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CLINICAL REVIEW

Genetics of restless legs syndrome: An update

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SUMMARY

A major role of genetic factors in the risk of developing restless legs syndrome (RLS) is supported by the high frequency of positive family history of RLS in patients affected with this disease, and the higher concordance rates in monozygotic twins compared with dizygotic ones in twin studies. In this review we have focused on those reports describing inheritance patterns of RLS, genetic anticipation, the results of studies performed on positivity of family history of RLS, twin studies, linkage studies in familial RLS, genome-wide association studies (GWAS), exome sequencing studies, and case–control association studies on candidate genes in RLS. Although to date the causative gene(s) has(ve) not been definitively identified, a number of variants of several genes, most of them through GWAS, have been associated with RLS risk, the strongest candidates being variants of *PTPRD*, *BTBD9*, and *MEIS1* genes. Despite results of several recent case–control association studies which have suggested a possible contribution of *heme-oxygenase 1 (HMOX1)* rs2071746 and *vitamin D3 receptor (VDR)* rs731236 variants, or the presence of allele 2 of the complex microsatellite repeat Rep1 within the *alpha-synuclein* (*SNCA*) gene promoter in modifying the risk for RLS, these studies need to be replicated in further studies involving different populations.

Introduction

Restless legs syndrome (RLS) is also currently known as Willis-Ekbom disease (WED) because the first description was attributed to Sir Thomas Willis in 1672, and a more detailed description was provided by Karl Ekbom in 1945, although some authors refer to a much older description by Xue Ji in a Chinese book edited in 1529 [1].

The International restless legs syndrome study group (IRLSSG) established the first diagnostic criteria for RLS/WED in 1995, which were updated in 2003 and later in 2014. The five essential criteria that must be met for the diagnosis of RLS/WED according to this latest update were essentially as follows [2]: A) an urge to move the legs usually but not always accompanied by, or felt to be caused by, uncomfortable and unpleasant sensations in the legs; B) the beginning or worsening of the urge to move the legs and an unpleasant sensation during periods of rest or inactivity (sitting or lying down); C) the partial or total relief of the urge to move and unpleasant sensations by movement (including walking or stretching) as long as the movement is maintained; D) the occurrence or worsening of the urge to move and unpleasant sensations during rest in the evening or at night; and E) the exclusion of other medical or behavioral conditions (which some authors have named "RLS-mimics") that can mimic these symptoms, such as myalgia, leg cramps, leg edema, venous stasis, arthritis, positional discomfort, or habitual foot tapping. These new RLS/WED criteria also included novel specifiers both for clinical course (chronic-persistent vs. intermittent) and for clinical significance of RLS/WED and merging of pediatric with adult diagnostic criteria [2]. The presence of periodic limb movements (PLMs) while awake (PLMW), during sleep (PLMS), as assessed by polysomnography or leg activity devices, responsiveness to dopaminergic treatment, family history of RLS/WED in first degree relatives, and lack of expected daytime sleepiness (due to the bad quality of sleep) were considered, as in previous versions of the IRLSSG criteria, as features supporting the diagnosis [2].

Despite the different inclusion criteria used in epidemiological studies, RLS/WED seems to be a high prevalence condition. In a

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Abbreviations

ATRN	attractin
BTBD9	BTB/POZ domain containing protein 9
cM	centiMorgans
CNV	copy number variations
CREB1	cAMP responsive element binding protein 1
DAT	dopamine transporter
DBH	dopamine-beta-hydroxylase
DMT1	divalent metal ion transporter 1
DRD	dopamine receptor
FAT2	FAT atypical cadherin
HCNR	highly conserved noncoding region
HMOX	heme-oxygenase
^{1}HMRS	proton magnetic resonance spectroscopy
HNMT	histamine-N-methyl transferase
iRLS	idiopathic restless legs syndrome
IRLSSG	International restless legs syndrome study group
KCNV2	potassium channel, subfamily V, member 2
KCTD18	BTB/POZ domain-containing protein KCTD18 or potassium channel tetramerisation domain containing 18
LCL	lymphoblastoid cell lines
LOD	score logarithm base 10 of odds
MAO A	monoamine-oxidase A
MAO B	monoamine-oxidase B
MAP2K5	mitogen-activated protein kinase 5
MAPT	microtubule associated protein tau
MPDZ	multiple PDZ domain crumbs cell polarity complex component 1
NOS1	nitric oxide synthase 1
NPL	nonparametric linkage
OR	odds-ratio
PCDHA3	protocadherin alpha
PD	Parkinson's disease
PLMs	periodic limb movements
PLMS	periodic limb movements during sleep
PLMW	periodic limb movements while awake
PTPRD	protein tyrosine phosphatase receptor type delta
RLS	restless legs syndrome
SCA	spinocerebellar ataxia
SNCA	alpha-synuclein
SGNP	stress granule and nucleolar protein gene
SKOR1 (or LBXSKOR1)	SKI family transcriptional corepressor 1
SLC1A1	solute carrier family 1, member 1
SLC1A2	solute carrier family 1, member 2
SLC11A2	solute carrier family 11 member 2
SPATSL	spermatogenesis-associated serine–rich protein 2-like
TDT	transmission disequilibrium test
TH	tyrosine-hydroxylase
TOX3	TOX high mobility group box family member 3
TRAPPC6	Trafficking protein particle complex 6 gene
VDR	vitamin D3 receptor
VNTR	variable number of tandem repeat
WED	Willis-Ekbom disease
WWC2	WW and C2 domain containing 2

recent review, Koo [1] identified two studies in Africa (both in Tanzania, with prevalence rates of 0.03 and 0.47%), 13 studies in Asia (five in South Korea, four in Japan, two in China, one in Taiwan and one in India: prevalence rates ranging from 0.9% to 12.1%); 22 studies in diverse countries of Europe, prevalences ranging from 3.2% in Turkey to 24.2% in France, 12 studies in North America (10 in the United States and two in Canada; prevalences ranging from 0.4 to 18.3%), and five studies in South America (two in Ecuador and single studies in Chile, Brazil and Argentina, prevalences ranging from 2.0% in Ecuador to 20.2% in Argentina). The differences in prevalence rates across studies should be related with the inclusion criteria used (in several studies the diagnosis of RLS was made by using only one question for the screening, in others three or four questions were used, although most studies used the current IRLSSG criteria). It must be taken into account that many patients who are diagnosed with RLS in epidemiological studies show mild symptoms only, and have not sought medical attention, but in cases of medium to high severity, RLS has an important impact on the quality of sleep.

From the etiological point of view, RLS can be classified into primary or idiopathic RLS or iRLS (it is thought that many patients with iRLS have a genetic predisposition and this diagnosis implies the exclusion or other associated conditions) and symptomatic or secondary RLS (related with anemia, uremia, iron deficiency, polyneuropathy, multiple sclerosis, several neurodegenerative diseases, etc).

The present review focuses on the main findings of the genetic aspects of iRLS, and updates previously reported information [3]. For this purpose, we describe and discuss the reported data on the frequency of positivity of family history of RLS in RLS patients, genetic anticipation, studies in twins, type of inheritance patterns in RLS, and results of the linkage studies in families with RLS, genome wide association studies (GWAS), exome sequencing studies, and case–control association studies in RLS. Because the presence of PLMs is a frequent event in RLS, we have included in this review the results of studies on RLS related genes in patients with PLMs with or without RLS. We also mention the results of case–control association studies reported for several secondary causes of RLS and for PLMs.

Search strategy

A search using PubMed Database from 1966 to July 13, 2017, was applied to identify references related with this issue. We crossed the term *"restless legs syndrome"* with "*genetics*" (306 items), "*genes*" (117 items), *"positive family history"* (65 items), *"genetic anticipation"* (six items), *"twin studies"* (seven items), *"linkage studies"* (27 items), *"genome wide association studies*" (51 items), "*exome sequencing studies*" (four items), "*case–control association studies*" (71 items) and "*polymorphisms*" (74 items), and selected the references of interest (the majority of these references were published after the year 2000).

Inheritance patterns in RLS

The majority of reports of families with RLS are consistent with a typical autosomal-dominant pattern of inheritance with variable expressivity [4–11]. Moreover, data from a large family study showed a vertical transmission suggesting a dominant mode or inheritance in 90% of the pedigrees [12]. However, pedigrees with a recessive pattern of inheritance have been reported [9,12]. Interestingly, 2.8% of families in a family report showed bilinear inheritance [12].

