

Latest Perspectives in Genetic Risk Factors for Restless Legs Syndrome

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Abstract

The high frequency of positive family history of restless legs syndrome (RLS) in patients with this disease and the observed high concordance rates in monozygotic compared with dizygotic twins support a major role of genetic factors in the development of RLS. Although a number of variants for several genes may increase the risk of RLS, no definitive causative genes have been identified to date. In this review, we summarise the studies performed on families with RLS, twin studies, linkage studies, genome-wide association studies, case-control association studies and exome sequencing in RLS. The strongest candidate genes are of *PTPRD*, *BTBD9* and *MEIS*.

Keywords

Restless legs syndrome, genetics, family history, linkage studies, genetic polymorphisms, risk factors

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Restless legs syndrome (RLS), first described by Willis in 1672 and in more detail by Ekbom in 1945 (hence 'Willis-Ekbom' disease), is a sensorimotor disorder with the primary sensory symptom consisting of a strong, often irresistible, urge to move the legs (and sometimes the arms as well), accompanied or caused by unpleasant sensations, that is brought on or made worse by rest, improves partially or totally by moving the legs (or arms) and worsens towards or only occurs in the evening and night time.¹ Positive family history for RLS, response to dopaminergic drugs and the presence of periodic limb movements (PLMs) while awake, during sleep, as assessed by polysomnography or leg activity devices, are considered supportive criteria for the diagnosis.¹ According to the 2011 Revised International RLS Study Group (IRLSSG) Diagnostic Criteria for RLS, it is also required that the above clinical features should not be related with another medical or behavioural condition, often referred to as 'RLS mimics', such as myalgia, venous stasis, leg oedema, arthritis, leg cramps, positional discomfort or habitual foot tapping, that have been commonly confused with RLS because they produce symptoms that meet or at least come close to meeting all of the above criteria. RLS is usually classified into idiopathic or primary (patients with a possible genetic predisposition and exclusion of other associated conditions) and symptomatic forms (secondary to iron deficiency, anaemia, uraemia, polyneuropathy, multiple sclerosis, several neurodegenerative diseases, etc.).²

In this review, we summarise the main findings related with genetic risk of RLS. The potential causes of secondary RLS are beyond the scope of this review.

Search Strategy

References for this review were identified by searching in PubMed from 1966 until 31 July 2013. The term 'restless legs syndrome' was crossed

with 'genetics', 'genes' and 'risk factors', and the related references were selected.

Family History of Restless Legs Syndrome

Family history of RLS is present in up to 60 % of the idiopathic RLS patients in classic studies.^{3–7} A recent family study of RLS involving 671 cases showed familial aggregation with a rate of 77.1 %.⁸ It has been reported that patients with definite hereditary RLS are younger at the age of onset than those with a negative family history.^{6,9}

Allen and colleagues¹⁰ reported a significantly higher risk of RLS in first- and second-degree relatives of patients diagnosed with RLS than in first- and second-degree relatives of controls with no clinical history of RLS and this risk was greater for first-degree relatives of early-onset than for relatives of late-onset RLS probands.

Genetic Anticipation in Restless Legs Syndrome

Genetic anticipation is a phenomenon whereby the symptoms of a genetic disorder become apparent at an earlier age as it is passed on to the next generation. In most cases, an increase in severity of symptoms is also noted. Although genetic anticipation could be related with different types of inheritance, it is more often reported with autosomal dominant diseases, such as Huntington's disease, myotonic dystrophy or several spinal cerebellar ataxias. Trenkwalder and colleagues¹¹ described a large German kindred of familial RLS with 20 affected subjects and involving four generations with a variety of clinical symptoms, which showed the variety of clinical RLS symptoms with decreasing age of onset in generations II–IV, suggesting the possibility of anticipation. Lazzarini and

Table 1: Susceptibility Loci for Familial Restless Legs Syndrome

Gene	Chromosome	Authors, Year, Reference	Gene Identity/OMIM	Inheritance
<i>RLS1</i>	12q12-q21	Desautels et al., 2001, ²³ 2005 ²⁶	192142/102300	Autosomal recessive
<i>RLS2</i>	14q13-q21	Bonati et al., 2003 ³²	450097/608831	Autosomal dominant
<i>RLS3</i>	9p24-p22	Chen et al., 2004 ³⁵	100188812/610438	Autosomal dominant
<i>RLS3*</i>	9p	Lohmann-Hedrich et al., 2008 ³⁸		Autosomal dominant
<i>RLS4</i>	2q33	Pichler et al., 2006 ⁴¹	100188813/610439	Autosomal dominant
<i>RLS5</i>	20p13	Levchenko et al., 2006 ⁴⁴	100188839/611242	Autosomal dominant
Other	19p13	Kemlink et al., 2008, ⁴⁶ Skehan et al., 2012 ⁴⁷		Autosomal dominant
	16p12.1	Levchenko et al., 2009 ⁴⁸		Autosomal dominant
	13q32.3-33.2	Balaban et al., 2012 ⁴⁹		Autosomal dominant

colleagues¹² described genetic anticipation in two out of five pedigrees with familial RLS.

