) males	34.6 (14.0)*	-	40.5 (18.0)*	-	28.8 (13.3)*	-
Positive family history	393 (100%)	-	418 (47.7%)	-	165/206 (80.1%)	-
Affymetrix 500K data	393	1602	-	-	-	-
Sequenom iPLEX data	393	-	875	836	211	241

Table includes only successfully genotyped samples. N, number; AaO= age at onset of the disease; GER, German; CAN, Canadian. KORAS3/F3 and KORAS4, controls drawn from KORA population-based cohort study, Germany.

AaO is unknown for 13 cases (7 females, 6 males) in CAN, 21 (15 females, 6 males) in GER1 and 39 cases (30 females, 9 males) in GER2.

Supplementary Table 9: Oligonucleotide sequences for replication and finemapping.

dbSNP ID	Sequence forward PCR primer	Sequence reverse PCR primer	Sequence extension primer
rs1000756	ACG TTG GAT GGA GTT ACT TTT CTC TGT TGGC	ACG TTG GAT GAC AAC ACT AAT CAA TTT AAC	TTC TCT GTT GGC TTT TTT TTT CCA
rs10184250	ACG TTG GAT GAC GCC TTA GGC AGA AGC TC	ACG TTG GAT GTG AGG GTA TCC GAA AGG CTG	AGC TCC TCA GGA TCA CTT
rs1026731	ACG TTG GAT GCA GAA ATG GTG CTA ACA TGC	ACG TTG GAT GAT CTT CCA CCC ACG GTG AC	ATC TAA CAT GCT TTC CGC
rs1026732	ACG TTG GAT GAT GGT GCA GCT CCC TGG AAC	ACG TTG GAT GGG GTG GAA GAT GCT CTT GAC	TGG AAC CCA GGC ACC AAT A
rs10456462	ACG TTG GAT GCC AGA CTG GCA AGT AAA CAC	ACG TTG GAT GAG TGC CAT TTT AGT GGA AAG	CAG CAA ACT CAA CAC AT
rs10518744	ACG TTG GAT GCA GAC CCT ACC AGA AGA AAC	ACG TTG GAT GCA ACG ATT TTG GTT AGC AAG	GGA GGA ACC CTT GGT TCT GTG
rs10816064	ACG TTG GAT GGA ATT TTT TTG CCC TCT ACT C	ACG TTG GAT GTT TCC CAC ATT GGG CTT CAG	CCC TCT ACT CTT TAA AAT CC
rs10865353	ACG TTG GAT GCA GTG TGC ATG GTA CTC AGG	ACG TTG GAT GAC ATG GGC ACA TAC ATA CGG	GTA CTC AGG CAT ACA CA
rs10947715	ACG TTG GAT GGG GAT CCC AGG AGA AGT AAC	ACG TTG GAT GAG CCA ATA CCC AGC TAG CTC	CCC CTA AGT AAC CCC AGC CT
rs10947716	ACG TTG GAT GAC ACC AGA CAG GCA CCA AGA	ACG TTG GAT GCA GGC AGC TGG AGA CAC AT	GCA CCA AGA GAG CCT
rs10947737	ACG TTG GAT GCA AGG ATG AAT CAA GAC TTG G	ACG TTG GAT GGA CCA CAT ATG AAG ACG ATG	GGG AGG AAT CAA GAC TTG GCA ACA A
rs10947740	ACG TTG GAT GAA TAA TCC TCA AGA ACA AG	ACG TTG GAT GGC ACT CCT GGT AAT ATG TCC	CCT CAA GAA CAA GTT TGG A
rs10947749	ACG TTG GAT GGC ATC AAA AAG ACC CAA TGA C	ACG TTG GAT GAA AGA GCC TGC CTA TCA GCC	CTC CAA TGA CTT TTA TAA AAG TAG AAG
rs11071959	ACG TTG GAT GAC AGT AAA TTT GCG CTC CAG	ACG TTG GAT GTA GAG TCT CTG AAC TCC TCG	TCA CAG GAA GGG AAA G
rs11126082	ACG TTG GAT GCA GAT GCC CAC TGT GAT CTC	ACG TTG GAT GTT CCC CCA AAA CTG AAT TGC	ATC TCC TCA CCT CTC CT
rs11630854	ACG TTG GAT GGA AGT CAT CTA TCT CAT TG	ACG TTG GAT GCA ACC TGG GCA ACA AAG CAA	GGA ATC TAT CTC ATT GAT GAG ATA ATT T
rs11635286	ACG TTG GAT GCA ATG AAA GAT GTG AAT TCT C	ACG TTG GAT GTC ACT ATA GTT TTT CTA GG	TGA ATT CTC AAA TTT AAA AAA AAC CA
rs11635424	ACG TTG GAT GTG TTA GGG CTG ATG TAC TGC	ACG TTG GAT GTG AGG GCT TGG ACA AGT TAG	CGC CTG CAC CTC ACA ACA TA
rs11637445	ACG TTG GAT GCT CTT GCC TGA CTG TGA TTC	ACG TTG GAT GGA GGA AAG TAG AAT GGG AGC	CTG TGA TTC AGG CAA ATG
rs11688578	ACG TTG GAT GTA TGG TTC TGT AGC ATA GGG	ACG TTG GAT GCA AAG GAA TAT AAG ATT TTT G	TAA TCC ATG CTC CCT TT
rs11692361	ACG TTG GAT GCC CAT GCC TAG GAC ATA AAG	ACG TTG GAT GGA CGT GGA GCG ATA CTT GAA	ACA TTT CCC AGC ATC AC
rs11692504	ACG TTG GAT GGT GAA CTT TCT CTG GAT CTG	ACG TTG GAT GTA AAA ACC ACC CCT GAA ATG	AAA TAC TTG GTA TTT GTT TTC TT
rs11751154	ACG TTG GAT GGC AGC TTA CAC ACA CAA CAG	ACG TTG GAT GTG TAG CCT TTT GAA TCT TGC	CAC ACA CAA CAG CAT GTA T
rs11752799	ACG TTG GAT GAC ACT GGG AGT CCC AGT TTG	ACG TTG GAT GTA TCA GGT CAT CCT CAC TCG	CAG GAA ATC ATT AAG ATT TTA TTA TTT G
rs11757846	ACG TTG GAT GGT TAA CAA ACG TCG AGG GAG	ACG TTG GAT GAC ATC AGA ACC AGT GTG GCT	GAG GTA GTC TTT AGT TTC AGC CC
rs11757985	ACG TTG GAT GTT CCA CTA AAC ACA TCC TGC	ACG TTG GAT GCC AGT TTA CAA TGG TTC AGC	CCC GGC TTT CAC ATC ATT GTA ACA
rs11856999	ACG TTG GAT GAT GAG TTT GTC GGA GGA AGC	ACG TTG GAT GCC ACC ACA GCC CCC TTT AA	ATT GGG AAT GGC AGA ATT GTG CCA CT
rs11857017	ACG TTG GAT GTT AAA GAC ACT TTG AGG CTG	ACG TTG GAT GGA GCC TTC TGT CAT GCT AAG	ATA CAC TTT GAG GCT GAT TTT TAT TT
rs12050749	ACG TTG GAT GCT TCC CTT TTC TGG GCA TAG	ACG TTG GAT GCC CCT GTT AGT GTG GTT TTA	GGT TTC TGG GCA TAG ATT TTT TTT
rs12055513	ACG TTG GAT GGT CTG CAT TAT CTT TTC CAG	ACG TTG GAT GGA CTC ATT TGT CTA CAT TCG	CAA GTT TAT CCA ATA AGC TAT GC
rs12148363	ACG TTG GAT GGG GGC CAT CAA TTT TGA TT	ACG TTG GAT GGT ACA TCT GAG AGA GGA GCA	GAT TGA TTG TTT GGG GAC
rs12196956	ACG TTG GAT GGG TTC GAT TCT GTG AGG TTG	ACG TTG GAT GAG AGA CAA CCC ACG CAG ATG	GGA CGC AGG TCC ACA GAT AAC