To date, two complex segregation analyses involving patients with RLS have investigated the mode of inheritance of this syndrome. The results of a study by Winkelmann et al. [8], who interviewed 238 RLS patients, 537 first-degree relatives, and 133 spouses, and stratified them according to age at onset (up to 30 vs over 30 y), suggest the action of a single major gene acting in an autosomal dominant fashion with a multifactorial component in the early-onset subgroup, and lack of evidence of the role of a major gene in the late-onset subgroup. In contrast, another segregation analysis reported by Mathias et al. [13], which involved 590 phenotyped subjects from 77 pedigrees (one proband per family, and first- and second-degree relatives) suggested a segregation pattern consistent with that of a single major locus, but showed lack of evidence for a major gene controlling age at onset; it raised the possibilities of a role of non-genetic causes in RLS, and that RLS should be considered a genetically complex disorder.

The possible occurrence of phenocopies, defined as individuals with a similar phenotype caused by different environmental, genetic or both determinants, is relatively frequent in RLS patients and in families with RLS [8,10,13], but such an occurrence could be explained by chance. Furthermore, some reported families seem to follow a non-mendelian pattern of inheritance because, despite an apparent autosomal dominant mode of transmission, the proportion of affected subjects (more than 50%) is higher than expected for a typical autosomal dominant inheritance. Zimprich [14] suggested the possible role of epigenetic factors in an attempt to explain both the non-mendelian features in the genetics of RLS and the presence of phenocopies in families with this disease.

Data supporting the role of genetic factors in idiopathic RLS

Family history of RLS

In classical studies, up to 60% of patients with iRLS reported a positive family history of RLS [15–19]. Similar frequencies have been reported in healthcare personnel [20], while lower frequencies, ranging from 15% to 26%, have been reported in children and adolescents [21] and in elderly people [22] from Turkey. In contrast, a study on Georgians showed 85% of positive family history of RLS [23].

Xiong et al. [12], in a family study involving 671 Canadian RLS patients found familial aggregation with a rate of 77.1%. Several reports showed a younger age at onset of RLS in patients with definite hereditary RLS as compared with RLS patients with a negative family history [18,24].

Finally, a study reported by Allen et al. [25] described, in patients diagnosed with RLS as compared with controls with no clinical history of RLS, a significantly higher frequency of history of RLS in first- and second-degree relatives. Moreover, this risk was greater for first-degree relatives of early-onset than for relatives of late-onset RLS probands.

Genetic anticipation in RLS

More typical, though not exclusive of autosomal dominant diseases, genetic anticipation is defined as an earlier age at onset of the symptoms (in many cases with an increase in the severity of symptoms as well) of a genetic disorder from one generation to successive generations. The presence of genetic anticipation supports an important role of genetic factors in a genetic disease. There are several descriptions of genetic anticipation in families with RLS. Trenkwalder et al. [26] described a large German kindred of familial RLS (four generations with 20 affected subjects), in which RLS symptoms showed decreasing age of onset in generations II-IV. Other large families with RLS showing genetic anticipation have been reported in Turkey (five generations with nine members affected) [11] and Brazil (three generations, five members affected) [9]. Finally, Lazzarini et al. [7] reported typical genetic anticipation in two out of five pedigrees with familial autosomal dominant RLS.

Findings of twin studies

The finding of a higher concordance rate in monozygotic twins than in dizygotic twins for a specific trait (that is, a higher than expected frequency of the same trait in both twins) gives support to the role of genetic factors for such a trait. The concordance rates of RLS between twins have been analyzed in a few studies.

The first study on this issue was reported by Ondo et al. [27] who found a high concordance rate (83.3%) for 12 monozygotic twins diagnosed with RLS, although authors did not include data from dizygotic twins in their study. Desai et al. [28], in an analysis of a questionnaire answered by 933 monozygotic and 1004 dizygotic twins from a national volunteer twin register, found a significantly higher concordance rates for monozygotic than for dizygotic twins (61% vs 45%, $p < 0.01$) for RLS symptoms. Xiong et al. [29], using a structured questionnaire answered by 272 twin pairs (140 monozygotic and 132 dizygotic) also found a higher concordance rate for monozygotic than for dizygotic twins (53.7% vs 15.4%).

Champion et al. [30] analyzed the concordance rates for growing pains (consisting of a mild to severe pain located primarily in the legs, more intense in the evening and during sleep, which has been related with RLS) in 1843 twin pairs, and found a higher concordance for this trait in monozygotic than in dizygotic twins (85% vs 36%). Twenty-three percent of the twins with growing pains met RLS criteria, while only 8% of those without growing pains did not, and 19% of twins with concordance for growing pains compared with 2% discordant for growing pains met diagnostic criteria for RLS. These data suggest a genetic relationship between RLS and growing pains.

Linkage studies in families with RLS

Linkage studies (defined as those which examine physical segments of the genome associated with certain traits) in families with RLS identified, to date, a minimum of eight susceptibility loci for familial RLS. However, a definitive identification of the responsible genes has not yet been established. Table 1 summarizes the susceptibility loci described to date for familial RLS.

RLS1

Despite the fact that most families with RLS show a typical autosomal dominant inheritance pattern, the first mapping of a locus conferring susceptibility to RLS was performed using a genome wide scan in a large French-Canadian family with autosomal recessive RLS, in which a significant linkage on chromosome 12q was found for a series of adjacent microsatellite markers with a maximum 2-point LOD score (logarithm base 10 of odds) of 3.42 at recombination fraction 0.05, and a final LOD score of 3.59 after multipoint linkage calculations [31]. A 14.71 centiMorgans (cM) region between D12S1044 and D12S78 was established as the genetic interval for the RLS-predisposing gene in a haplotype analysis [31].

Other authors failed to confirm this susceptibility locus for RLS on chromosome 12q in families from other populations [19,32,33], and some authors noted that an autosomal recessive model with very high allele frequency was used by the single French Canadian family [8]. However, Desautels et al. [34], after analyzing 276 individuals from 19 families (five of them with a confirmed linkage to chromosome 12q) confirmed the presence of the major locus for RLS-susceptibility designated as *RLS1* at chromosome 12q. These

Table 1
Studies on susceptibility loci for familial RLS.