Twin Studies

Twin concordance means a higher than expected frequency of the same trait in both twins. A concordance rate of a trait which is higher in monozygotic than in dizygotic twins supports a role of genetic factors. To date, only three studies have analysed the concordance rates of RLS between twins.

Ondo and colleagues¹³ reported a high concordant rate (83.3 %) for RLS in a study involving 12 monozygotic twins, in which one of them was diagnosed with RLS. Desai and colleagues¹⁴ reported higher concordance rates for monozygotic than for dizygotic twins for RLS symptoms after analysing a questionnaire responded by 1,937 twin pairs (933 monozygotic and 1,004 dizygotic) from a national volunteer twin register. Xiong et al.¹⁵ reported higher concordance rates for monozygotic than for dizygotic twins as well using a questionnaire responded by 272 twin pairs (140 monozygotic and 132 dizygotic).

Inheritance Patterns in Restless Legs Syndrome

Investigations of families with RLS have suggested an autosomal-dominant mode of inheritance with variable expressivity.^{12,16–20} Most of the pedigrees (90 %) of a large family study showed a vertical transmission compatible with a dominant-like inheritance pattern, although some pedigrees were compatible with recessive inheritance, and 2.8 % families had bilinear inheritance.⁸

Winkelmann and colleagues,¹⁹ in a complex segregation analysis with RLS families stratified according to the mean age at onset of the disease within these families, suggested an autosomal dominant mode of inheritance in early age at onset families, while in late age at onset families they found no evidence for a major gene. A further segregation analysis involving 590 phenotyped subjects from 77 pedigrees did not show evidence for a major gene controlling age at onset and suggested that non-genetic causes of RLS may exist and that RLS should be a genetically complex disorder.²¹

The possible presence of phenocopies in families with RLS^{19–21} must be taken into account. This term defines individuals with a similar phenotype, which can be caused by different genetic or environmental determinants. The occurrence of phenocopies is frequent in common diseases such as RLS and can be explained by chance. In some RLS families with an apparent autosomal dominant mode of transmission, the proportion of affected individuals was higher than the expected 50 % and therefore suggests a non-Mendelian inheritance in some cases. In an attempt to provide answers to the presence of phenocopies in families

with RLS and to explain the non-Mendelian features in the genetics of this disease, Zimprich²² suggested the hypothesis of a possible role of epigenetic factors (heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence or functionally relevant modifications to the genome that do not involve a change in the nucleotide sequence) in the inheritance of RLS.

Linkage Studies

Linkage studies look at physical segments of the genome that are associated with given traits. Linkage studies in families with RLS identified at least eight susceptibility loci for familial RLS, although the responsible genes have not been clearly identified.

1 – *RLS1* (Chromosome 12q12-q21, OMIM 102300)

The first mapping of a locus conferring susceptibility to RLS was described by Desautels and colleagues,²³ who conducted a genome-wide scan in a large French-Canadian family with autosomal recessive RLS. They found a significant linkage on chromosome 12q for a series of adjacent microsatellite markers with a maximum two-point logarithmic odds (LOD) score of 3.42 at recombination fraction 0.05, whereas multipoint linkage calculations yielded a LOD score of 3.59. Haplotype analysis refined the genetic interval, positioning the RLS-predisposing gene in a 14.71 cm region between D12S1044 and D12S78.

Although some authors could not confirm the susceptibility locus for RLS on chromosome 12q in the families they studied^{24,25} and other authors noted that the study of the single French-Canadian family used an autosomal recessive model with very high allele frequency,¹⁹ Desautels and colleagues²⁶ confirmed the presence of a major RLS-susceptibility locus on chromosome 12q, designated as *RLS1* and also suggested that at least one additional locus may be involved in the origin of this prevalent condition, in a study involving of 276 individuals from 19 families (five of whom showed linkage to chromosome 12q). Desautels and colleagues²⁶ noted that autosomal dominant inheritance is suggested clinically in RLS but stated that their LOD scores were higher under a recessive model. Moreover, Winkelmann and colleagues²⁷ confirmed evidence for linkage on chromosome 12q.