rs12198616	ACG TTG GAT GTG TCG TTG CCA CTG CAC GAG	ACG TTG GAT GCT GTG AAC TTG ATT CTA CGG	CCC CGC ACA TAC ATA CAC ACG
rs12206905	ACG TTG GAT GCT GCT TTC AAA CAA CCA GG	ACG TTG GAT GGA ATG TGG GCA CAT TTA GAC	AGG TTA CAT ATG AAC TGT CAG AGC
rs12208647	ACG TTG GAT GAA ACT GCC GTT GTC AAG GTC	ACG TTG GAT GTG AAG CAA GAC AAG GAC TCG	ACC ACA GTG CTG AGC
rs12212721	ACG TTG GAT GCC TTC AAT ATG TCT GCG TTG	ACG TTG GAT GAA GTT CGG AAG TTC ACA CTG	CCC CTT TCC TGG TCA TGT GAT TCT T
rs12212820	ACG TTG GAT GCG AGA GTC GTC CTC CAC TG	ACG TTG GAT GTT CTT CCC CTG GCA AAC AGT	GAG CCA TGG GAA GTT
rs12373638	ACG TTG GAT GTT TTA CCC TTT AGC GGA GGA	ACG TTG GAT GAA GAC ATG GAG AGT CAG AGC	GGG GAG CAG TAA ATT CA
rs12441598	ACG TTG GAT GAA CAC CAG GGT TTA GCC ATC	ACG TTG GAT GCA TAT GTG CTG CTG ATC ACC	GAG CTA GGA GGT GCT
rs12469063	ACG TTG GAT GGA ACA TTC AAA AGC AAT TCA C	ACG TTG GAT GTT AAT GTC CCT ACA GAC TGC	AGC AAT TCA CTG CAT CA
rs12471916	ACG TTG GAT GAT GCT GAT GGA AGA GTG TGG	ACG TTG GAT GGG TCT TTC TTT TGA TGG GAC	CTT ACG AGT GTG GGT CAG GAA T
rs12592315	ACG TTG GAT GGA TTG CAA CAT TAA GCC CAG	ACG TTG GAT GCA AAA GGC TGT TTA CGA TTC	TTA GTG TTC ACA ATC TAG CA
rs12593664	ACG TTG GAT GTG TTA ACT ATA ACA GCA GCC	ACG TTG GAT GCA TGC ATG TGC TTA TTA GTC	CCC AAG CAG CCA AAA ATA ATG TAA
rs12593813	ACG TTG GAT GAG ACA CCA GCT ATA GCT TTC	ACG TTG GAT GTT CCA GAC AAG AGC TGC AGG	CTT TTC TCT TTT ACT CTC TGA AAT TA
rs12614369	ACG TTG GAT GTA CAG CAC TCA CCA CCT TAC	ACG TTG GAT GTG ATC TAG TAA GGC AGA ACC	CCC CAT CTA TAA ACA AGG C
rs12619205	ACG TTG GAT GCC ACT TCA GTG AGG CAT TCA	ACG TTG GAT GAG GTT CTG GAG AAT CTT CCC	ATT CAT GTG CAC CCA
rs12660215	ACG TTG GAT GGC AGG TGA ATA TGG ACT CCG	ACG TTG GAT GGC TGT CGG TAC ACT TAC TAC	CTA GGG ACT CCG GTG TTT CTG TTC AG
rs12664020	ACG TTG GAT GTC TAC CGA CCT GCA AAA AAC	ACG TTG GAT GCT GCT TTC TCG GTT ATT CTG	GAA ACA ACT ATT AGC ACC TGC TAG
rs12713566	ACG TTG GAT GGT GAG TGG CAC TAC AAA TTC	ACG TTG GAT GAT CAG TGA TTG CAT CTG ACC	GTA GGG GCA CTA CAA ATT CAC AAA AGA A
rs12713567	ACG TTG GAT GAG AGT TCA TGG TCA GTT TCC	ACG TTG GAT GGC AGG GAT TGT GAG GAA ACA	ATT CTG CCT CCC CTC
rs12898654	ACG TTG GAT GTA GAG TCC TTT GCG CTG GG	ACG TTG GAT GTC CTT TGT GTC CGC TCT GGT	GGG TTC CCA GGA TGT TCC GA
rs12901985	ACG TTG GAT GTG CAC ACA GAT GTG AAA GGC	ACG TTG GAT GCT GGC TAG TGT GGC TAT TAG	GGG TGA AAG GCA GCT TAT ATC
rs12905175	ACG TTG GAT GCT CAC TCC TTT CTT GAG AAC	ACG TTG GAT GAA TCA CCA ATG TTG GGG ATG	GAT TTA GAA CTC CAC CTC CTG TT
rs12905371	ACG TTG GAT GCC CGG CCT CAA CTA TTT CTT	ACG TTG GAT GGT GAC TTT TTT TTC TGA ACC	GAG ATT CCA TTT ATT GGT TCG
rs12917587	ACG TTG GAT GCT CCA ATG TCA CCT TCT CTC	ACG TTG GAT GGC TGT GAA AGT GTG GCA TAG	ATG AAG TGG CCA TCA TC
rs13005707	ACG TTG GAT GGA AAC AAG TAG CAA AAG AG	ACG TTG GAT GTG GCC ACA CGT CAC ACA GT	AAC ATC TCT CCT ACC TTG
rs13193103	ACG TTG GAT GGG CTT GGT ACA TAT AAA TCT C	ACG TTG GAT GCC ACC AGT ATA TGG CTA CC	CAG ATA AAT CTC AAT TAG ACA GTG TTT
rs13194038	ACG TTG GAT GCC CTT ATT CTC ATT GTG CAG	ACG TTG GAT GGC AAA ATC ACA TCT GCT AAT C	TTG TGC AGT TTC CCT AAA
rs13196708	ACG TTG GAT GCC ACT TTC TTA CAC GTA AAG C	ACG TTG GAT GGT ATA CAA TGC CTT TCT AC	CAC TTT AGC CCT TTG ATT TTC A
rs13205736	ACG TTG GAT GCT AAA ATT CAG GAA AAC AG	ACG TTG GAT GTA GAG ATA GCC TTT TCA AG	GAT TCA GGA AAA CAG ATG TTC A
rs13206817	ACG TTG GAT GAA AAA CTT CAC CTC CAA TG	ACG TTG GAT GGT TGG TCC TTT GTT TTG GTG	CAA CCT TCA CCT CCA ATG CAT TCA
rs13213112	ACG TTG GAT GTC AAC TTG GAA TGG CTC CCT	ACG TTG GAT GCT TGT ATG TGG GAG GGT AG	CCC CCG AAT GGC TCC CTC ACA GAG
rs13219887	ACG TTG GAT GAG GAC CAA GGC TTG AAA GTG	ACG TTG GAT GCA GTA GGT GTA GCG AAT AGC	TTA TAC ATC ACT ACC ACT CA
rs14429	ACG TTG GAT GTG GGC TTT CAG TGC CCT GC	ACG TTG GAT GTG GTG TCC ATG TGG GTG TG	TTG CGG GCT CCG CCT CTC CTC C
rs16890428	ACG TTG GAT GTC TGC CAT ACT GGC TTA CAC	ACG TTG GAT GGG GTT CTG GGA CAT TAT TTG	CCT CCT GGC TTA CAC ATA TTT CCA
rs16890541	ACG TTG GAT GGT TAG CCC TCA TGA GAA TAG	ACG TTG GAT GGT TTT ATA TGC TGC TGC CCC	GGA GTG AGA ATA GCT GCA TTT CTG TGA C
rs16890826	ACG TTG GAT GGA AAA TTG ATA GTC TGT CAT C	ACG TTG GAT GTC TAA TGT TGG TGA GGG GAG	CAA TTG ATA GTC TGT CAT CAA AAT C
rs16951060	ACG TTG GAT GGC TGT ATC TGC AAA GGG CAC	ACG TTG GAT GTT TCT CAG GCT TAG AAG CTC	GCC CTG AAT GGG TGA CTG
rs1699018	ACG TTG GAT GCT TAT TTC CTG TGG CTG CCT	ACG TTG GAT GGC TTT GCA GGT TAT ACA ATC	GCT GCC TTT AGG CTC
rs17032119	ACG TTG GAT GGC AGA AGA CAA ATA GTT AAA	ACG TTG GAT GGA CTT TGG ATA TGT AAG TGC	ACA CAA ATC ACT GGG A
rs17241403	ACG TTG GAT GAA CCC ACT AGG CTG CAA TAA	ACG TTG GAT GAG ACA GTC TCA TAT TCT GA	GGA TAA ATT GTT AAA CAT AGT CTT TCT