Gene	Chromosome	Gene ID/MIM	Inheritance	Study	Country	Main findings
RLS1	12q12q21	192142/102300	Autosomal recessive	Desautels et al., 2001 [31]	Canada	Description of *RLS1* locus in a large French-Canadian family in a 14.71 cM region between D12S1044 and D12S78
				Desautels et al., 2005 [34]	Canada	Confirmation of *RLS1* locus in a study of 276 individuals from 19 families (five with a confirmed linkage to chromosome 12q)
				Winkelmann et al., 2006 [35]	Germany	Confirmation of *RLS1* locus in a study involving 70 affected family members from 12 families.
				Vogl et al., 2006 [19]	Italy	Lack of confirmation of *RLS1* locus in a study involving 530 adults from an isolated population in the South Tyrolean Alps
				Kock et al., 2002 [32]	Italy	Lack of confirmation of *RLS1* locus in two large South Tyrolean families with autosomal dominant RLS (one with nine out of 26 subjects, and the other with 10 out of 44 affected with RLS)
				Lohmann-Hedrich et al., 2008 [33]	Germany	Lack of confirmation of *RLS1* locus in a four-generational German RLS family with 37 family members including 15 cases affected with RLS
RLS2	14q13-q21	450097/608831	Autosomal dominant	Bonati et al. [41]	Italy	Description of *RLS2* locus in a large three-generational Italian family composed of 30 members in a critical region span 9.1 cM, between markers D14S70 and D14S1068
				Levchenko et al., 2004 [42]	Canada	Confirmation of *RLS2* locus in a study involving 14 large French Canadian families with many affected members at D14S1068 region
				Kemlink et al., 2007 [43]	Europe[a]	Confirmation of *RLS2* locus in a study involving 159 European RLS trios at markers D14S1014 and D14S1017
				Vogl et al., 2006 [19]	Italy	Lack of confirmation of *RLS2* locus in a study involving 530 adults from an isolated population in the South Tyrolean Alps
				Winkelmann et al., 2006 [35]	Germany	Lack of confirmation of *RLS2* locus in a study involving 70 affected family members from 12 families.
				Lohmann-Hedrich et al., 2008 [33]	Germany	Lack of confirmation of *RLS2* locus in a four-generational German RLS family with 37 family members including 15 cases affected with RLS
RLS3	9p24-p22	100188812/610438	Autosomal dominant	Chen et al., 2004 [44]	USA	Description of *RLS3* locus in 15 large and extended multiplex pedigrees consisting of 453 subjects (134 affected with RLS)
				Liebetanz et al., 2006 [46]	Germany	Confirmation of *RLS3* locus in a large family of Bavarian origin (16 subjects affected with RLS, nine with early-onset RLS, nine unaffected and five with uncertain status) using transmission disequilibrium tests and affected-only linkage analysis. Narrowing of the region containing the *RLS3* locus to 11.1 cM (16.6 Mbp) for the marker D9S1810
				Winkelmann et al., 2006 [35]	Germany	Lack of confirmation of *RLS3* locus in a study involving 70 affected family members from 12 families.
				Vogl et al., 2006 [19]	Italy	Lack of confirmation of *RLS3* locus in a study involving 530 adults from an isolated population in the South Tyrolean Alps
				Kemlink et al., 2007 [43]	Europe[a]	Lack of confirmation of *RLS2* locus in a study involving 159 European RLS trios, with the exception of marginal association of certain markers in two subsets of patients
RLS3*	9p		Autosomal dominant	Lohmann-Hedrich et al., 2008 [33]	Germany	Description of a haplotype flanked by D9S974 and D9S1118 in a 9.9-Mb centromeric region to *RLS3* in 12 patients (11 of them carried a common haplotype extending telomeric to D9S2189) in a four-generational German RLS family with 37 family members including 15 cases affected with RLS
RLS4	2q33	100188813/610439	Autosomal dominant	Pichler et al., 2006 [49]	Italy	Description of *RLS4* locus in a study involving 37 RLS patients belonging to a population isolate (530 individuals) from the South Tyrol in Italy, with linkage between markers D2S311 and D2S317 (11.7 cM)
				Lohmann-Hedrich et al., 2008 [33]	Germany	Lack of confirmation of *RLS4* locus in a four-generational German RLS family with 37 family members including 15 cases affected with RLS

(*continued on next page*)

Table 1 (*continued*)

Gene	Chromosome	Gene ID/MIM	Inheritance	Study	Country	Main findings
RLS5	20p13	100188839/611242	Autosomal dominant	Levchenko et al., 2006 [52]	Canada	Description of *RLS5* locus in a French Canadian pedigree with 17 affected individuals at D20S849 marker
				Sas et al., 2010 [53]	Netherlands	Confirmation of *RLS5* locus in a large multigenerational Dutch family with early-onset RLS and restriction of the critical region
				Lohmann-Hedrich et al., 2008 [33]	Germany	Lack of confirmation of *RLS5* locus in a four-generational German RLS family with 37 family members including 15 cases affected with RLS
Other	19p13		Autosomal dominant	Kemlink et al., 2008 [54]	Italy	Description of a new locus between markers rs754292 and rs273265 in a large RLS family of Italian origin with 12 affected members in three generations, and confirmation in a family-based association study in a set of 159 trios of European origin.
				Skehan et al., 2012 [55]	Ireland	Confirmation of linkage at 19p13 chromosome (maximum form marker D19S878), and definition of a region of 6.57 cM on chromosome 19p13.3 in a Irish RLS pedigree with 11 affected members,
	16p12.1		Autosomal dominant	Levchenko et al., 2009 [56]	Canada	Description of linkage on chromosome 16p12.1 (10 markers) in a French-Canadian pedigree
	13q32.3–33.2		Autosomal dominant	Balaban et al., 2012 [57]	Turkey	Description of linkage on chromosome 13q32.3 –33.2 in nine of 10 family members affected with RLS and two unaffected subjects belonging to a RLS family from Turkey

cM, centimorgans; ID, identity; MIM, mendelian inheritance in man; RLS, restless legs syndrome.
[a] Germany, Czech Republic, Finland, Italy, Netherlands, Austria, France, Greece.

authors suggested the involvement of at least one additional locus in the origin of RLS, and found that the LOD scores were higher under a recessive model. Moreover, further evidence for linkage of RLS on *RLS1* locus at chromosome 12q was obtained by other authors [35].

After a description of *RLS1*, several studies addressed the possible relationship between genes mapped at chromosome 12q with the risk for RLS. The neurotensin gene (MIM 162650, gene ID 4922), which is important in the modulation of the dopaminergic neurotransmission, has been the focus of two scale gene-based case–control and family-based genetic association studies, which failed to show such an association [36,37]. Another case–control association study did not show association between the *solute carrier family 11 member 2* or *divalent metal ion transporter 1* gene (*DMT1* or *SLC11A2*; MIM 600423, gene ID 4891), which is very important as an iron transporter, and the risk for RLS [38].

Winkelmann et al. [39] reported association between the rs7977109 single nucleotide polymorphism (SNP) in the *neuronal nitric oxide synthase* (*NOS1*) gene and the risk for RLS, with an odds-ratio (95% confidence intervals) (OR (95%CI) of 0.76 (0.64–0.90)), as the result of a three-stage association study, which included an explorative study, a replication study, and a high-density mapping (with correction for multiple testing), in two Caucasian RLS case–control samples from a total of 918 independent cases and controls. They screened 1536 SNPs in 366 genes in a 21 Mb region encompassing the *RLS1* critical region on chromosome 12 in 367 cases and 367 controls in the explorative study, and genotyped the most significant SNPs found at that initial stage in a replication study involving 551 cases and 551 controls. In contrast, a replication study involving 205 RLS patients and 328 healthy controls in a Spanish Caucasian population did not confirm this association, and showed a lack of association between the rs693534 SNP in the *NOS1* gene and the risk for RLS as well [40].

RLS2

The first evidence of linkage to a locus in familial autosomal dominant RLS was reported by Bonati et al. [41], who described linkage to a critical region spanning 9.1 cM, between markers D14S70 and D14S1068, with the maximum two-point LOD score value (3.23) for marker D14S288, on chromosome 14q13-21 in a large three-generation Italian family composed of 30 members. This was designated as *RLS2* locus.

The existence of the *RLS2* locus was confirmed by Levchenko et al. [42] in 14 large French Canadian families with many affected members. After genotyping four microsatellite markers (D14S70, D14S301, D14S266, and D14S1068) they found that all the maximum LOD scores peaked at the fourth marker, D14S1068 (LOD 1.46 for the autosomal dominant model, LOD 2.51 for the autosomal recessive model, and nonparametric linkage (NPL) 5.31 for the nonparametric analysis).

A further family-based association study involving 159 European RLS trios (a trio consists of a patient diagnosed with RLS and their parents) found a significant association with a haplotype formed by markers D14S1014 and D14S1017 on chromosome 14q13-21 as well [43].

In contrast, other studies failed to find linkage of *RLS2* with RLS in other populations [19,33].

RLS3

The locus designated as *RLS3* was reported in a study by Chen et al. [44] involving 15 large and extended multiplex pedigrees with autosomal mode of inheritance, and consisting of 453 subjects, 134 of them affected with RLS. This new susceptibility locus for RLS on chromosome 9p22-p24 had a multipoint NPL score of 3.22, it was validated after model-based linkage analysis in two of these families, with a two-point LOD score of 3.77 (and a multipoint LOD score of 3.91), and it was defined to a critical interval by further fine mapping. In addition, the authors looked for (although they did not find evidence of) the presence of pathogenic mutations in three possible candidate genes at this RLS locus: *potassium channel, subfamily V, member 2* gene (*KCNV2*, MIM 607604; gene ID 169522; this gene encodes a potassium-channel subunit that mediates voltage-dependent potassium-ion permeability of excitable

membranes), *multiple PDZ domain crumbs cell polarity complex component 1* gene (*MPDZ* or *MUPP1*; MIM 603785, gene ID 8777; this gene is related with serotonergic transmission), and *solute carrier family 1, member 1* (*SLC1A1* or *EAAC1*, MIM 133550, gene ID, 6505; this gene has an important role as transporter of L-glutamate and L- and D-aspartate both in neurons and in epithelium).