One scale gene-based case-control association study²⁸ and one family-based genetic association study²⁹ did not show association between the *neurotensin* gene (chromosome 12q, OMIM 162650, important in the modulation of the dopaminergic transmission)^{28,29} and the risk of RLS. Other case-control association study did not show association between the divalent metal ion transporter 1 gene (*DMT1* or *SLC11A2*; chromosome 12q, OMIM 600423; an important iron transporter),³⁰ and the risk of RLS.

Winkelmann and colleagues³¹ performed a three-stage association study (explorative study, replication study, high-density mapping) in two Caucasian RLS case-control samples of altogether 918 independent cases and controls. In the explorative study (367 cases and controls, respectively), they screened 1,536 single nucleotide polymorphisms (SNPs) in 366 genes in a 21 Mb region encompassing the *RLS1* critical region on chromosome 12. They found three genomic regions that were significant ($p < 0.05$). In the replication study (551 cases and controls, respectively) they genotyped the most significant SNPs from Stage 1. After correction for multiple testing, association was observed with SNP rs7977109, with an odds ratio (OR) (95 % confidence interval [CI]) equal to 0.76 (0.64–0.90), this SNP is located at the neuronal nitric oxide synthase (*NOS1*) gene.

2 – *RLS2* (Chromosome 14q13-q21, OMIM 608831)

Bonati and colleagues,³² showed significant evidence of linkage to a new locus for RLS on chromosome 14q13-21 region in a 30-member, three-generation Italian family. This was the second RLS (*RLS2*) locus identified and the first consistent with an autosomal dominant inheritance pattern. The new RLS critical region spans 9.1 cM, between markers D14S70 and D14S1068. The maximum two-point LOD score value (3.23) was obtained for marker D14S288.

Levchenko and colleagues³³ found support for this locus in a French-Canadian population and Kemlink and colleagues³⁴ described a significant association with a haplotype formed by markers D14S1014 and D14S1017 of this chromosome in a family based association study of 159 European RLS trios (an affected patient and their parents). On the other hand, Vogl and colleagues²⁵ did not find linkage to *RLS2* in a South Tyrolean (Italy) population.

3 – *RLS3* (Chromosome 9p24-p22, OMIM 610438)

Chen and colleagues³⁵ characterised 15 large and extended multiplex pedigrees, consisting of 453 subjects (134 affected with RLS) and identified one novel significant RLS susceptibility locus on 9p24-p22 with a multipoint nonparametric linkage (NPL) score of 3.22. Model-based linkage analysis, with the assumption of an autosomal-dominant mode of inheritance, validated the 9p24-22 linkage to RLS in two families (two-point LOD score of 3.77; multipoint LOD score of 3.91) and further fine mapping confirmed the linkage result and defined this novel RLS disease locus to a critical interval. These authors did not find pathogenic mutations in three possible candidate genes at this RLS locus: *multi-PDZX domain protein 1* (*MUPP1*; OMIM 603785, related with serotonergic transmission), *solute carrier family 1, member 1* (*SLC1A1*, also known as EAAC1, OMIM 133550, an important transporter of L-glutamate and L- and D-aspartate both in neurons and in epithelium) and potassium channel, sub-family V, member 2 (*KCNV2*, OMIM 607604; encodes a potassium-channel subunit that mediates voltage-dependent potassium-ion permeability of excitable membranes).

Ray and Weeks³⁶ concluded, contrary to the findings of Chen and colleagues,³⁵ that there is no convincing evidence of linkage for RLS on 9p. However, Liebetanz and colleagues³⁷ confirmed this locus, and narrowed the region containing the autosomal dominant *RLS3* locus to 11.1 cM (16.6 Mbp) for marker D9S1810, through analysis of transmission disequilibrium tests (TDTs) and affecteds-only linkage analysis in one large family of Bavarian origin, taking into account age at onset.

Kemlink and colleagues³⁴ found no significant association in the sample of all families and only marginally significant associations were detected, 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 ($p = 0.0086$ and 0.0077 , respectively) of this chromosome in a family-based association study of 159 European RLS trios. Vogl and colleagues²⁵ did not find linkage to *RLS3* in a South Tyrolean population.