Supplementary Table 9: continued

dbSNP ID	Sequence forward PCR primer	Sequence reverse PCR primer	Sequence extension primer
rs17244601	ACG TTG GAT GTC CCT GCG GCT TTT CCC TAC	ACG TTG GAT GGA CAC AAT ACA TGC TGA AGG	GCT TTT CCC TAC TCT TCG
rs17300363	ACG TTG GAT GGC TAG CTA TAG AGA TTA TGG	ACG TTG GAT GTA CCT TAT TCA GGT CTT GGG	ATG GTT AAA AAG AGA CTG CTT ATA T
rs17542411	ACG TTG GAT GTG AAG CTC TCC TGC TAG TCG	ACG TTG GAT GGA ATA AAA AGT CTG TCT CAA G	ATG CTG TCC AGG GAT A
rs17543178	ACG TTG GAT GCT AGG TTA TAA TTT TGA TAG	ACG TTG GAT GAA ACA CCA AGA CAT CCT CAG	TTG AAC CTA AGA ATA TGT CTC TG
rs17614684	ACG TTG GAT GGC CTT AAT TTG GCC TAC TGG	ACG TTG GAT GGC CAG CAG GGT ATA TGA AAC	GAC TTG GCA GGC TCC
rs17620389	ACG TTG GAT GTC GTT GAC AGC TGG TTA GTG	ACG TTG GAT GCA ATA GAC TAC AAT GAC ACT G	GGT TAG TGC TGA TTC TCA
rs17757272	ACG TTG GAT GGG AAA AGG TAG GAG GGA AAC	ACG TTG GAT GCC AAA AAA TAT ATT TAA TGT	AGG GAA ACA TTT CAG TCT ACA
rs1931762	ACG TTG GAT GCT TCT GTT AGC TGC TTT AC	ACG TTG GAT GAC ATA TAG ATG GTT TAA CTA	CTT GTG ATA GTT ATT TTT TGT TCT
rs1983167	ACG TTG GAT GGC ACC TTT GAG ATT TAC TGC	ACG TTG GAT GTT GTG CTT AGT CTG CCA CTG	AAA CTG AGA TTT ACT GCA GTG TAT
rs2061845	ACG TTG GAT GCA GCC TCC TTC ACT TCC TTG	ACG TTG GAT GCT GGA TAT TAA TCC AAC GGC	GTT GGG CCA GGG CCG C
rs2110974	ACG TTG GAT GGA AAA TAA ACA TGC ACA GAT G	ACG TTG GAT GGA AAA TAT TTA TAG AAA TCA C	TAA TGC ACA GAT GAT AAT TGA TA
rs2139246	ACG TTG GAT GGG AAC TGG CTA ATT CAA AGG	ACG TTG GAT GGT ACT TCA TGT TTT CCC AAG	CGG TGT TAT GAG GAA CAA G
rs2192954	ACG TTG GAT GCA AAT CAA ACA TGG TAT TGT C	ACG TTG GAT GGT CCC TCA AGT AAC TAA AAG	ATG GTA TTG TCA TTA TTT GTC AAA C
rs2246023	ACG TTG GAT GAA TGC CAC TGT TTA GAC CTC	ACG TTG GAT GAA GCC TGC TGA GAA AAG ATG	GTC ACT GTT TAG ACC TCT AGT TTG
rs2280334	ACG TTG GAT GAA GTA GTT GAG ACT CAA CGC	ACG TTG GAT GGT ACC TCC AAT CCA GAG AAC	CCC CGA CTC AAC GCT TCC CTC
rs228181	ACG TTG GAT GGA TAA CAC AGC AAG TGA GAC	ACG TTG GAT GGC TTT TGT ATC TAG CAT GAG G	AGT TAA CAA TTG CAA ACA GT
rs2300477	ACG TTG GAT GGG GAG ACC ATC AAT TTT GCG	ACG TTG GAT GAA AAC TGT CTC AAC ATC AGC	GCG TGC ACA TCT AGA TTC AT
rs2300478	ACG TTG GAT GGC ATT TCT CTG ACC AGA TAC	ACG TTG GAT GGC ACA ACT TGT TGC AAA TCC	CCC CAT GAC CAG ATA CTT ACA GAC
rs2300480	ACG TTG GAT GGA AAT GCA GGG ACA GGT CG	ACG TTG GAT GAG GAC ACT GTC GCC CAA ATC	GGA CAG GTC GCT GCA ATC AAA ACA
rs2300483	ACG TTG GAT GAA ATA AAG AGT TCC AAC AG	ACG TTG GAT GAT CTC CCA TGT AAC TCT CTG	AAG AGT TCC AAC AGA AAA ATT TC
rs2395694	ACG TTG GAT GTT CAG CCG TGA GGC AGT GAG	ACG TTG GAT GGA TTT CTC TCC TTA TAG GGC	TTG AAG GAG GCG AGG ACC G
rs2589985	ACG TTG GAT GCC TTT CCT CAA CCA AAA AAC	ACG TTG GAT GTG AGC CAC TGT ACC CAC GAA	AAC CAA AAA ACA GAG CTA ATA A
rs2748153	ACG TTG GAT GAC AAT ATT GCT ACA GTC CCC	ACG TTG GAT GCA CTC ATT AAA TCA AAA GGC	TAA AGT CCC CTC CAG TAC
rs2748160	ACG TTG GAT GGG TAT TGA CAA TAT GAC ATG G	ACG TTG GAT GTG AAG GTC TTC CAC CCA ATC	GAG GAC ATG GAA TTT TAT GCT ATG
rs2748169	ACG TTG GAT GAT GCA CGC GTT TCC CAG TTC	ACG TTG GAT GCA CAC ACA CAT CCA GAA TAC	GGT GCC CAG TTC TGA ACA GAT GAT
rs2748174	ACG TTG GAT GTT GTG TGC ACA CAG GTG TTT	ACG TTG GAT GAG AGT ATG GAA ATG CCA CTG	GTG GGC ACA CAG GTG TTT TCA TAA
rs28580436	ACG TTG GAT GTG CTT CTG AAG TTC CAG TGT	ACG TTG GAT GGA AGA TTA AAA AAT ATT AAT AC	GTA ATA TCT AGT AGT ATG ACC TAA ATA
rs28730807	ACG TTG GAT GGA ACT GAA AAA AAT ACT AGC C	ACG TTG GAT GCT GGA CGG TAA TAT GTT ATT	AAA AAT ACT AGC CAA TGG CC
rs28730811	ACG TTG GAT GAG TCC GAG GAC TGC CTG TCA	ACG TTG GAT GTT GAA CTG CAC GAT GAA CGG	GGG ATG TCA CAG CCT CCT CCT CTT CCC
rs2901863	ACG TTG GAT GTA CCT GGT GCT CTA ATT CCC	ACG TTG GAT GAT AAT CCT GAT GGA CAG CCC	CAT GAC CGT CCA TTA CGA
rs34118838	ACG TTG GAT GCT CTC AAG CAC AGC GTA CAC	ACG TTG GAT GTG GTG GAG AAA GAT ATC GAG	AAT GTT TCA CAA ATT TAT CCT CAC CT
rs34841627	ACG TTG GAT GTG GAG ATT CCA TTG GAT TAG	ACG TTG GAT GCT GAT TAT AAA AGC CAG AC	CCC TTT CAA TTA CCT TGT AGT GAT AA
rs35067867	ACG TTG GAT GCA ACT ACT TAA CCT GCT GCC	ACG TTG GAT GCT CCT CTG TCA ATG ACG CTT	CCT ACC CAT AAA TGG CA
rs35101671	ACG TTG GAT GTC ACC TTG GCC GGC GCG G	ACG TTG GAT GGC GGC TAC GAG CTG CGA GA	GGG GTG CCG GCG CGG GGC CTC CTA GGG
rs3784692	ACG TTG GAT GGT CAA TCC AAG CTA ACA TTT C	ACG TTG GAT GTT GTT TAT GGA TGA ATG TG	CTC ACA TTT TCA ATT AAC AGA T