Despite criticisms raised by Ray & Weeks [45] who concluded that there was lack of convincing evidence of linkage for RLS with chromosome 9p, other authors, using an analysis of transmission disequilibrium tests (TDTs) and affected-only linkage analysis in a large family of Bavarian origin, not only confirmed association with this locus, but they were also able to narrow the region containing the autosomal dominant *RLS3* locus to 11.1 cM (16.6 Mbp) for the marker D9S1810 [46].

Kemlink et al. [43], in a family-based association study of 159 RLS European trios, found no significant association in the samples of all the families, and they found only marginally significant associations with a haplotype involving markers D9S1846-D9S171 in a subset of South European trios and with a haplotype at D9S156-D9S157 in a subset of Central European trios on this locus. In addition, a study by Vogl et al. [19], in a South Tyrolean population, failed to find linkage to the *RLS3* locus.

Finally, a linkage analysis of a four-generational German autosomal dominant RLS family with 37 family members including 15 affected cases, with disease onset mainly in childhood or adolescence, excluded linkage to the *RLS1*, *RLS2*, *RLS4*, and *RLS5* loci [33]. However, in the same study, a likely new *RLS* gene locus on chromosome 9p with a maximum LOD score of 3.60 generated by a model-based multipoint linkage analysis was identified, and was named *RLS3** by the authors. All the 12 investigated patients shared a haplotype flanked by D9S974 and D9S1118 in a 9.9-Mb region centromeric to *RLS3*, and 11 of them carried a common haplotype extending telomeric to D9S2189 (located within *RLS3* as well) [33].

With regard to studies on candidate genes in the region of the *RLS3* locus, in a GWAS reported by Schormair et al. [47], involving 2458 affected individuals and 4749 controls from Germany, Austria, Czechia and Canada, an association was identified between risk of RLS and two SNPs (rs4626664 and rs1975197) in the *protein tyrosine phosphatase receptor type delta* gene (*PTPRD*; chromosome 9p23-24; MIM 601598, gene ID 5789; protein tyrosine phosphatases – PTPs – which act as signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation) (link http://www.ncbi.nlm.nih.gov/gene/5789). A replication study including a family-based study (144 family members from 15 families) and a case–control association study (189 RLS patients and 560 controls) confirmed this association [48].

RLS4

The description of the locus designated as *RLS4* appeared in a genome wide linkage study involving 37 RLS patients belonging to a population isolate (530 individuals) from the South Tyrol in Italy by Pichler et al. [49]. The *RLS4* locus was found using both nonparametric and parametric analyses, on chromosome 2q33, with nominal evidence of linkage on chromosomes 5p and 17p. There was significant evidence of linkage between markers D2S311 and D2S317 (11.7 cM), with a nonparametric LOD score of 5.5. A common 7-marker haplotype in the chromosome 2q33 region was found to be identical in the 15 affected members from three families which descended from a common founder couple (10 generations before). A two-point linkage analysis gave a maximum LOD score of 4.1 at theta = 0.0 at the marker D2S2242.

Co-occurrence of heterozygous *PARKIN* mutations in some patients carrying the *RLS4* haplotype, which was associated with anticipation of RLS onset age of 16.9 y, but not with disease severity, was reported by the same group [50].

Finally, this group restricted the candidate region to 46.9 Kb over the exons 10–13 of the *spermatogenesis-associated serine-rich protein 2-like* or *stress granule and nucleolar protein* gene (*SPATS2L*, *SGNP*; MIM 613817, gene ID 26010) and the potassium channel-related gene *KCTD18* (*BTB/POZ domain-containing protein KCTD18*; potassium *channel tetramerisation domain containing 18*; Gene ID 130535) using fine-mapping of the *RLS4* locus in the affected members of the three linked families with a next generation sequencing approach [51]. In contrast, Lohmann-Hedrich et al. [33] found no linkage to the *RLS4* locus in the multigenerational German family they studied.

RLS5

The locus designated as *RLS5* was found in a 5.2-Mb candidate region on chromosome 20p13, with a maximum multipoint LOD score of 3.86 at D20S849 marker, in a genome wide linkage analysis performed on a French Canadian pedigree segregating autosomal dominant RLS with 17 affected individuals [52]. This locus was thereafter confirmed in a large multigenerational Dutch family with early-onset RLS (average age at onset 18 y), in which the critical region was reduced from 5.2 to 4.5 megabases with a maximum multipoint LOD score of 3.02 [53]. In contrast, Lohmann-Hedrich et al. [33] found no linkage to *RLS5* locus in their multigenerational German family.

Other described RLS loci

Two genome-wide linkage analyses have shown significant linkage on chromosome 19p13:

A) A study by Kemlink et al. [54] using 5861 single nucleotide polymorphisms on a large RLS family of Italian origin with 12 affected members over three generations was performed under an autosomal-dominant model with complete penetrance. This study showed a maximum multipoint LOD score of 2.61 between markers rs754292 and rs273265. These authors replicated this finding in a family-based association study in a set of 159 trios of European origin.
B) A study by Skehan et al. [55] on an Irish autosomal dominant RLS pedigree with 11 affected members found this linkage for a series of microsatellite markers, with a maximum two-point LOD score of 3.59 at $\theta = 0.0$ for marker D19S878. In addition, in this study the authors defined a genetic region of 6.57 cM on chromosome 19p13.3, corresponding to an interval of 2.5 Mb, using recombination events and identification by haplotype analysis.

A study by Levchenko et al. [56] on a French-Canadian pedigree with autosomal dominant RLS found linkage with chromosome 16p12.1 which spanned 1.18 megabases with maximum multipoint LOD scores of 3.5 from the total of 10 markers.

Finally, a whole genome linkage analysis in nine family members affected and two unaffected with RLS, belonging to an RLS family from the eastern part of central Turkey with 10 patients suffering from this syndrome, showed linkage on chromosome 13q32.3–33.2 with a theoretical maximum LOD score of 3.29 [57].

Genome-wide association studies (GWAS)

GWAS have shown significant association between RLS and variants of several genes:

PTPRD (protein tyrosine phosphatase receptor type delta, chromosome 9p23-24, MIM 601598, gene ID 5789)

Described above, in the section on the *RLS3* locus, are the initial data on GWAS related with this gene.

Kim et al. [58], in a case–control association study involving 320 RLS patients and 320 healthy controls of Korean origin, described a significant interaction between the rs4626664 SNP in the *PTPRD* and rs3923809 SNP in the *BTBD9* genes, with an OR = 2.05 (P < 0.0001) using the additive model, 1.80 (P = 0.002) using the dominant model and 2.47 (P = 0.004) using the recessive model. However, the rs4626664 and rs1975197 SNPs in the *PTPRD* gene did not show, individually, association with the risk for RLS.

Moore et al. [59] reported that increased risk for periodic limb movements (PLMs), which is associated with RLS, was related with the rs1975197 SNP in *the PTPRD* gene (OR = 1.31, P = 6.3×10^{-3}), in a population of 1090 subjects from the Wisconsin sleep cohort.

Although Schormair et al. [60] found no association between several SNPs in the *PTPRD* gene and the risk for RLS in end stage renal disease, Lin et al. [61] reported a modest association between the *PTPRD* variant rs4626664 and uremic RLS (OR = 1.52, 95% CI = 1.03–2.23, P = 0.03).

BTB/POZ domain containing protein 9 (BTBD9, MIM 611237, gene ID 114781, RLS6-MIM 611185, gene ID 100502312; chromosome 6p21)

This gene encodes a BTB/POZ domain-containing protein, which is known to be involved in protein–protein interactions (link http://www.ncbi.nlm.nih.gov/gene/114781).

The first description of association between the rs9296249 and rs9357271 SNPs in the *BTBD9* gene (related with the *RLS6* locus) and the risk of RLS was reported by Winkelmann et al. [62] in a GWAS involving 401 patients with familial RLS and 1644 controls, this association being confirmed in two independent replication studies involving 903/891 and 255/287 RLS patients/controls, respectively.