Lohmann-Hedrich and colleagues,³⁸ in a linkage analysis of a four-generational German RLS family with 37 family members including 15 affected cases, with a mode of inheritance compatible with an autosomal dominant pattern, and disease onset mainly in childhood or adolescence, excluded linkage to the *RLS1*, *RLS2*, *RLS4* and *RLS5* loci. However, they identified a likely new RLS gene locus (named *RLS3* by the authors) on chromosome 9p with a maximum LOD score of 3.60 generated by model-based multipoint linkage analysis. A haplotype flanked by D9S974 and D9S1118 in a 9.9 Mb region, centromeric to *RLS3*, was shared by all 12 investigated patients. In addition, 11 of them carried a common haplotype extending telomeric to D9S2189 that is located within *RLS3*.

Schormair and colleagues³⁹ identified association of RLS with rs4626664 and rs1975197 SNPs in the *protein tyrosine phosphatase receptor type delta* (*PTPRD*) gene (chromosome 9p23-24; OMIM 601598) in a genome-wide association study (GWAS) involving 2,458 affected individuals and 4,749 controls from Germany, Austria, Czech Republic and Canada. This finding was confirmed in a replication study in a family-based study including 144 family members from 15 families and in a case-control association study involving 189 RLS patients and 560 controls.⁴⁰ Protein tyrosine phosphatases (PTPs) are known to be signalling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle and oncogenic transformation.

4 – *RLS4* (Chromosome 2q33, OMIM 610439)

Pichler and colleagues,⁴¹ in a genome-wide linkage scan of patients with RLS ($n = 37$) assessed in a population isolate ($n = 530$) of South Tyrol, found a novel locus on chromosome 2q, with nominal evidence of linkage on chromosomes 5p and 17p, using both non-parametric and parametric analyses. Follow-up genotyping yielded significant evidence of linkage (non-parametric LOD score 5.5) between markers D2S311 and D2S317 (11.7 cM). They found that a common seven-marker haplotype in the chromosome 2q33 region was shared identical by descent by all 15 affected members of three families (these families descended from a common founder couple 10 generations ago). Two-point linkage analysis of this large genealogy resulted in a maximum LOD score of 4.1 at $\theta = 0.0$ at marker D2S2242.

The same group described co-occurrence of heterozygous parkin mutations in some of their patients with *RLS4* haplotype and found that this was associated with anticipation of RLS onset age of 16.9 years, but not with disease severity⁴² and after a fine-mapping of *RLS4* locus in the affected members of the three linked families using a next generation sequencing approach, they restricted the shared candidate region to 46.9 Kb over the potassium channel-related gene *BTB/POZ domain-containing protein KCTD18* (*KCTD18*); *potassium channel tetramerisation domain containing 18*, Gene ID 130535) and exons 10–13 of *spermatogenesis-associated serine-rich protein 2-like or stress granule and nucleolar protein* (*SPATS2*; OMIM 613817) genes.⁴³

5 – *RLS5* (Chromosome 20p13, OMIM 611242)

Levchenko and colleagues,⁴⁴ by genome-wide linkage analysis in a French-Canadian pedigree segregating RLS (17 affected individuals, autosomal dominant pattern of inheritance), identified a 5.2 Mb candidate region, referred to as *RLS5*, on chromosome 20p13 (maximum

Table 2: Results of Case-control Association Studies of Possible Candidate Genes for Restless Legs Syndrome