rs3784709	ACG TTG GAT GTT CCT CAT TGG CCA TGA CTC	ACG TTG GAT GGG AGA GGA GGC TCT TTT AGG	CCA TTG GCC ATG ACT CAG CTC A
rs3784711	ACG TTG GAT GCT CTC AAC ACT AGC AGC TC	ACG TTG GAT GAT CTA GGT TAG GAT CCA GGC	GGA GCA GCT CAT CAG AGT
rs3890755	ACG TTG GAT GCC CTA TCA AAA ATT AGC TC	ACG TTG GAT GTG GCA GTT ACA TGT AAG GG	TAT CAA AAA TTA GCT CTT TTA CAT A
rs3923809	ACG TTG GAT GGT CCT ACT GAA TTG CAG ATG	ACG TTG GAT GTG ACA GAA TGC CAT GTC TTC	ACT GAA TTG CAG ATG GAT AAA
rs41306690	ACG TTG GAT GCA AGA TCC CAA ATA GTG GCG	ACG TTG GAT GGC ACA TCC CTG AAG AGT AAC	GAC TGG ACA GTG CAC TC
rs4131034	ACG TTG GAT GGG AAA TCC AAG GGC ATG GTG	ACG TTG GAT GTT GCC CTT CTG CTT TTC ACC	GAG ATT CTG GCA AGG GCT TTT TTA CC
rs4140443	ACG TTG GAT GGT TCT ATC TTT ATT TCA CCC	ACG TTG GAT GAA TTG TAA ACA TAT ATG AAA	TCA CCC ATA TTC TTG AAA AT
rs4236060	ACG TTG GAT GCA TGG AAT ATG AAT AAC AC	ACG TTG GAT GGA AGA GAA TAC ACC ATG GAA	GGT GCC TTC ATT TTG CTA CCC A
rs4300815	ACG TTG GAT GCT CCT GAT GTG TGA GCA CTT	ACG TTG GAT GTA GCC AAG TTG CCC ACA CTC	CCC GTC ACT TCA GTA TTG CTC A
rs4302899	ACG TTG GAT GTT CAG GGC ACT TCT TTG AGC	ACG TTG GAT GTG GCC ATG TTC TCC AAA CTC	ACT TCT TTG AGC TCA CTG CAT CA
rs4430927	ACG TTG GAT GAG GCC ATG GTC TAG TGA AAG	ACG TTG GAT GTG TCC GTA GAC GAA TTG TAG	GAC AGG GTC TAG TGA AAG AGG CCA AC
rs4489954	ACG TTG GAT GTG TCT CTA ATG CCT CTT TCG	ACG TTG GAT GGC TTC ACT GTG CCT TGA AAC	GTG TTT TAT TGG ACT GTC ATC
rs4537967	ACG TTG GAT GTC AGC AGC ACT GAC ACT GAG	ACG TTG GAT GAT GAG CAG AGG GTA AAG TGG	CCG GGG GAA AGA CAA GTC TTG AAG C
rs4544423	ACG TTG GAT GCA CCA GCT CAT AAA GAA ACC	ACG TTG GAT GTC TGC CTC CCA GCT TAA ATT	GAA ACC ACA GCA GAG C
rs4605359	ACG TTG GAT GAG GCT CGG GCA TTA TAA GAC	ACG TTG GAT GCC AGG TAT TGC CAA TTA AGG	CCC AAA TCT GCT TTG ATA CGA TTA TC
rs4623233	ACG TTG GAT GCA TTG TTT CAT GGA ACG ACC	ACG TTG GAT GAG TGC CTG GCA TAG AGT TAC	ACC CTA GTC ACA CTG ATA
rs4626419	ACG TTG GAT GCC CAA GAT TTA AAA TTG TCG	ACG TTG GAT GCC TGA CTT TCG GGA CAT TTG	AAG ATT TAA AAT TGT CGT ATT GCT ATA
rs4626664	ACG TTG GAT GGG TTG TGA ATC AAG GCA CTG	ACG TTG GAT GGA CTT TTC CAA TGA TCT TAC	AAT GGA AAT AAT AAA TCA ATT TTG AA
rs4671730	ACG TTG GAT GTC CCC ACA CAC TTG CTA ATC	ACG TTG GAT GGT CCA GGT AGA TTT CTT TGC	CAT TTC ACA ATC TTC TTC CA
rs4671737	ACG TTG GAT GCT TTT CCT TTC TTC GCT TTC	ACG TTG GAT GAA GTT TCA GAA ATC TAC CCG	CGC TTT CTT TTT TCT CTT TTT CTT TT
rs4711549	ACG TTG GAT GTA CCA TGG CAT AAA CGA CCC	ACG TTG GAT GAA TGA GAT GGT GCT GAA GAG	AGA CCC AAC TGG TCT C
rs4711550	ACG TTG GAT GGC TGG CTT CTA GAA TAA CCC	ACG TTG GAT GTT TCA TGC AGA GAG AGC GTC	CCC ATC CCA GAG CTA A
rs4714146	ACG TTG GAT GTG AAT TGC TGA TGC CAT CTC	ACG TTG GAT GCC AAA TGC ACG GAT TCT CTC	GAT TCC ATC TCT CCC CCA GTA GCC ACA
rs4714156	ACG TTG GAT GTT TGT GGA AAG GCA GCA AGC	ACG TTG GAT GGG TTC TGC ATA TTC TGA GGG	CAG CAA GCA AAA GGG AGA CTT GTC
rs4714165	ACG TTG GAT GAT CAG CAT TGA TTC TTT CC	ACG TTG GAT GTC TGT CAC CCT AGT GTT ATC	GCA TTG ATT CTT TCC TAT TCC C
rs4776970	ACG TTG GAT GAA GTG CTT ATG TGC TTC ACC	ACG TTG GAT GGG AAA GGG GAT AAT AGT GAC	CAC ATT ATT TGC CTT TTT ACA AAA ACA G
rs4776982	ACG TTG GAT GAA CTG GCT TAG CAG CGT TGA	ACG TTG GAT GAG CCA AGT CAT CGT TGG GAG	GGC AGC GTT GAG AGC GT
rs6494696	ACG TTG GAT GTG AAG GTC TGA GAG GCC TG	ACG TTG GAT GTC GCC CAC TCA CTT TCT AAC	AGG CTG CCT CCA GTG AGG GTT T
rs6500961	ACG TTG GAT GGG GCC TTG TAG ACA AGA TG	ACG TTG GAT GCA GAA GAG GAT CTT GCA CT	AAC AGA AGA TCG GGG CT
rs6520824	ACG TTG GAT GTC TCT CCA TTT TCC CAT GCG	ACG TTG GAT GGT TAG TTT GTC TCA TAG AGG G	GTC CCA TGC GAG AAG CCT GTA
rs6546232	ACG TTG GAT GGA CGA CTG CAT GCT TTA ACC	ACG TTG GAT GAG GAA AAT TTG TGT GTA GG	CAT GAT GTG CCA GAT TAC C
rs6610746	ACG TTG GAT GTG TAG GTT CAG CTA ACT TGG	ACG TTG GAT GCA GAT GGC TTG TTT ACT GC	CAC ACA AGG TCC ACT CTC
rs6705285	ACG TTG GAT GCT CAC ACC ACC ACC CTT ATG	ACG TTG GAT GTG TCA GGC CTT AGG TTA TTC	TGG TGG AGC AGA GAT T
rs6705647	ACG TTG GAT GGA AAC AAA CAC TGG ACT ACC	ACG TTG GAT GTT GTG CTA AGC TCT GGG ATG	GGC ATT GAG GAC AGG GAT TGG TCT TAC T
rs6710341	ACG TTG GAT GGG TAA ATG ATA TAC AAT TGG C	ACG TTG GAT GGT ATA TGG TGA TGA GTG TTC	TTG GCT TCA TGA AAT AAA ATG GT
rs6711787	ACG TTG GAT GAT GTT GCC TAC GGA TGG AAG	ACG TTG GAT GAC TGA AAA CTT ACA TGC ACG	TGG AAG TTA ATG CAC CAT