Stefansson et al. [63] described association between the rs3923809A allele in the *BTBD9* gene with the specific risk for PLMs in RLS patients in a study involving two independent samples of 306 and 123 Icelandic subjects, and another sample of 188 patients from the US, respectively (OR = 1.9). In contrast, lack of association of the rs3923809A allele with the risk for RLS was observed in 229 patients with RLS without PLMs. The authors suggested that they had identified a genetic determinant of PLMs.

The association between the rs3923809A allele with the risk for PLMs was confirmed recently by Moore et al. [59] in a sample of 1090 subjects from the Wisconsin sleep cohort, by Winkelmann et al. [64] in 2356 white male participants in the osteoporotic fractures in men sleep study cohort (OR = 1.43, 95%CI = 1.26–1.63), and by Haba-Rubio et al. [65] in a population study involving 2162 subjects. In addition, Winkelmann et al. [64] described association of PLMs with the rs9357271T allele in the same gene (OR = 1.38, 95%CI = 1.20–1.58).

A study of 244 patients with RLS (121 with sporadic RLS and 123 familial probands) and 497 controls, confirmed an association between the risk for RLS and the rs9296249 and rs3923809 SNPs (the latter was found only for familial probands), and found an association with rs4714156 and rs9357271 [66]. Another study involving 649 RLS patients and 1230 controls from three European populations (Czech Republic, Austria, and Finland), also replicated the association between RLS and rs3923809 in the *BTBD9* gene, both in familial and in sporadic cases [67].

The association between the rs9357271 SNP in the *BTBD9* gene was replicated in a population based case–control association study involving 189 RLS patients and 560 controls from the US, with an OR = 1.59 but not in a family-based study involving 38 RLS families from the US [68].

As we commented previously, Kim et al. [58] reported interaction between the rs3923809 SNP in the *BTBD9* genes and the rs4626664 SNP in the *PTPRD* genes in Korean individuals, although neither rs3923809 nor rs9296249 nor rs9357271 in the *BTBD9* gene showed, individually, any association with the risk for RLS.

Schormair et al. [60] described an association of the 3923809 SNPs in the gene *BTBD9* with the risk for RLS in end stage renal disease, but this finding was not confirmed by Lin et al. [61].

Interestingly, *BTBD9* gene dysfunction has been found in two experimental models of RLS. The loss of the Drosophila homolog *CG1826* (*dBTBD9*) gene causes disruption of sleep with concomitant increases in waking and motor activity, thus resembling RLS symptoms [69]. *BTBD9* mutant mice showed a clinical phenotype similar to that in RLS patients, including motor restlessness, sensory alterations probably limited to the rest phase (which improved with ropinirole), and decreased sleep and increased wake times during the rest phase which, in addition, showed altered serum iron levels and monoamine neurotransmitter systems [70].

MEIS1 (MIM 601739, gene ID 4211, RLS7-MIM 612853, gene ID 100302561, chromosome 2p14p13)

The *MEIS1* gene encodes a homeobox protein belonging to the 'three amino acid loop extension' (TALE) family of homeodomain-containing proteins. Homeobox genes are very important in normal development (link http://www.ncbi.nlm.nih.gov/gene/4211). Interestingly, a reduction of MEIS1 expression in human cells cultured under iron-deficiency conditions has been described, suggesting a link between *MEIS1* gene and iron metabolism [71], with brain and iron insufficiency (together with dopaminergic dysfunction) being one of the main pathophysiological mechanisms for RLS [72].

The association between the *MEIS1* gene (related with the *RLS7* locus) and the risk for RLS was first reported in a GWAS by Winkelmann et al. (details of this study are described in the section on *BTBD9*) [62] and consisted of the association of the rs2300478G allele (OR = 1.74, 95%CI = 1.57–1.92) with this risk.

A study of 244 patients with RLS (121 with sporadic RLS and 123 familial probands) and 497 controls, confirmed a strong association between the risk for RLS and the rs12469063G allele (more marked in familial probands), and a milder association between the rs6710341 SNP and RLS risk in familial probands [66].

A study by Kemlink et al. [67] involving 649 RLS patients and 1230 controls from three European countries (Czech Republic, Austria, and Finland), replicated the association between RLS and rs2300478 in the *MEIS1* gene as well, with an OR of 1.47, both in familial cases or in the combined sample of familial and sporadic cases. Another group also confirmed the association between RLS risk and the rs2300478 SNP in the gene *MEIS1* in a population based case–control association study involving 189 RLS patients and 560 controls from the US, with an OR = 1.65, but failed to confirm association in a family-based study involving 38 pedigrees with RLS [68]. However, the association between this SNP and the risk for RLS was not found in a case–control association study involving 320 RLS patients and 320 healthy controls of Korean origin [58].

Moore et al. [59] described an association between the rs12469063G and rs2300478G alleles, and a lack of association between the rs6710341G allele and the risk for PLMs in their population of 1090 subjects from the Wisconsin sleep cohort. Winkelmann et al. [64] confirmed an association between the rs2300478G allele and PLMs in 2356 white male participants in the osteoporotic fractures in men sleep study cohort (OR = 1.31, 95%

CI = 1.14–1.51), and Haba-Rubio et al. [65] also confirmed this association in their population study.

Vilariño-Güell et al. [73] identified an Arg272-to-His (p.R272H) substitution in the *MEIS1* gene in one of 71 familial probands with RLS, but did not find additional mutations in 378 additional RLS patients and in 853 unrelated controls. This mutation was segregated with RLS in three additional affected family members, though it was also found in one out of three unaffected members. The phenotype in this family was highly variable, with different ages at onset and varied severity. This group did not identify any novel variants in the *BTBD9* gene in their 71 familial probands.

Xiong et al. [74] did not identify any causative coding, or exon–intron junction, mutations after sequencing the 13 *MEIS1* exons and their splice junctions in 285 RLS familial probands with confirmed clinical diagnosis of RLS. However, in an analysis of brain samples from 28 RLS patients and 140 controls, they found a risk haplotype (rs12469063/2300478 GT/GG 43% vs 25%, p = 0.0095). This group also found in the brain tissues and in lymphoblastoid cell lines (LCLs) from RLS patients homozygous for the intronic RLS risk haplotype both a significant decrease in MEIS1 mRNA expression (assessed by quantitative real-time polymerase chain reaction) and decreased MEIS1 protein, as compared with those individuals homozygous for the non-risk haplotype. These data suggest that reduced expression of the *MEIS1* gene could predispose to RLS.

Schulte et al. [75] described three novel variants in a discovery series composed of 188 RLS patients with the rs2300478 GT/GG haplotype (they also analyzed two replication samples including 735 German RLS patients and 735 German controls, and 279 Czech RLS patients), and confirmed the presence of the variant p.R272H in two of their RLS patients, through a screening of the coding regions and exon-intron boundaries of *MEIS1* for variants. Furthermore, this group genotyped 3000 RLS cases and 3000 controls, in search of rare variants of the *MEIS1* gene, and reported that a significant total genetic burden of rare *MEIS1* variants existed in RLS patients which were linked to a MEIS1 loss of function.

Schormair et al. [60] described an association between some SNPs in the *MEIS1* gene (rs12469063 and rs2300478) and the risk for RLS in end stage renal disease, a finding that was not confirmed by Lin et al. [61].

Spieler et al. [76] analyzed the role of highly conserved non-coding regions (HCNRs) in the *MEIS1* gene (which can function as cis-regulatory modules), for allele-dependent enhancer activity in zebrafish and mice. They found, in the murine model, that the risk allele of the lead SNP rs12469063 reduced enhancer activity in the Meis1 expression domain at the embryonic ganglionic eminences. CREB1 (cAMP responsive element binding protein 1) bound this enhancer and rs12469063 affected its binding *in vitro*. Moreover, heterozygous Meis1-deficient mice exhibited hyperactivity, thus resembling the RLS phenotype.

Finally, a recent study by Salminen et al. [77] involving *Meis1* knockout mice showed in this animal model a pattern of circadian hyperactivity, compatible with that of human RLS, and a replicable prepulse inhibition deficit with hyposensitivity to the prepulse inhibition deficit reducing effect of D2/D3 dopamine receptor agonists, which suggests a role of *Meis1* gene in the dopaminergic system.