Gene Group	Gene (Identity)	Chromosome	OMIM	Authors, Year, Reference	Allelic Variant	RLS Patients N (Frequency)	Controls N (Frequency)	OR (95 % CI)	p Value
Nitric oxide synthase neuronal	<i>NOS1</i> (4842)	12q24.2-q24.31	163731	Winkelmann et al., 2008 ³¹	rs7977109	918 (NAD)	918 (NAD)	0.76 (0.64–0.90)	NAD
DRD	<i>DRD1</i> (1812)	5q35.1	126449	Desautels et al., 2001 ⁶⁴	-48A/G	92 (0.403)	182 (0.381)	1.10 (0.64–1.90)	0.712
	<i>DRD2</i> (1813)	11q23	126450	Desautels et al., 2001 ⁶⁴	Ser311Cys	92 (0.007)	182 (0.033)	0.32 (0.01–2.76)	0.274
	<i>DRD3</i> (1814)	3q13.3	126451	Desautels et al., 2001 ⁶⁴	DRD3Gly (rs6280)	298 (0.319)	506 (0.328)	0.96 (0.77–1.20)	0.723
				Jiménez-Jiménez et al., 2013 ⁶⁵					
	<i>DRD4</i> (1815)	11p15.5	126452	Desautels et al., 2001 ⁶⁴	-521C/T	92 (0.553)	182 (0.560)	0.98 (0.57–1.67)	0.924
Dopamine transporter	<i>DRD5</i> (1816)	4p16.1	126453	Desautels et al., 2001 ⁶⁴	Pro326Pro	92 (0.377)	182 (0.304)	1.42 (0.81–2.48)	0.194
	<i>SLC6A3, DAT</i> or <i>DAT1</i> (6531)	5p15.3	126455	Desautels et al., 2001 ⁶⁴	VNTR40bp	92 (0.219)	182 (0.266)	0.78 (0.41–1.46)	0.402
	<i>TH, DYT14</i> or <i>DYT5b</i> (7054)	11p15.5	191290	Desautels et al., 2001 ⁶⁴	Val81Met	92 (0.364)	182 (0.342)	1.08 (0.62–1.89)	0.767
Tyrosine-hydroxylase									
Dopamine-beta-hydroxylase	<i>DBH</i> (1621)	9q34	609312	Desautels et al., 2001 ⁶⁴	444G/A	92 (0.511)	182 (0.517)	0.98 (0.57–1.67)	0.930
MAO	<i>MAOA</i> (4128)	Xp11.3	309850	Desautels et al., 2001 ⁶⁴	VNTR*	96 (0.176)	200 (0.265)	0.60 (0.31–1.14)	0.096
	<i>MAOB</i> (4129)	Xp11-23	309860	Desautels et al., 2001 ⁶⁴	GT**	96 (0.149)	200 (0.172)	0.83 (0.40–1.72)	0.598
COMT	<i>COMT</i> (1312)	22q11.21	116790	Mylius et al., 2010 ⁶⁹	Val158Met	298 (0.471)	135 (0.500)	0.89 (0.58–1.36)	0.560
MAPT	<i>MAPT</i> (4137)	17q21.1	157140	Roco et al., 2013 ⁷⁵	rs1052553G	205 (0.268)	324 (0.272)	0.98 (0.74–1.31)	0.906
Glutamate related genes	<i>Solute carrier family 1-glial affinity glutamate transporter-member 2 (SCL1A2, EATT2</i> or <i>GLT-1)</i> (6506)	11p13-p12	600330	Jiménez-Jiménez et al., 2013 ⁷⁷	rs3794087T	205 (0.251)	328 (0.252)	1.00 (0.74–1.34)	0.991

*Variable number of tandem repeats (VNTR), frequency of short alleles (three repeats); **GT frequency of 1–5 repeats of the sequence GT. CI = confidence interval; COMT = catechol-ortho-methyl-transferase; DRD = dopamine receptors; MAO = monoamine oxidases; MAPT = microtubule-associated protein tau; NAD = non-available data; OR = odds ratio; RLS = restless legs syndrome.

multipoint LOD score of 3.86 at D20S849). Sas and colleagues⁴⁵ confirmed this locus in a large multigenerational Dutch family with RLS of early onset (average 18 years old) and reduced its critical region from 5.2 to 4.5 Mb with a maximum multipoint LOD score of 3.02.

6 – Other Described Restless Legs Syndrome Loci

Kemlink and colleagues,⁴⁶ in a genome-wide linkage analysis in a large RLS family of Italian origin with 12 affected members in three generations using 5,861 single nucleotide polymorphisms, performed under an autosomal-dominant model with a complete penetrance, described linkage on chromosome 19p13 with maximum multipoint LOD score of 2.61 between markers rs754292 and rs273265. This locus was replicated in a family based association study in a set of 159 trios of European origin.

Skehan and colleagues⁴⁷ found significant linkage on chromosome 19p13 as well, in a genome-wide linkage analysis in an Irish autosomal dominant RLS pedigree with 11 affected members. This linkage was found for a series of microsatellite markers, with a maximum two-point LOD score of 3.59 at $\theta=0.0$ for marker D19S878 and 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.

Levchenko and colleagues⁴⁸ described an autosomal-dominant locus for RLS in a French-Canadian pedigree on chromosome 16p12.1 that spanned 1.18 Mb with a maximum multipoint LOD scores of 3.5 over the total of 10 markers.

Balaban and colleagues⁴⁹ identified an RLS family from the eastern part of central Turkey which has 10 patients suffering from this syndrome. Whole genome linkage analysis in nine family members affected and

two unaffected identified a theoretical maximum LOD score of 3.29 at chromosome 13q32.3–33.2. The susceptibility loci for familial RLS described to date are summarised in *Table 1*.