Supplementary Table 9: continued.

dbSNP ID	Sequence forward PCR primer	Sequence reverse PCR primer	Sequence extension primer
rs6721499	ACG TTG GAT GCA TCC TGA GGA TCC CAT TTG	ACG TTG GAT GGG CAC ATA AGT GTG TCT AAC	CAT TTG GTT AAG CCT TAC TA
rs6727352	ACG TTG GAT GTA CAT GAT GGC AGA GAC GTG	ACG TTG GAT GTC TGA TTA TGG TTA GGC GGG	AAG GAA GAG ACG TGT GGC TTT C
rs6917654	ACG TTG GAT GGT CTA CTC TTT GCC AGT TAC	ACG TTG GAT GGT CTC TAA AAA TCC TGA AAA C	ACT CTT TGC CAG TTA CTA TTT T
rs6923737	ACG TTG GAT GTC ACT AGC TAC TAA GCT CTC	ACG TTG GAT GGG TGC GTC AAG TAG TAC TTT	TAC TAA GCT CTC TCT CTT CT
rs6932235	ACG TTG GAT GAG GCC AAG AGC ACA ATC TAC	ACG TTG GAT GCT TTA GAG GAA TAC TGT GTC	GGT GTA ACC TGA TAA AAG GGA
rs7162980	ACG TTG GAT GGC TCC AGA TTC AAT TAT GAG	ACG TTG GAT GAG GTG CAG TAC TAG GAA GAC	GTT TAA CAA CTA GAC TCT AAG T
rs7180716	ACG TTG GAT GAA CCC TAT GTG GGA AAG GTG	ACG TTG GAT GGC ACA GTC TTA TTA CTA CTG C	GGT GAA GCC AAG ACA C
rs7181869	ACG TTG GAT GGG CGG CGG CGC CAT GTT CT	ACG TTG GAT GAC TGC CGC TGC GTC CTT G	GAT GTT CTG GGG GCA TCA
rs7194617	ACG TTG GAT GTC ATG CCC AAT TTT CAC AGG	ACG TTG GAT GAT GGG ACA TGG GCA AGT ATC	CCC CAA TTT TCA CAG GGC AAA GCA TC
rs726160	ACG TTG GAT GTA AAT CCA GCT TCT TGC CAC	ACG TTG GAT GTC GGT ACA GTC CTC TTT AGC	GAA CTC CAG CTT CTT GCC ACT ATA CCA
rs737172	ACG TTG GAT GGT CTA GGG CCG AGG CTT TG	ACG TTG GAT GGG GAA CGA GAT AGC AGC TTG	ATT TTC AGA TGC GAG GC
rs745213	ACG TTG GAT GCA TGT TTC TTT TCT TGT AGC C	ACG TTG GAT GGG TCA AGG CAG CCA AAA AAC	TTT CCT TGT AGC CTC TTC GG
rs7497457	ACG TTG GAT GGG GAT CAG CGT TTG AGT AAG	ACG TTG GAT GTT TTG CCT GCG CGT CTT TCC	TTG AAC CCT CCG CGC C
rs7563565	ACG TTG GAT GCA CAG ATA CAT TTT AAT GC	ACG TTG GAT GCA GGT TGA TTC TTA ATA TAG	TTC ATG CAA TAA AAT CCT AAA GTG TG
rs7579466	ACG TTG GAT GTC CAT TTG TAC CTT GTA AC	ACG TTG GAT GAA AAT GCC ATG TTT AAT AAG	TTT GAA CAG TTA TAT GGG TTA A
rs7586211	ACG TTG GAT GCA AAT GGC TGG CTA GTG TTC	ACG TTG GAT GAA ACA AAA GTG CCC ATG GAC	TGG ATG GCT AGT GTT CAG TTA GGT G
rs7603236	ACG TTG GAT GTT GCC AAG TTT GAA CCT CAC	ACG TTG GAT GAG AAT GAG GGC AAC ATT ACC	CTC ACC TCA CAA TTA CTG GT
rs7740763	ACG TTG GAT GAC TAC TGA AGA AAG AAA ATT	ACG TTG GAT GAC TGA ACA CTA CAG CTG AC	GGA GAA TAA ACT TTG TGT AGC CTA A
rs7745176	ACG TTG GAT GTT ACT GAT ACT CAG TAC AT	ACG TTG GAT GCC ATC CCT TGA AAT GGA ATG	TTA CTG ATA CTC AGT ACA TAA TTT A
rs7763775	ACG TTG GAT GTG ACC TTT TCC TTT AAG GAG	ACG TTG GAT GCA GGC ATT ACG TGC TTC TTG	CCC TTT TTC CTT TAA GGA GAT ACT AAG A
rs7769186	ACG TTG GAT GAA ACC AGG ACC TTC CAC TAA	ACG TTG GAT GGA AGA ATC CGA AGA AGC AGG	CAG GAC CTT CCA CTA ATA TTC C
rs7872553	ACG TTG GAT GAA CAC CTC AAA TTG CTT CAG	ACG TTG GAT GAC TCC AGC AGA CTC TAA ACC	ATC AAA TTG CTT CAG TTT GAG TGA TT
rs7881785	ACG TTG GAT GCA TTC ATA ATA GCC AAA CAC	ACG TTG GAT GCG TGA ATA TAC TCA ATT CAT	CAT CAC TCA GAT ATT CAT TAG TC
rs8025526	ACG TTG GAT GCA CTT AAG TTA GAG CAT TC	ACG TTG GAT GGG GAA TAA GAA TAA TCC TT	TTA TCT CAT TTG ACC TTC AC
rs8025790	ACG TTG GAT GAT GTT TTA TGT TTA GAT CC	ACG TTG GAT GGA ATC CAG AAA GGA TCC TGT	GTT ATG TTT AGA TCC ATT TCT CT
rs884202	ACG TTG GAT GAT TAA ATG AGC TCA CCC TCG	ACG TTG GAT GGC ACA GGT AGG AAT TGC TGA	CCT CGT GGG AAG TCT CCT G
rs909997	ACG TTG GAT GCC GTT TAT TGT TAT TTT ATC	ACG TTG GAT GGA AAC TTT AGA AGT AAA TCC	CGT TTA TTG TTA TTT TAT CAG TAA AAG T
rs910516	ACG TTG GAT GTA CCA CAA CAT GTC TGA CTC	ACG TTG GAT GGT AGT GAT AAC TGC AGT GTC	GCT GTC TTG CTT AAT TCT GA
rs915161	ACG TTG GAT GAT TAG CCT ACC AGA TCC ACG	ACG TTG GAT GAG AGT TGG TGT TCT CGG AGC	CCC CAG ATC CAC GCT CAA AC
rs922493	ACG TTG GAT GTC CCA CGG GAA TGT TGT GTC	ACG TTG GAT GTT CAT TTG CCA AAC ATG CTG	GGG CCT GGC ACT TAG
rs926564	ACG TTG GAT GCC CTT AAT GTT ATA TTG GGC	ACG TTG GAT GGA TAA AGT TCA TGG CAA TGT C	TTA TAT TGG GCA AAA TTA TAT ATA AAT G
rs9296249	ACG TTG GAT GAG TGG GCA GAT CAT GAA AGG	ACG TTG GAT GTC TCA GGG CTC CTC TTC ACC	GCT GTG GAT CTT GGA CTT TA
rs9302245	ACG TTG GAT GGC AAA TAA GTG TTG TAT TAC	ACG TTG GAT GCC ATG TTT GTG ATG CAT CTG	CCT GTT GTA TTA CAC ATA CTA ATT TAT G
rs9349073	ACG TTG GAT GCT TAA GCA ATT CAA TCC AGG	ACG TTG GAT GGC TAT ATC ACC TTA GCT GCC	GGC CCG GAG ATT TAA AAA ACT GCA ATA
rs9357271	ACG TTG GAT GGT GGA TCT TGG ACT TTA TGC	ACG TTG GAT GCG AAC GAA GTC ATG TCA CTC	GGA GCC CTG AGA AGT TT
rs9366950	ACG TTG GAT GCT GGA TGT GGG TCA TTT GTC	ACG TTG GAT GTA AAT CGA GGA GAA CTG GGC	CCC CGT CAT TCA TCC TGC TCA GTT
rs9369064	ACG TTG GAT GTA TAT GAG CAT CCA ACA CTG	ACG TTG GAT GGC CTA CAG TTG GGC AAA ATC	CCT CGC ATC CAA CAC TGT TTA TGG ATC A
rs9380739	ACG TTG GAT GCT TTT TAG TGT GTC TAA CAG G	ACG TTG GAT GCG TCA ATC TAG TTG GTT TAG	TCG TGT GTC TAA CAG GAT AAA ATG
rs9380755	ACG TTG GAT GGC TTT CCA AGT AAC CTC CTC	ACG TTG GAT GGA GGA AGT TTG TAG TCT CCG	CTC CTC TTT AAC TGA TGA TG
rs9394492	ACG TTG GAT GCT AAC ATA TCA TAC ACT GG	ACG TTG GAT GTT TCT CAA GAT CTA CAG GGC	AAC TCA ACC AAC TAG ATT GAC GAA
rs9394507	ACG TTG GAT GTC CTC CCT GTC TCT TGA CC	ACG TTG GAT GCA AGG ATC ATC TGT GTA ATG	CTA TTG ACC CCT CAG ACA
rs9462409	ACG TTG GAT GAG ATG CGC ATC TGA TGA ACG	ACG TTG GAT GTA AAA TGG GGC CAT GAT GGG	CTC TGC CAA CAG CCC T
rs9462426	ACG TTG GAT GTT AGC TCT AGC TGA GGA ATC	ACG TTG GAT GAA TCC CCC TTG CAC TGA ATC	GCA GTA TTT GCC TGC TGC A
rs9462433	ACG TTG GAT GAA ACA AGT GAG TTG TAT GG	ACG TTG GAT GGC ACT ATT TTT TGT AAC AGC	TGA GTT GTA TGG TAT ATA AAC G
rs9470822	ACG TTG GAT GGA AAA CTA TAA AAG TAA ACC	ACG TTG GAT GCA ATT GTT TAT GAC CTG GTG	ACT ATA AAA GTA AAC CAA CCG AT
rs9470888	ACG TTG GAT GTT GTG ATT CGT GAG AGG TGG	ACG TTG GAT GTG CAG CAA TCC AGT CAT ATC	TAT GCG TGA GAG GTG GTC AAA ATA

VI. Discussion

Four studies have been presented that explored issues of diagnosis, treatment and pathophysiology in RLS. While one of the studies is currently in press, the three other studies have already been published and in the following the results of these studies will be discussed taking into account new empirical results that have become available since the original publications.