MAP2K5/SKOR1 (MIM 602520/611273, gene ID 5607/390598; chromosome 15q23)

The *mitogen-activated protein kinase 5/SKI family transcriptional corepressor 1* (*MAP2K5/SKOR1* or *MAP2K5/LBXCOR1*) gene encodes a dual specificity protein kinase that belongs to the MAP kinase family. This kinase specifically interacts with and activates MAPK7/ERK5. This kinase itself can be phosphorylated and activated by MAP3K3/MEKK3, as well as by atypical protein kinase C isoforms (aPKCs). The signal cascade mediated by this kinase is involved in growth factor stimulated cell proliferation and muscle cell differentiation (link http://www.ncbi.nlm.nih.gov/gene/5607).

Winklemann et al. [62], in their GWAS study (described in detail in the *BTBD9* section) found a significant association between several variants in the *MAP2K5/SKOR1* gene (rs12593813, rs11635424, rs469954, rs3784709, rs1026732, and rs6494696, with OR ranging from 1.50 to 1.53) and the risk for RLS. Kemlink et al. [67] found an association between rs11635424, rs3784709, rs1026732, and rs65890259 SNPs in this gene and the risk for RLS in their study involving three European populations. Yang et al. [68] found an association of rs1026732 with RLS risk both in a family-based and in a case–control association study, while Kim et al. [58] found a lack of association of this SNP with the risk for RLS in a case–control association study involving 320 RLS patients and 320 healthy controls of Korean origin. Finally, Vilariño-Güell et al. [66] found a lack of association of RLS with four SNPs in the *MAP2K5/SKOR1* gene (rs11635424, rs884202, rs3784709, and rs6494696) in a study involving 244 patients with RLS, including 123 familial probands.

Moore et al. [59] described an association between rs6494696G allele and the risk for PLMs (OR = 1.24) in their population study, while Winkelmann et al. [64] found an association between rs1026732 and the risk for PLMs in their cohort (OR = 1.16, 95% CI = 1.02–1.31).

No SNPs in the gene *MAP2K5/SKOR1* were found to be associated with the risk for RLS in end stage renal disease in two studies [60,61].

Other genes and loci

Winkelman et al. [78], in a GWAS for RLS involving 922 RLS patients and 1526 controls, performed an analysis of 301.406 SNPs, followed by a replication study of 76 candidate SNPs in 3935 RLS patients and 5754 controls of European origin, and identified six RLS susceptibility loci of genome-wide significance. Four of them had been previously described and the other two were novel: an intergenic region on chromosome 2p14 (rs6747972, OR = 1.23) and a locus on 16q12.1 (rs3104767, OR = 1.35) in a linkage disequilibrium block containing the 59-end of *TOX3* and the adjacent non-coding RNA *BC034767*.

The *TOX high mobility group box family member 3* gene (*TOX3*, MIM 611416, gene ID 27324, chromosome 16q12.1) encodes a protein which contains an HMG-box, is involved in bending and unwinding of DNA and alteration of chromatin structure, and seems to play a role in the development of the nervous system. One variant of this gene has been associated with the risk for breast cancer (link http://www.ncbi.nlm.nih.gov/gene/27324).

Moore et al. [59] showed an association for the rs3104767G, rs3104774G, and rs3104788T variants in the *TOX3/BC034767* genes with the risk for PLMs (OR between 1.27 and 1.32). Haba-Rubio et al. [65] confirmed association between rs3104788 SNP and the risk for PLMs.

Finally, Lin et al. [61] found a non-significant trend for association of the rs3104767 SNP to the with the risk for RLS in end stage renal disease (OR = 1.74, 95%CI = 0.97–3.11, P = 0.06).

Exome sequencing studies

Whole exome sequencing is a very efficient (and less costly than GWAS) technique for a selective sequencing of all the expressed genes in a genome (known as the exome). To date, only three whole exome sequencing studies have been reported in familial RLS.

The first study, reported by Weissbach et al. [79], which was performed on a German family with autosomal dominantly inherited RLS in seven definitely and two possibly affected members, identified three novel missense and one splice site variant in the *protocadherin alpha 3* (*PCDHA3*, chromosome 5q31, MIM 603130), *WW and C2 domain containing 2* (*WWC2*; chromosome 4q35.1, gene ID 80014), *attractin* (*ATRN*, chromosome 20p13, MIM 603130) and *FAT atypical cadherin* (*FAT2*, chromosome 5q33.1, MIM 604269) genes that segregated with RLS in the studied family. The most plausible candidate, the *PCDHA3* gene containing four exons, (related with the *RLS8* locus, MIM 615197), was sequenced in 64 unrelated RLS patients and 250 controls, and the analysis revealed three additional rare missense variants (frequency <1%) of unknown pathogenicity in two patients and one control.

The second study, reported by Gan-Or et al. [80], was performed on seven RLS families, focusing on six known genetic loci (*MEIS1*, *BTBD9*, *PTPRD*, *MAP2K5/SKOR1*, *TOX3*, and rs6747972). In four of these families the p.E111A and the promoter c.-7C>T GLO1 variants (both within the *BTBD9* locus) were identified as co-segregated with the disease. These variants were genotyped in two case–control cohorts (French-Canadian and US) involving a total of 627 RLS patients and 410 controls, and in a familial cohort composed of 718 subjects, the GLO1 p.E111A variant being associated with RLS in both case–control cohorts (OR = 1.28, p = 0.009, for the combined analysis), but not in the familial cohort. They also found a strong association of the *BTBD* rs9357271 SNP with the risk for RLS, and a lack of association of GLO1 p.E111A variant with conditional haplotype analysis controlling for the effect of *BTBD* rs9357271. Finally, they demonstrated a lack of co-segregation with RLS of several variants in the *SKOR1* (p.W200R and p.A672V) and *PTPRD* (p.R995C, p.Q447E, p.T781A, p.Q447E, and c.551-4C > G) genes.

Lastly, Aridon et al. [81], in an exome sequencing study performed in their previously described *RLS2* family, described 15 variations in the 14q region, and found a c.485G > A transition of the *trafficking protein particle complex 6B* gene (*TRAPPC6B*; gene identity 122553; MIM 610397; encoding a component of TRAPP complexes involved in vesicle transport) which segregated with the *RLS2* haplotype and was absent in 200 local controls.

Studies on candidate genes

Genes associated with dopaminergic neurotransmission

Taking into account the possible role of the dopaminergic dysfunction in the pathogenesis of RLS as one of the main pathogenetic theories of RLS [72], it seems reasonable to investigate genes involved in dopaminergic neurotransmission in patients with RLS as possible candidates for the risk of this disease.

The first case–control association study on this issue was made by Desautels et al. [82], who found a lack of association of the most common variants in the *DRD1*, *DRD2*, *DRD3*, *DRD4*, *DRD5*, *dopamine transporter* (*DAT*), *tyrosine-hydroxylase* (*TH*), and *dopamine-beta-hydroxylase* (*DBH*) genes, and risk for RLS in an association study involving 92 RLS patients and 182 controls. Our group confirmed lack of association between the rs6280 SNP in the *DRD3* gene and the risk for RLS in a case–control association study involving 206 RLS patients and 324 controls, together with the lack of relation of these SNPs with the positive family history of RLS and with the response of RLS symptoms to dopaminergic drugs [83]. The genotype and allele frequencies of the most common SNPs in the *DRD1*, *DRD2*, *DRD3*, and *DRD4* genes were similar in a series of 96 Korean schizophrenic patients with and 94 without RLS symptoms induced by neuroleptic drugs [84].

The possible role for the *monoamine-oxidase A* (*MAOA*) and B (*MAOB*) genes A in the risk for RLS was investigated in a population-study involving 96 RLS patients and 200 controls by Desautels et al. [85]. Although the authors did not find association between variable numbers of tandem repeat (VNTR) polymorphism in the *MAOA* gene and GT polymorphism in the *MAOB* gene, they concluded that high activity allele of the *MAOA* gene might represent a modifying factor involved in the severity of RLS manifestations exclusively in females. The genotype and allele frequencies of the most common SNPs in *MAOA* and *MAOB* genes did not differ significantly between a group of 96 Korean schizophrenic patients with and another 94 without RLS symptoms induced by neuroleptic drugs [86].

Li et al. [87] found no association between 16 candidate genes related to dopaminergic transmission and iron metabolism with RLS risk in a Han RLS family.