Genome-wide Association Studies

GWAS showed significant association between RLS and variants of several genes.

1 – Protein Tyrosine Phosphatase Receptor Type Delta (*PTPRD*, OMIM 601598)

Data on GWAS related with this gene have been described in the *RLS3* locus section. SNPs in the gene *PTPRD* are not associated with the risk of RLS in end-stage renal disease.⁵⁰

2 – BTB/POZ Domain Containing Protein 9 (*BTBD9*, OMIM 611237, *RLS6*-OMIM 611185, Chromosome 6p21)

This gene encodes a BTB/POZ domain-containing protein, which is known to be involved in protein–protein interactions.

The association of this gene (related with *RLS6*) with RLS risk were first described by Winkelmann and colleagues,⁵¹ who found a significant association with the SNPs rs9296249 and rs9357271 in the *BTBD9* gene. These findings were confirmed in two independent replication studies of 875 and 211 patients, respectively.

Stefansson and colleagues⁵² described association between the rs3923809A allele and the risk of RLS with PLMS in two independent samples of 306 and 123 Icelandic subjects, and another sample of US patients, respectively, with an OR of 1.9. However, they did not find

association between the A allele of rs3923809 among 229 individuals with RLS without periodic limb movement, suggesting that the authors had identified a genetic determinant of PLMs.

Vilariño-Güell and colleagues⁵³ confirmed an association with several SNPs in the *BTBD9* gene, including rs3923809, in a study of 244 patients with RLS, including 123 familial probands.

In a study including 649 RLS patients and 1,230 controls from the Czech Republic, Austria and Finland, Kemlink and colleagues⁵⁴ replicated association between RLS and rs3923809 in the *BTBD9* gene, with an OR of 1.58, both in familial and sporadic cases.

Yang and colleagues⁵⁵ described association between RLS risk and rs9357271 SNP in the gene *BTBD9* in a population-based case-control association study (189 RLS patients/560 controls) from the US (OR=1.59) but not in a family based (38 RLS families) study.

Interestingly, some SNPs in the gene *BTBD9* have been found associated with the risk of RLS in end-stage renal disease.⁵⁰

Finally, two experimental models of RLS have been reported related with *BTBD9* gene impaired function. The loss of the drosophila homologue CG1826 (*dBTD9*) disrupts sleep with concomitant increases in waking and motor activity.⁵⁶ A *BTBD9* mutant mice showed motor restlessness, sensory alterations likely limited to the rest phase (which improved with ropinirol) and decreased sleep and increased wake times during the rest phase, and had altered serum iron levels and monoamine neurotransmitter systems.⁵⁷

3 – *MEIS1* (OMIM 601739, *RLS7*-OMIM 612853, Chromosome 2p14p13)

This gene encodes a homeobox protein belonging to the three amino acid loop extension (TALE) family of homeodomain-containing proteins. Homeobox genes play a crucial role in normal development. In addition, some experimental data suggest a link between the RLS *MEIS1* gene and iron metabolism, such as the description that human cells cultured under iron-deficient conditions show reduced *MEIS1* expression.⁵⁸

Winkelmann and colleagues⁵¹ described association of this gene (related with *RLS7*) with RLS. This finding was confirmed in two independent replication studies of 875 and 211 patients, respectively. These authors found a significant association between RLS risk and the rs2300478G allele in the *MEIS1* gene, with an OR of 1.74.

Vilariño-Güell and colleagues⁵³ confirmed an association of RLS with two SNPs in the *MEIS1* gene in a study of 244 patients with RLS, including 123 familial probands. The most significant association was observed for the G allele of rs12469063 in familial cases.

In a study including 649 RLS patients and 1,230 controls from the Czech Republic, Austria and Finland, Kemlink and colleagues⁵⁴ replicated association between RLS and rs2300478 in the *MEIS1* gene, with an OR of 1.47, both in familial cases or in the combined sample of familial and sporadic cases.

Vilariño-Güell and colleagues⁵⁹ identified an Arg272-to-His (R272H; rs61752693) substitution in the *MEIS1* gene in one of 71 probands with RLS, which was segregated with RLS in three additional family members, but was also found in one unaffected member. The phenotype in this

family was highly variable, with different ages at onset and differing severity. This variant was not found among 378 additional RLS cases or 528 controls from North America, but it was found in one of 325 European controls, suggesting reduced penetrance.