Cognitive functioning in RLS

Our study on cognitive functioning in RLS has shown deficits in short-term attention and verbal fluency in unmedicated subjects with clinically significant RLS. In particular, performance in the Stroop task, the d2-cancellation task and phonemic and semantic fluency was found decreased in RLS subjects. These results have been discussed in relation to the four fully published studies[1-4] in this area in Chapter II but beside these, three other research groups have presented preliminary results about cognitive functioning in RLS at various scientific meetings. Trygvadottir and the research group from Reykjavic[5] presented an abstract describing cognitive function testing in 19 RLS patients (5 males, 14 females, 22-55 years, mean age 41) and 18 controls matched for age, sex, and occupation. Sixteen of the patients were drug naïve and no information was available regarding drug status of the remaining three or severity and aetiology of RLS (idiopathic versus secondary RLS). Tests included the Stroop task, verbal fluency, and the sub-tests matrix reasoning and similarities from the Wechsler Adult Intelligence Scale. In addition, five computerised tests from the CANTAB expedio system, presumably focusing on planning, and tasks assessing selective and sustained attention, flexibility of attention, spatial working memory, and reaction time were given but were not described in detail. The RLS patients performed significantly worse than the healthy controls on the tests of verbal fluency, reaction time, and sustained and selective attention. The authors also noted a trend towards worse performance on other tests of executive functioning.

Ferini-Strambi and co-workers[6] conducted at study exploring attention, memory, executive functions, visuoconstructive and motor abilities in twelve patients (4 males, 8 females, 31 to 62 years, mean age 49) with idiopathic RLS and documented PLMS and 12 matched healthy controls. In addition, patients and controls did not differ in years of education, subjective daytime sleepiness (ESS) or depressed mood (BDI). Polysomnography in both groups revealed decreased total sleep time and sleep efficacy, and increased sleep onset latency in RLS patients compared to controls. Both the number of PLM and PLM associated arousal per hour of sleep was higher in patients than in controls while the number of arousals per hour of sleep did not differ. Ferini-Strambi assessed sustained attention (TR2) and short-term attention (number cancellation, TMT-A and B, Stroop task, digit and block span). In addition, working memory was tested with the digit span backward, and visual-spatial learning (Corsi supraspan), verbal long-term memory, verbal fluency, visuoconstructive abilities (copy of Rey's complex figure), and motor abilities (Purdue Pegboard) were explored. Compared to controls RLS patients showed decreased performance in the test of sustained attention, in the Corsi supraspan test, in the digit span forward, and in the Purdue Pegboard task. In the same study, also a comparable group with PLMS but no RLS had been included and these patients showed decreased performance in very same tasks. Overall, however, performance was lowest in the RLS group which lead the author to speculate that the effect of sleep deprivation and sleep fragmentation (RLS and PLMS) causes greater neurocognitive consequences than sleep fragmentation alone (PLMS).

Weniger et al.[7] from Göttingen evaluated psychiatric co-morbidity and neuropsychological function in 28 RLS patients and 28 healthy controls. Age, gender, or treatment status of the participants was not stated in the meeting abstract, but twelve of the RLS patients had at least one psychiatric diagnosis. Neuropsychological tasks evaluated general cognitive functioning, different aspects of attention, and explicit and implicit visual and verbal memory but not details as to the specific tasks were given. RLS patients did not differ from controls in attention tasks but both RLS patients with and without psychiatric co-morbidity showed impairments in explicit visual and verbal memory.

Taking all studies together, it is apparent that the area of attention and executive functioning has received most of the attention while memory, motor function, or visuoconstructive performance have only been assessed in a few of the studies. For the memory domain not enough evidence has accumulated to draw any conclusions. Even given the focus on the attention tasks, there is no single task that has been applied in more than two studies. Nevertheless, with the exception of one study[7], all other studies reported at least one attention task with reduced performance of RLS subjects when compared to controls. Converging results were obtained for simple reaction time and sustained attention. In addition, while the more central executive functions such as planning and concept formation did not differ between RLS patients and controls there is now consistent indication that verbal fluency is an area with decreased performance in RLS patients.

Placebo effect in RLS treatment studies

Recent years have seen the emergence of a number of substances for the treatment of RLS[8] and with several pivotal studies conducted it soon became apparent that the improvement of RLS symptoms in the placebo groups was substantial[9,10]. Our meta-analysis has quantified the magnitude of the placebo response in RLS treatment studies by combining results from 24 studies conducted during the last 25 years (Chapter III). The pooled placebo response rate for all studies was 40% (vs. 68% in the treatment groups) with response being defined as a physician rating of “much” or “very much improved” in the majority of trials. Meta-regression of placebo response rates revealed a strong effect of study duration on response rates that were increasing over time. The estimated placebo response rate was 22% at the end of the first week and expected to increase by 3% for each additional week. At the time of the study conduction no placebo-controlled trials longer than twelve weeks were available. In the meantime, results from a double-blind placebo-controlled trial with 26 weeks duration have become available[11]. In this study with 205 RLS patients in the placebo group and 196 patients receiving ropinirole, the response rate after 12 weeks was 52% in the placebo group and 68% in the treatment group. After 26 weeks response rates rose to 64% and 84%, suggesting that the placebo response is also increasing over periods longer than 12 weeks, although the rate of improvement over time appears to be attenuated for longer periods.

In addition, there was a large placebo effect in RLS severity as measured by the IRLS while other scores of RLS severity had a lower – small to moderate – placebo effect that was lower in cross-over trials than in parallel group trials. This difference between cross-over and parallel group trials could not be analyzed for the IRLS since 13 of the 14 trials that employed the IRLS were parallel group trials. Recent studies, however, have provided a striking example for the magnitude of this effect. In the last year, two short-term studies have explored the effect of gabapentin enarcabil on RLS symptoms[12,13]. Gabapentin enarcabil is a precursor to gabapentin that allows for the rapid absorption through the gastrointestinal tract before being converted into gabapentin and overcomes gabapentin’s unfavorable pharmacokinetic profile and dose-dependent bioavailability. Both studies had a highly similar study design including a study length of 14 days with the only major difference being that one

study[12] was a cross-over design while the other[13] was conducted with a parallel group design. The cross-over study enrolled 38 subjects and found that mean change after 14-days of placebo was only -1.9 points on the 40-point IRLS scale (vs. -12.1 for gabapentin enarcabil). In the parallel-group study 29 subjects received gabapentin enarcabil and 33 received placebo and the mean change in IRLS after 14 days was -8.9 (vs. -16.1 for the active treatment). Similar, response rates were 14.7% (vs. 79.5%) in the cross-over trial but 48.5% (vs. 81.3%) in the parallel-group trial.

While the magnitude of the placebo response in RLS seems substantial it is not out of proportion when compared to other disorders of the central nervous system with which it shares some features. For example, a recent meta-analysis[14] involving 11 studies and 858 patients with *Parkinson's Disease* (PD) showed an overall response rate of 16% with a range of 0 to 55%. However, considering only surgical trials, a positive placebo response was observed in 42% of PD patients. Migraine like RLS is episodic and sensory in nature. Here a meta-analysis[15] showed an average headache placebo response rate of 30%. Although no overall response rates are available for insomnia, a meta-analysis[16] has demonstrated a substantial placebo effect in both subjective and objective sleep parameters and this placebo response also increases with time[17] as with RLS. Finally, in depression the proportion of patients who respond to placebo has been estimated as 30%[18]. While the 40% placebo response rate in RLS seems in the upper range compared to these disorders, it has to be taken into account that treatment response rates vary correspondingly. For example, the 30% placebo response rate is matched with a 50% treatment response rate in depression[18], and with a 60% rate in migraine patients[15]. These are ratios of placebo to treatment response of 0.6 and 0.5 which again is comparable to the ratio of 0.58 for RLS (40% placebo response, 68% treatment response).