As we commented previously in the subheading corresponding to the *RLS1* locus, two scale gene-based case–control and family-based genetic association studies did not find association between neurotensin gene and the risk of developing RLS [36,37]. Finally, a study involving 298 RLS patients and 135 controls found a lack of association between the Val(158)Met (rs4680) polymorphism in the *catechol-orthomethyl-transferase* (*COMT*) gene and the risk for RLS [88].

Heme-oxygenases 1 and 2

Because of the important role of iron deficiency in the pathophysiology of RLS, the fact that heme-oxygenases (HMOX) are related with iron metabolism (they intervene in the degradation of heme to biliverdin, carbon monoxide, and free iron), and the possible relation of several SNPs in the *HMOX1* (chromosome 22q13.1, MIM 141250, gene ID 3162) and *HMOX2* (chromosome 16p13.3, MIM 141251, gene ID 3163) genes with the risk for Parkinson's disease (PD), our group addressed a case–control association study (205 RLS patients/405 controls) on the possible role of the *HMOX1* rs2071746, *HMOX1* rs2071747, *HMOX2* rs2270363, and *HMOX2* rs1051308 SNPs, and the presence of copy number variations (CNVs) of these genes in the risk for RLS [89]. *HMOX1* rs2071746TT genotype and *HMOX1* rs2071746T allele were marginally associated with decreased risk for RLS (OR = 0.61, 95% CI = 0.36–1.01, and OR = 0.71, 95%CI = 0.55–0.94, respectively for genotype and allele frequency), although none of the four *HMOX* SNPs mentioned were related with age at onset or severity of RLS, family history of RLS, serum ferritin levels, or response to dopaminergic agonist, clonazepam or GABAergic drugs.

Vitamin D3 receptor genes

Because several recent works suggest a possible role of vitamin D deficiency in the etiology of RLS, with decreased serum 25-hydroxyvitamin D levels in females with iRLS, which was inversely correlated with disease severity, increased prevalence of RLS in patients with “musculoskeletal symptoms” with relatively lower vs higher serum 25-dihydroxyvitamin levels, increased cerebrospinal fluid vitamin D binding protein levels in proteomic analysis in RLS patients, and improvement in the severity of RLS symptoms with administration of vitamin D supplements to patients with vitamin D deficiency, our group analyzed the possible relationship of two common SNPs (rs2228570 and VDR rs731236) in the *vitamin D3 receptor* gene (*VDR*, *NR1I1* or *PPP1R163*; chromosome 12q13.11; MIM 601769; Gene ID 7421) with the risk for iRLS in a case–control association study (205 RLS patients/445 controls) [90]. This gene encodes the nuclear hormone receptor for

Table 2
Results of case–control association studies of possible candidate genes for RLS.

Gene group	Gene	Gene ID/MIM	Chromosome	Study	Country	Allelic variant	RLS patients N (MAF)	Controls N (MAF)	OR (95%CI)	P value
Nitric oxide synthase neuronal	*NOS1*	4842/163731	12q24.2-q24.31	Winkelmann et al., 2008 [39]	Germany	rs7977109	918 (NAD)	918 (NAD)	0.76 (0.64–0.90)	NAD
				Jiménez-Jiménez et al., 2015 [40]	Spain	rs7977109	205 (0.485)	328 (0.483)	1.01 (0.78–1.30)	0.946
						rs693534	205 (0.359)	328 (0.393)	0.86 (0.66–1.12)	0.256
Dopamine receptors (DRD)	*DRD1*	1812/126449	5q35.1	Desautels et al., 2001 [82]	Canada	rs4532	92 (0.403)	182 (0.381)	1.10 (0.64–1.90)	0.712
	DRD2	1813/126450	11q23	Desautels et al., 2001 [82]	Canada	rs1801028	92 (0.007)	182 (0.033)	0.32 (0.01–2.76)	0.274
	DRD3	1814/126451	3q13.3	Desautels et al., 2001 [82]	Canada	rs6280	92 (0.301)	182 (0.308)	0.98 (0.55–1.76)	0.955
				Jiménez-Jiménez et al., 2013 [83]	Spain	rs6280	206 (0.330)	324 (0.342)	0.95 (0.73–1.25)	0.723
	DRD4	1815/126452	11p15.5	Desautels et al., 2001 [82]	Canada	rs1800955	92 (0.553)	182 (0.560)	0.98 (0.57–1.67)	0.924
	DRD5	1816/126453	4p16.1	Desautels et al., 2001 [83]	Canada	rs6283	92 (0.377)	182 (0.304)	1.42 (0.81–2.48)	0.194
Dopamine transporter	*SLC6A3*, *DAT* or *DAT1*	6531/126455	5p15.3	Desautels et al., 2001 [82]	Canada	VNTR40bp	92 (0.219)	182 (0.266)	0.78 (0.41–1.46)	0.402
Tyrosine-hydroxylase	*TH*, *DYT14 or DYT5b*	7054/191290	11p15.5	Desautels et al., 2001 [82]	Canada	rs6356	92 (0.364)	182 (0.342)	1.08 (0.62–1.89)	0.767
Dopamine-beta-hydroxylase	*DBH*	1621/609312	9q34	Desautels et al., 2001 [82]	Canada	rs1108580	92 (0.511)	182 (0.517)	0.98 (0.57–1.67)	0.930
Monoamine oxidases (MAO)	*MAOA*	4128/309850	Xp11.3	Desautels et al., 2002 [85]	Canada	VNTR*	96 (0.176)	200 (0.265)	0.60 (0.31–1.14)	0.096
	MAOB	4129/309860	Xp11-23	Desautels et al., 2002 [85]	Canada	GT**	96 (0.149)	200 (0.172)	0.83 (0.40–1.72)	0.598
Catechol-ortho-methyl-transferase (COMT)	*COMT*	1312/116790	22q11.21	Mylius et al., 2010 [88]	Germany	rs4680	298 (0.471)	135 (0.500)	0.89 (0.58–1.36)	0.560
Heme-oxygenases	*HMOX1*	3162/141250	22q13.1	Jiménez-Jiménez et al., 2015 [89]	Spain	rs2071746	205 (0.402)	445 (0.479)	0.73 (0.58–0.94)	0.010
				Jiménez-Jiménez et al., 2015 [89]	Spain	rs2071747	205 (0.032)	445 (0.045)	0.69 (0.34–1.36)	0.259
	HMOX2	3163/141251	16p13.3	Jiménez-Jiménez et al., 2015 [89]	Spain	rs2270673	205 (0.288)	445 (0.310)	0.90 (0.69–1.17)	0.425
				Jiménez-Jiménez et al., 2015 [89]	Spain	rs1051308	205 (0.322)	445 (0.354)	0.87 (0.67–1.12)	0.265
Vitamin D receptor	*VDR*	7421/601769	12q13.11	Jiménez-Jiménez et al., 2015 [90]	Spain	rs2228570	205 (0.363)	445 (0.335)	1.13 (0.88–1.46)	0.314
				Jiménez-Jiménez et al., 2015 [90]	Spain	rs731236	205 (0.420)	445 (0.345)	1.37 (1.07–1.76)	0.010
Microtubule associated protein tau (MAPT)	*MAPT*	4137/157140	17q21.1	Roco et al., 2013 [97]	Spain	rs1052553	205 (0.268)	324 (0.272)	0.98 (0.74–1.31)	0.906
Histamine-N-methyl transferase	*HNMT*	3176/605283	2q22.1	Jiménez-Jiménez et al., 2016 [99]	Spain	rs11558538	205 (0.093)	410 (0.120)	0.75 (0.50–1.14)	0.157
Solute carrier family 1 – glial affinity glutamate transporter-, member 2 (EATT2 or GLT-1)	*SLC1A2*	6506/600330	11p13-p12	Jiménez-Jiménez et al., 2014 [101]	Spain	rs3794087	205 (0.251)	328 (0.252)	1.00 (0.74–1,34)	0.991

OR odds-ratio (OR); 95%CI 95% confidence intervals (CI); NAD = non-available data; MAF = minor allele frequency, * VNTR variable number of tandem repeats, frequency of short alleles (three repeats). **GT frequency of 1–5 repeats of the sequence GT.

vitamin D3 which shares structural similarities with thyroid and steroid receptors, and their main functions include regulation of mineral metabolism and other metabolic pathways involved in cancer and in the immune response. We found a decreased risk for RLS in carriers of the *VDR* rs731236AA genotype (OR = 0.61, 95%CI 0.42–0.88) and the *VDR* rs731236A allelic variant (major allele; OR = 0.73, 95%CI = 0.57–0.93). Despite finding, additionally, that RLS patients carrying the allelic variant rs731236G had an earlier age at onset, and those carrying the rs731236GG genotype had higher severity of RLS, the statistical significance disappeared after multivariate analyses (these used a linear regression under the standard additive model, which included in a single model the genotypes, gender, age, age at onset, relatively low but normal ferritin levels – 30–50 ng/l, and IRLSSGRS score). *VDR* rs2228570 SNP was not associated with the risk for RLS, and none of the two SNPs studied was related with the positivity of family history of RLS.