Xiong and colleagues⁶⁰ sequenced all 13 *MEIS1* exons and their splice junctions in 285 RLS probands with confirmed clinical diagnosis and did not identify any causative coding or exon-intron junction mutations, but an analysis of two RLS-associated SNPs, rs12469063 and rs2300478, showed a GT/GG risk haplotype (43 versus 25 %; $p=0.0095$) in 28 RLS compared with and 140 control brain samples. They also detected a significant decrease in *MEIS1* mRNA expression by quantitative real-time polymerase chain reaction in lymphoblastoid cell lines (LCLs) and brain tissues from RLS patients homozygous for the intronic RLS risk haplotype compared with those homozygous for the non-risk haplotype and decreased *MEIS1* protein levels in the same batch of LCLs and brain tissues from the homozygous carriers of the risk haplotype compared with the homozygous non-carriers. The authors suggested that these data mean that reduced expression of the *MEIS1* gene, possibly through intronic cisregulatory element(s), predisposes to RLS.

Schulte and colleagues⁶¹ screened the coding regions and exon-intron boundaries of *MEIS1* for variants in a discovery series of 188 RLS patients with rs2300478 GT/GG haplotype and in two replication samples including 735 German RLS patients and 735 German controls, and 279 Czech RLS patients. They described three novel variants in their discovery series and confirmed the presence of the variant pR272H in two of their RLS patients.

Yang and colleagues⁵⁵ described association between RLS risk and rs2300478 SNP in the gene *MEIS1* in a population-based case-control association study (189 RLS patients/560 controls) from the US (OR=1.65) but not in a family-based (38 RLS families) study.

Schormair and colleagues⁵⁰ described association between some SNPs in the gene *MEIS1* and the risk of RLS in end-stage renal disease.

4 – Other Genes and Loci

Some authors described association between *mitogen-activated protein kinase kinase 5/SKI family transcriptional corepressor 1* (*MAP2K5/SKOR1*, chromosome 15q23, OMIM 602520/611273) gene and the risk of RLS.^{51,54,55} SNPs in the in the gene *MAP2K5/SKOR1* have not been found associated with the risk of RLS in end-stage renal disease.⁵⁰ The protein encoded by this gene is 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.

Winkelman and colleagues,⁶² in a GWAS for RLS in 922 cases and 1,526 controls (using 301,406 SNPs) followed by a replication of 76 candidate SNPs in 3,935 cases and 5,754 controls, all of European ancestry, identified six RLS susceptibility loci of genome-wide significance, two of them novel: an intergenic region on chromosome 2p14 (rs6747972, OR=1.23) and a locus on 16q12.1 (rs3104767, OR=1.35).

Exome Sequencing Studies

Whole exome sequencing is an efficient strategy to selectively sequence the coding regions of the genome. The first whole exome sequencing

published in familial RLS was performed in a German family with autosomal dominantly inherited RLS in seven definitely and two possibly affected members, and identified three novel missense and one splice site variant in the *protocadherin alpha 3* (*PCDHA3*, chromosome 5q31, OMIM 603130), *WW and C2 domain containing 2* (*WWC2*; chromosome 4q35.1 gene identity 80014), *attractin* (*ATRN*, chromosome 20p13, OMIM 603130) and *FAT atypical cadherin* (*FAT2*, chromosome 5q33.1, OMIM 604269) genes that segregated with RLS in the family. All four exons of the *PCDHA3* gene, the most plausible candidate (related with the *RLS8* locus, OMIM 615197), were sequenced in 64 unrelated RLS cases and 250 controls, revealing three additional rare missense variants (frequency <1 %) of unknown pathogenicity in two patients and one control.⁶³

Studies on Candidate Genes

1 – Genes Associated with Dopaminergic Neurotransmission

Because the possible role of the dopaminergic system in the pathogenesis of RLS, it seems reasonable to investigate candidate genes involved in dopaminergic neurotransmission in patients with RLS.

Desautels and colleagues⁶⁴ described lack of association of *DRD1*, *DRD2*, *DRD3*, *DRD4*, *DRD5*, dopamine transporter (*DAT*), tyrosine-hydroxylase (*TH*) and dopamine-beta-hydroxylase (*DBH*) genes and the risk of RLS in an association study involving 92 RLS patients and 182 controls. These findings were confirmed for the *DRD3* gene by our group in an association study involving 206 RLS patients and 324 controls.⁶⁵ Kang and colleagues⁶⁶ found no significant differences in the genotype and allele distribution of the most common SNPs in *DRD1*, *DRD2*, *DRD3* and *DRD4* genes, between 96 schizophrenic patients with and 94 without RLS symptoms induced by neuroleptic drugs.