Certainly the most pressing question at the moment is that of the "real" size of the placebo effect. So far, the placebo response has been conceptualized as the before-after difference observed in the placebo group. Without a proper control condition, however, there is no way to distinguish the effect of the placebo from the natural course of the disease, regression to the mean or other factors that operate in a time-dependent manner[19]. So far, we have no valid estimates about what is the expected rate of spontaneous significant improvement or remission in RLS. This rate, however, will critically determine the size of true placebo effect and hence effective ways to cope with it. Conceptually, beside the inclusion of a no-treatment control group there are also other less obvious paradigms to obtain an estimate of the true placebo effect such as the open and hidden administration of a treatment[20]. In this latter paradigm a patient either fully views a treatment or receives it in a "hidden" manner without any cues for example by means of a computer-programmed drug infusion pump with no clinician present and the patient unaware that treatment is being administered[21]. With respect to pain, the difference in medication needed for analgesia between open and hidden injections reflects the placebo analgesic effect. It is important to stress that the size of the real placebo effect is more than a purely academic question given the seminal and provocative work of Hróbjartsson and Gøtsche[22] that showed that in many instances a placebo effect is no longer apparent when compared to a non-treatment control condition in several conditions. A notable exception to this is experimental pain or pain disorders where placebo exerts a powerful effect even compared to no-treatment control groups.

Other questions concern potential moderators of the placebo response. For most of the outcome parameters in RLS trials there was significant between-study heterogeneity. Moderators of the placebo response were trial duration and study design. Nothing is known so far about other important variables that might explain within study variability such as severity of RLS, pre-medication status, familiar vs. sporadic RLS and other subject-specific characterizations. Also, the placebo response in special populations such as the elderly or

children with RLS has not been explored so far. Although there are only a very limited number of double-blind placebo-controlled treatment trials in secondary RLS the placebo responses appear to be considerably smaller at least in uremic RLS[23,24].

It is important to stress that the existence or magnitude of a placebo effect does neither say anything about RLS being a “real” vs. “imagined” condition, nor necessarily about the quality of the trial. Indeed, the conceptualization of the placebo effect has shifted from the focus on the inert content of a physical placebo agent to the overall simulation of a therapeutic intervention including for example the interaction between patient and medical personnel. In recent years, research has identified many types of placebo responses driven by different mechanisms depending on the particular context wherein the placebo is given[20]. Some placebo responses, such as analgesia, are initiated and maintained by expectations of symptom change and changes in motivation/emotion. Placebo factors have neurobiological underpinnings and actual effects on the brain and body. They are not just response biases. Other placebo responses result from less conscious processes, such as classical conditioning in the case of immune, hormonal, and respiratory functions[20]. Intriguingly, the two systems that play a crucial role in the physiological response to a placebo are the dopamine and the opioid systems[25]. The unique responsiveness of RLS to both dopaminergic agents and opioids places it right at the crossroad of these two systems.

Prevalence of RLS in transsexual patients

Epidemiological studies have shown a markedly higher prevalence of RLS in women[26,27] and this increased risk for RLS in females has been related to the number of pregnancies[27]. A recent study[28] found markedly elevated estradiol levels in pregnant women with RLS during late-term pregnancy but not after delivery when subjects were symptom-free. This has prompted us to elucidate the role of gender and steroid hormones as a risk factor for RLS by exploring the prevalence of RLS symptoms in a large group of transsexual patients treated with either testosterone or estrogens. Contrary to our hypothesis there was no statistically significant difference in RLS prevalence between the two groups and the numerically higher prevalence in the estrogen-treated group could be explained by the higher average age in this group. In addition, we did not observe a systematic relationship between the onset of RLS and the onset of hormonal treatment.

While the study was most likely underpowered to reveal significant differences and neglected to obtain information about transient RLS symptoms, another explanation for this lack of findings could be the dosage of hormonal treatment in this group. In male-to-female transsexuals estradiol levels range in general from 100 to 200 pg/ml, dependent on the dosage and time of substitution, as compared to 50-500 pg/ml found during the menstrual cycle. This is considerably lower than estradiol levels found during pregnancy and in the study [8] on pregnant females with and without RLS levels of estradiol were up to 100 times higher (15,000 to 45,000 pg/ml) than those found during the menstrual cycle.

This is corroborated by studies involving hormone replacement therapy (HRT) where estradiol levels are increased but considerably lower than during pregnancy. Here, two epidemiological studies[29,30] failed to find a more frequent use of HRT in women with RLS. RLS symptoms are not routinely included into the assessment during HRT trials and it is therefore unknown what would be the incidence of new-onset RLS in subjects undergoing HRT. However, In a one year trial[31] only one of the 73 (1.4%) women dropped out because of the new onset of RLS, which would suggest a low incidence of RLS during HRT. Although in a sleep lab population one study[32] found that HRT was more frequent in patients with RLS (74%) than in patients without RLS (48%), several other polysomnographic investigations[33-35]

of HRT failed to find an influence on periodic leg movements, a closely associated characteristic of RLS.

Taken together, at the present moment the available evidence does not argue conclusively for a role of low-dose estrogens in the pathophysiology of RLS. However, while estrogens might not be a necessary and/or sufficient factor to elicit and maintain RLS, it is still conceivable that estrogens may be a trigger in subjects with a pre-existing, probably genetically-based, vulnerability to RLS.

Genome-wide association study in RLS

Our genome-wide association study found that genetic variants in MEIS1, BTBD9, and LBXCOR1/MAP2K5 were associated with an increased risk for developing RLS[36]. Previous linkage studies in families with RLS had identified eight loci that are associated with RLS but no causally related sequence has yet been identified[37]. These loci, named RLS1 to RLS8, are located on chromosomes 12q, 14q, 9p, 20p, 2q, 4q, 17p and 19p, respectively. In these studies the apparent genetic heterogeneity of RLS has made linkage studies notoriously difficult which favors association approaches. Our initial genome-wide screen for common variants in 400 cases and 1,600 controls enabled us to detect risk alleles with odds ratios > 1.5 and the substantially increased sample size in the replicate study has and provided unequivocal evidence for the signals. A particular feature of our study design was the use of a control group from the general population. This provided us with very accurate estimates of the genotype frequencies and it avoided any bias to which a disease-negative population is prone.

At the time of publication, BTBD9 was independently identified in another genome-wide association study of an Icelandic population[38]. Their phenotypic assessment included the measurement of PLM and, interestingly, the association with BTBD9 was found in subjects with periodic leg movements (PLM) without RLS but not in subjects with RLS without PLMs. Interestingly, in the Icelandic study a 13% further reduction of serum ferritin levels was found in the RLS group, implicating BTBD9 in iron storage. Since then, the three genetic variants MEIS1, BTBD9 and LBXCOR1/MAP2K5 have been independently replicated in three European populations[39] and a further genome-wide association study has identified PTPRD (protein tyrosine phosphatase receptor type delta) as another genetic variant associated with RLS[40]. Studies in other disorders have found recently an association between BTBD9 and Tourette's syndrome in a candidate gene study[41].

Apart from one association with body iron stores[38], in the genome-wide association studies no genes could be identified that were related to the iron, dopamine or endogenous opioid systems. The functions of the five genes that were identified—MEIS1, BTBD9, MAP2K5/LBXCOR1, and PTPRD—are largely related to embryonic neuronal development, and no functional relationships with RLS have so far been established. At this point one can only speculate about possible relations between the genetics and RLS. The genome-wide association studies are not exhaustive in identifying genetic factors contributing to RLS and further investigations are expected to contribute towards a better picture of what constitutes the genetic architecture of the complex phenotype of restless legs syndrome.