Fragile X premutation

Summers et al. [91] reported a significantly increased risk for RLS in carriers of the fragile X premutation (OR = 1.9, 95% CI = 1.1–3.2, p = 0.025) in a study involving 127 carriers and 86 age matched controls. In addition, the fragile X premutation carriers with RLS had a higher IRLSSGRS, insomnia severity index and Pittsburgh sleep quality index than controls with RLS.

Alpha-synuclein promoter Rep1

Lahut et al. [92] showed a decreased risk for RLS in 258 patients carrying the longest size variant (allele 2) of the complex microsatellite repeat Rep1 within the *alpha-synuclein* (*SNCA*) gene promoter (associated to risk for PD) compared with 235 healthy controls (OR for the longest allele = 0.58, 95%CI = 0.35–0.96; P = 0.028).

Other candidate genes

Several studies have shown a relatively high prevalence of RLS symptoms in patients with spinocerebellar ataxias (SCA) [93,94]. For this reason, two studies analyzed CAG repeat expansions in *SCA* locus. Both studies, one by Desautels et al. [95] (125 RLS patients/188 controls, analyzing the frequency of CAG repeat expansions at the *SCA3* locus), and another by Konieczny et al. [96] (215 patients with RLS and PLMs, analyzing the frequency of CAG repeat expansions at the *SCA1*, *SCA2*, *SCA3*, *SCA6*, *SCA7*, and *SCA17* loci), reported a lack of association of CAG repeat expansions with idiopathic RLS.

The *microtubular associated protein tau* (*MAPT*) *H1* discriminating haplotype SNP (rs1052553), which has been related with the risk for PD, was the subject of a case–control association study (205 RLS patients/324 healthy controls) that found a lack of association [97]. The rs11558538 in the *histamine-N-methyl-transferase* gene (*HNMT*, chromosome 2q22.1, MIM 605283, gene identity 3176), which has also been associated with the risk of developing PD in a recent meta-analysis [98], has not been found to be associated with the risk for RLS in a case–control association study involving 205 RLS patients and 410 controls [99].

Despite the finding of a significant increase in thalamic glutamate concentrations in RLS patients, as compared with controls, using proton magnetic resonance spectroscopy (^{1}HMRS) could suggest a possible role of the glutamatergic system in the pathophysiology of RLS [100]; a case–control association study (205 RLS cases/328 controls) reported by our group found a lack of association between RLS risk and the rs3794087 SNP in the *solute carrier family 1, member 2* gene (*SLC1A2*, also known as *EATT2* or *GLT-1;* chromosome 11p13-p12; MIM 600330; Gene Identity 6506; this gene encodes a member of a family of solute transporter proteins, which is the main transporter clearing the excitatory neurotransmitter glutamate) [101].

As previously mentioned, the SNP rs7977109 in the *NOS1* gene was associated with the risk for RLS in a three-stage association study [39], but this was not confirmed in a case–control replication study [40]; the *DMT1* gene was not associated with the risk for RLS [38].

The results of case–control association studies on candidate genes for RLS are summarized in Table 2. Several genes were selected as candidates because of their relationship with PD. Although the possible relationship between PD and RLS has not been clearly established and has been a matter of controversy, a recent prospective study on a large cohort of patients has shown a significantly higher risk for incident PD in subjects with RLS as compared to those without RLS [102]. However, to date, none of the polymorphisms found associated with the risk for PD have shown association with the risk for RLS. On the other hand, two case–control cohorts from Tel-Aviv and New-York, including 1133 PD patients and 867 controls found a lack of association between four RLS-related SNPs in the *MEIS1*, *BTBD9*, *PTPRD* and *MAP2K5/SKOR1* genes with the risk for PD [103].

Conclusions

The role of inheritance in the etiology of RLS is supported by the high frequency of family history of RLS found in patients diagnosed with this syndrome, by data obtained from family studies, and by the higher concordance rates in monozygotic twins than in dizygotic ones. While the majority of pedigrees reported are consistent with an autosomal dominant pattern of inheritance, others did follow an autosomal recessive pattern (such as the family of the original description of *RLS1* locus) or non-mendelian patterns. The possible role of epigenetic factors in RLS remains to be determined. At least eight genes/loci have been identified through linkage studies as responsible for RLS in a low number of families (thus explaining a small percentage of RLS heritability), most of them with apparently autosomal dominant RLS. Several GWAS found a strong association of certain variants of *PTPRD*, *BTBD9*, and *MEIS1* genes with the risk for RLS, and others found association with variants of *MAP2K5/SKOR1* and *TOX3* genes. A strong association of the *BTBD9* rs9357271 SNP with the risk for RLS has been described in an exome sequencing study as well. Despite the fact that genetic association of these variants with RLS seems clear, it is important to note that some of the studies, especially those related with *BTBD9* and *MEIS1*, showed more association for PLMs than for RLS. The possible role of *PCDHA3* and *TRAPPC6B* genes on RLS risk found in exome sequencing studies in two families needs to be elucidated. Among the case–control association studies, a *NOS1* gene variant was slightly associated with the risk for RLS in a single study, but was not confirmed in another. Other single studies showed a modest association between some variants in the *HMOX1*, *VDR*, and *SNCA* genes and the risk for RLS in certain populations, but they deserve replication in further studies.

Practice points

A) There is considerable evidence suggesting the role of genetic factors (it is likely that this syndrome is a genetically complex disorder) in the etiology or RLS.
B) Because both autosomal dominant (the most frequently reported), autosomal recessive, and non-mendelian patterns of inheritance of RLS have been reported, as well as the presence of phenocopies, the role of epigenetic factors in RLS genetics should also be considered.
C) Most of the genes/loci (at least eight) identified in familial RLS using linkage studies involved families with apparently autosomal dominant RLS.
D) Certain variants of *PTPRD*, *BTBD9*, *MEIS1*, *MAP2K5/SKOR1* and *TOX3* genes have shown association with RLS, and some of them with PLMs (both in patients with RLS and without RLS).
E) A strong association of the *BTBD* rs9357271 variant with RLS risk has been shown in an exome sequencing study. The results of other exome sequencing studies in families, indicating the possible role of *PCDHA3* and *TRAPPC6B* genes on *RLS* risk deserve further studies.
F) The search for candidate genes through case–control association studies is still limited because they are usually based on short series and because there is a lack of replication studies in other populations.

Research agenda

We suggest that ideal studies on the genetics of RLS should fulfill at least the following conditions:

A) The index patients included in genetic studies should be diagnosed with RLS according to standardized criteria, and have a positive family history for RLS.
B) Index patients should participate in both case–control association studies and in family studies.
C) Because the prevalence of RLS is high, it is important to interview the patients selected as "healthy controls" in case–control association studies investigating the presence of RLS symptoms. In addition, "healthy controls" who report a positive family history of RLS should be excluded from these studies.
D) Clinical interview and examination, including IRLSSGRS and other standardized scales for RLS, should be performed at least on all available first-degree relatives of each index patient.
E) It would be ideal to obtain blood from the index patients, their relatives, and healthy controls in order to determine the potential role of genetic factors in RLS.
F) The design of multicentre prospective long-term studies, including clinical follow-up of the index patients and their relatives, to explore the development of PD or the development of RLS in relatives who did not fulfill RLS criteria at the time of enrollment in the study would be desirable to avoid misdiagnoses of RLS.

Conflict of interest statement

The authors do not have any conflicts of interest to disclose.

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