Desautels and colleagues⁶⁷ investigated the possible role for the monoamine-oxidase A (*MAOA*) and B (*MAOB*) genes in RLS using a population-based association study involving 96 patients and 200 controls. They concluded that high activity allele of the *MAOA* gene might represent a modifying factor involved in the severity of RLS manifestations in females, although they did not find association between variable number of tandem repeat (VNTR) polymorphism in the *MAOA* gene and GT polymorphism in the *MAOB* gene when analysing the whole series of RLS patients and controls.⁶⁷ Kang and colleagues⁶⁸ found no significant differences in the genotype and allele distribution of the most common SNPs in *MAOA* and *MAOB* genes between 96 schizophrenic patients with and 94 without RLS symptoms induced by neuroleptic drugs, although the authors found that the haplotype frequencies differed between the male schizophrenic patients with and without RLS symptom and the interaction between the two polymorphisms had a significant influence on the RLS scores of patients with schizophrenia.

Mylius and colleagues⁶⁹ found no association between catechol-O-methyltransferase (*COMT*) Val(158)Met polymorphism and RLS in a study involving 298 RLS patients and 135 controls.

2 – Other Candidate Genes

Li and colleagues⁷⁰ found no association between 16 candidate genes related to dopaminergic transmission and iron metabolism with RLS risk in a Han restless legs family.

Because a relatively high prevalence of RLS symptoms was reported for patients with spinocerebellar ataxias (SCA).^{71,72} Desautels and colleagues⁷³ analysed CAG repeat expansions at the *SCA3* locus in 125 RLS patients and 188 controls, and Konieczny and colleagues⁷⁴ analysed the frequency of CAG repeat expansions *SCA1*, *SCA2*, *SCA3*, *SCA6*, *SCA7* and *SCA17* loci in 215 patients with RLS and periodic leg movements in sleep. Both studies failed to find association of CAG repeat expansions with idiopathic RLS.

As was previously mentioned, the SNP rs7977109 in the *NOS1* gene was associated with the risk of RLS,³¹ while the *DMT1* gene was not associated with the risk of RLS.

Our group found lack of association between MAPT H1-discriminating haplotype SNP (rs1052553), related with the risk of Parkinson's disease, and the risk of RLS in a case-control association study involving 205 patients with RLS and 324 healthy controls.⁷⁵

Because a recent study with proton magnetic resonance spectroscopy (1HMRS) described a significant increase of thalamic glutamate concentrations in RLS patients compared with controls,⁷⁶ suggesting a possible role of the glutamatergic system in the pathophysiology of RLS, *solute carrier family 1, member 2* (*SLC1A2*, also known as *EAT2* or *GLT-1*; chromosome 11p13-p12; gene identity 6506, OMIM 600330; this gene encodes a member of a family of solute transporter proteins, which is the main transporter clearing the excitatory neurotransmitter glutamate) should be a candidate gene to modify RLS risk. Our group showed lack of association between the SNP rs3794087 in the *SLC1A2* gene and the risk of RLS.⁷⁷

The results of association studies on candidate genes for RLS are summarised in Table 2.

Conclusions

The high frequency of family history of RLS found in patients with this syndrome, data from family studies and concordance rates which are higher in monozygotic than in dizygotic twins, support a role of inheritance in the aetiology of RLS. Despite the fact that some family reports suggest an autosomal dominant pattern of inheritance, there have been reports of some RLS families with a higher frequency of relatives with RLS than that expected for a typical autosomal dominant inheritance, suggesting a non-mendelian pattern.

Linkage studies have identified at least eight genes/loci in a low number of families with apparently autosomal dominant RLS (with the exception of *RLS1* locus found in families with autosomal recessive RLS), but the responsible genes have not been clearly identified and they only explain a small percentage of RLS heritability.

Several variants of *PTPRD*, *BTBD9* and *MEIS1* genes have been found to be associated with the risk of RLS in GWAS. The possible role of the *PCDHA3* gene on RLS risk found in an exome sequencing study in a large German family remains to be elucidated. The possible association of RLS with genes mutated in other degenerative diseases such as SCAs has not been found. Among the case-control association studies, only a *NOS1* gene variant seemed to modestly increase the risk of RLS in a single study.³¹ ■

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