References

1. Saletu, B. et al. Sleep laboratory studies in restless legs syndrome patients as compared with normals and acute effects of ropinirole. 1. Findings on objective and subjective sleep and awakening quality. *Neuropsychobiology* **41**, 181-189 (2000).
2. Pearson, V.E. et al. Cognitive deficits associated with restless legs syndrome (RLS). *Sleep Med.* **7**, 25-30 (2006).
3. Driver-Dunckley, E. et al. No evidence for cognitive dysfunction or depression in patients with mild restless legs syndrome. *Mov. Disord.* **24**, 1843-1847 (2009).
4. Celle, S. et al. Prevalence and clinical correlates of restless legs syndrome in an elderly French population: the synapse study. *J. Gerontol. A Biol. Sci. Med. Sci.* **65**, 167-173 (2010).
5. Tryggvadottir, H.B., Jonsdottir, M.K., Kristjansson, K., Rye, D. & Sigmundsson, T. Cognitive function in restless legs syndrome. *J. Sleep Res.* **13**, 732 (2004).
6. Ferini-Strambi, L. Cognitive functions in RLS patients with PLM and in PLMD patients without RLS. *Sleep Med.* **6**, S31 (2005).
7. Weniger, G., Trenkwalder, C., Dörnte, M. & Irle, E. Psychiatric comorbidity and neuropsychological changes in patients with restless-legs-syndrome. *Pharmacopsychiatry* **38**, A249 (2005).
8. Fulda, S. & Wetter, T.C. Emerging drugs for restless legs syndrome. *Expert Opin. Emerg. Drugs* **10**, 537-552 (2005).
9. Allen, R. et al. Ropinirole decreases periodic leg movements and improves sleep parameters in patients with restless legs syndrome. *Sleep* **27**, 907-914 (2004).
10. Walters, A. et al. Ropinirole is effective in the treatment of restless legs syndrome. TREAT RLS 2: a 12-week, double-blind, randomized, parallel-group, placebo-controlled study. *Mov.Disord.* **19**, 1414-1423 (2004).
11. GlaxoSmithKline Study ID: ROR104836. *Clinical Trial Register* at <http://www.gsk-clinicalstudyregister.com/files/pdf/21020.pdf>
12. Kushida, C.A. et al. A Randomized, Double-Blind, Placebo-Controlled, Crossover Study of XP13512/GSK1838262 in the Treatment of Patients With Primary Restless Legs Syndrome. *Sleep* **32**, 159-168 (2009).
13. Walters, A.S. et al. Gabapentin enacarbil in restless legs syndrome: a phase 2b, 2-week, randomized, double-blind, placebo-controlled trial. *Clin. Neuropharmacol.* **32**, 311-320 (2009).
14. Goetz, C. et al. Placebo response in Parkinson's Disease: comparisons among 11 trials covering medical and surgical interventions. *Mov. Disord.* **23**, 690-699 (2008).
15. Bendtsen, L., Mattson, P., Zwart, J. & Lipton, R. Placebo response in clinical randomized trials of analgesics in migraine. *Cephalalgia* **23**, 487-490 (2003).
16. Bélanger, L. et al. Meta-analysis of sleep changes in control groups of insomnia treatment trials. *J. Sleep Res.* **16**, 77-84 (2007).
17. Perlis, M., McCall, W., Jungquist, C., Pigeon, W. & Matteson, S. Placebo effects in primary insomnia. *Sleep Med.Rev.* **9**, 381-389 (2005).
18. Walsh, B., Seidman, S., Sysko, R. & Gould, M. Placebo response in studies of major depression. Variable, substantial, and growing. *JAMA* **287**, 1840-1847 (2002).
19. Hróbjartsson, A. What are the main methodological problems in the estimation of placebo effects? *J. Clin. Epidemiol.* **55**, 430-435 (2002).
20. Price, D., Finniss, D. & Benedetti, F. A comprehensive review of the placebo effect: recent advances and current thought. *Annu. Rev. Psychol.* **59**, 565-590 (2008).
21. Levine, J. & Gordon, N. Influence of the method of drug administration on analgesic response. *Nature* **312**, 755-756 (1984).
22. Hróbjartsson, A. & Gøtzsche, P.C. Is the placebo powerless? An analysis of clinical trials comparing placebo with no treatment. *N. Engl. J. Med.* **344**, 1594-1602 (2001).
23. Ausserwinkler, M. & Schmidt, P. Erfolgreiche Behandlung des "restless legs"-Syndroms bei chronischer Niereninsuffizienz mit Clonidin. *Schweiz.Med.Wochenschr.* **119**, 184-186 (1989).
24. Thorp, M., Morris, C. & Bagby, S. A crossover study of gabapentin in treatment of restless legs syndrome among hemodialysis patients. *Am. J. Kidney Dis.* **38**, 104-108 (2001).

25. Colloca, L., Lopiano, L., Benedetti, F. & Lanotte, M. Placebo response in conditions other than pain. *Semin. Pain Med.* **3**, 43-47 (2005).
26. Allen, R.P. et al. Restless legs syndrome prevalence and impact: REST general population study. *Arch. Intern. Med.* **165**, 1286-1292 (2005).
27. Berger, K., Luedemann, J., Trenkwalder, C., John, U. & Kessler, C. Sex and the risk of restless legs syndrome in the general population. *Arch. Intern. Med.* **164**, 196-202 (2004).
28. Dzaja, A., Wehrle, R., Lancel, M. & Pollmächer, T. Elevated estradiol plasma levels in women with restless legs during pregnancy. *Sleep* **32**, 169-174 (2009).
29. Rothdach, A.J., Trenkwalder, C., Haberstock, J., Keil, U. & Berger, K. Prevalence and risk factors of RLS in an elderly population: the MEMO study. Memory and Morbidity in Augsburg Elderly. *Neurology* **54**, 1064-1068 (2000).
30. Wesström, J., Nilsson, S., Sundström-Poromaa, I. & Ulfberg, J. Restless legs syndrome among women: prevalence, co-morbidity and possible relationship to menopause. *Climacteric* **11**, 422-428 (2008).
31. Popp, A.W. et al. Prevention of postmenopausal bone loss with long-cycle hormone replacement therapy. *Maturitas* **53**, 191-200 (2006).
32. Brown, L.K., Dedrick, D.L., Doggett, J.W. & Guido, P.S. Antidepressant medication use and restless legs syndrome in patients presenting with insomnia. *Sleep Med.* **6**, 443-450 (2005).
33. Polo-Kantola, P., Rauhala, E., Erkkola, R., Irjala, K. & Polo, O. Estrogen replacement therapy and nocturnal periodic limb movements: : A randomized controlled trial. *Obstet. Gynecol.* **97**, 548-554 (2001).
34. Montplaisir, J., Lorrain, J., Denesle, R. & Petit, D. Sleep in menopause: differential effects of two forms of hormone replacement therapy. *Menopause* **8**, 10-16 (2001).
35. Hachul, H. et al. Effects of hormone therapy with estrogen and/or progesterone on sleep pattern in postmenopausal women. *Int. J. Gynaecol. Obstet.* **103**, 207-212 (2008).
36. Winkelmann, J. et al. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. *Nat. Genet.* **39**, 1000-1006 (2007).
37. Trenkwalder, C., Högl, B. & Winkelmann, J. Recent advances in the diagnosis, genetics and treatment of restless legs syndrome. *J. Neurol.* **256**, 539-553 (2009).
38. Stefansson, H. et al. A Genetic Risk Factor for Periodic Limb Movements in Sleep. *N. Engl. J. Med.* **357**, 639-647 (2007).
39. Kemlink, D. et al. Replication of restless legs syndrome loci in three European populations. *J. Med. Genet* **46**, 315-318 (2009).
40. Schormair, B. et al. PTPRD (protein tyrosine phosphatase receptor type delta) is associated with restless legs syndrome. *Nat. Genet.* **40**, 946-948 (2008).
41. Rivière, J. et al. Association of intronic variants of the BTBD9 gene with Tourette syndrome. *Arch. Neurol.* **66**, 1267-1272 (2009).

VII. Appendix

Publications

Fulda S, Beitinger ME, Reppermund S, Winkelmann J, Wetter TC (2010) Short-term attention and verbal fluency is decreased in restless legs syndrome patients. Movement Disorders 2010; 25: 2641-2648

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Fulda S, Wetter TC (2008) Where dopamine meets opioids: a meta-analysis of the placebo effect in RLS treatment studies. Brain; 131: 902-917

Impact factor: 9.603

Fulda S, Stalla GK, Wetter TC (2007) Prevalence of the restless legs syndrome in transsexual patients: the hormonal hypothesis revisited. J Neurol; 254: 1748-1749

Impact factor: 2.477

Winkelmann J, Schormaier B, Lichtner P, Ripke S, Xiong L, Jalilzadeh S, **Fulda S**, Pütz B, Eckstein G, Hauk S, Trenkwalder C, Zimprich A, Stiasny-Kolster K, Oertel W, Bachmann CG, Paulus W, Peglau I, Eisensehr I, Montplaisir J, Turecki G, Rouleau G, Gieger C, Illig T, Wichmann HE, Holsboer F, Müller-Myhsok B, Meitinger T. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Nat Genet 2007; 39(8): 1000-1006.

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