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 Ekborn in 1945 (hence ‘Willis-Ekborn’ 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. 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. 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.).

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 or RLS is present in up to 60 % of the idiopathic RLS patients in classic studies. A recent family study of RLS involving 671 cases showed familial aggregation with a rate of 77.1 %. It has been reported that patients with definite hereditary RLS are younger at the age of onset than those with a negative family history.

Allen and colleagues 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, myotonict dystrophy or several spinal cerebellar ataxias. Trenkwalder and colleagues 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
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 colleagues\(^1\) 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 colleagues\(^2\) 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.\(^3\) 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.\(^4\) 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 bicharacteristic inheritance.\(^5\)

Winkelmann and colleagues,\(^6\) in a complex segregation analysis with RLS families stratified according to the 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.\(^7\)

The possible presence of phenocopies in families with RLS\(^8\) 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, Zimprich\(^9\) 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,\(^10\) 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\(^11\) and other authors noted that the study of the single French-Canadian family used an autosomal recessive model with very high allele frequency,\(^12\) Desautels and colleagues\(^13\) 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 276 individuals from 19 families (five of whom showed linkage to chromosome 12q). Desautels and colleagues\(^14\) 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 colleagues\(^15\) confirmed evidence for linkage on chromosome 12q.

One scale gene-based case-control association study\(^16\) and one family-based genetic association study\(^17\) did not show association between the neurotensin gene (chromosome 12q, OMIM 162650, important in the modulation of the dopaminergic transmission)\(^18\) and the risk of RLS. Other case-control association studies did not show association between the divergent metal ion transporter 1 gene (DMT1 or SLC11A2; chromosome 12q, OMIM 600423; an important iron transporter),\(^19\) and the risk of RLS.

### Table 1: Susceptibility Loci for Familial Restless Legs Syndrome

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Authors, Year, Reference</th>
<th>Gene identity/OMIM</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLS1</td>
<td>12p12-p21</td>
<td>Desautels et al., 2001, 2005(^a)</td>
<td>192142/102300</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>RLS2</td>
<td>14q13-q21</td>
<td>Bonati et al., 2003(^b)</td>
<td>45097/608831</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>RLS3</td>
<td>9p24-p22</td>
<td>Chen et al., 2004(^c)</td>
<td>10018812/610438</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>RLS5</td>
<td>9p</td>
<td>Lohmann-Hedrich et al., 2008(^d)</td>
<td></td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>RLS4</td>
<td>2q33</td>
<td>Pichler et al., 2006(^e)</td>
<td>10018813/610439</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>RLS5</td>
<td>20p13</td>
<td>Lecherek et al., 2006(^f)</td>
<td>10018839/112142</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Other</td>
<td>19p13</td>
<td>Kemlink et al., 2008, Skehan et al., 2012(^g)</td>
<td></td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Other</td>
<td>16p12.1</td>
<td>Levchenko et al., 2009(^h)</td>
<td></td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Other</td>
<td>13q32.3-33.2</td>
<td>Balaban et al., 2012(^i)</td>
<td></td>
<td>Autosomal dominant</td>
</tr>
</tbody>
</table>
Movement Disorders  Restless Legs Syndrome

Winkelmann and colleagues performed a three-stage association study (exploratory study, replication study, high-density mapping) in two Caucasian RLS case-control samples of altogether 918 independent cases and controls. In the exploratory 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 (651 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 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 D14S570 and D14S1068. The maximum two-point LOD score value (3.23) was obtained for marker D14S288.

Levchenko and colleagues found support for this locus in a French-Canadian population and Kemlink and colleagues 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 parent and their parents). On the other hand, Vogl and colleagues did not find linkage to RLS2 in a South Tyrolean (Italy) population.

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

Chen and colleagues 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 score (NPL) 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: \textit{multi-PDZ domain protein 1} \( (MUPP1; \text{OMIM } 603785, \text{related with serotonergic transmission}) \), \textit{solute carrier family 1, member 7} \( (SLC1A7, \text{also known as EAAC1, OMIM } 133550, \text{an important transporter of L-glutamate and L- and D-aspartate both in neurons and in epithelium}) \), and \textit{potassium channel, sub-family V, member 2} \( (KCNV2, \text{OMIM } 607604; \text{encodes a potassium-channel subunit that mediates voltage-dependent potassium-ion permeability of excitable membranes}) \).

Ray and Weeks concluded, contrary to the findings of Chen and colleagues, that there is no convincing evidence of linkage for RLS on 9p. However, Liebetanz and colleagues confirmed this locus, and narrowed the region containing the autosomal dominant RLS3 locus to 11.1 cm \( (16.6 \text{ Mbp}) \) 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 colleagues 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 \( (p=0.0086 \text{ and } 0.0077, \text{respectively}) \) of this chromosome in a family-based association study of 159 European RLS trios. Vogl and colleagues did not find linkage to RLS3 in a South Tyrolean population.

Lohmann-Hedrich and colleagues performed 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 Rs132 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 colleagues identified association of RLS with rs4526664 and rs1975197 SNPs in the \textit{protein tyrosine phosphatase receptor type delta (PTPRD)} gene (chromosome 9p23-24; OMIM 60159B) 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. \textit{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 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 severity \( (p=0.043) \) 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 \textit{TBX2P02 domain-containing protein KCTD18} \( (KCTD18) \), \textit{potassium channel tetramerisation domain containing 18}, \textit{Gene ID } 130353 \( ) \text{ and exons } 10-13 \text{ of spermatogenesis-associated serine-rich protein 2-like or stress granule and nucleolar protein (SPATS2)} \text{, OMIM } 613817 \text{ genes} \).

5 – RLS5 (Chromosome 20p13, OMIM 611242)

Levchenko and colleagues, by genome-wide linkage analysis in a French-Canadian pedigree segregating RLS \( (17 \text{ affected individuals, autosomal dominant pattern of inheritance}) \), identified a 5.2 Mb candidate region, referred to as RLS5, on chromosome 20p13 (maximum
multipoint LOD score of 3.86 at D20S849). Sas and colleagues\(^6\) 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,\(^6\) 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 colleagues\(^5\) 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 α=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 colleagues\(^5\) 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 colleagues\(^5\) 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.

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

<table>
<thead>
<tr>
<th>Gene Group</th>
<th>Gene (Identity)</th>
<th>Chromosome</th>
<th>OMIM</th>
<th>Authors, Year, Reference</th>
<th>Allelic Variant</th>
<th>RLS Patients N</th>
<th>Controls N</th>
<th>OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitric oxide synthase neuronal</td>
<td>NOS1 (4842)</td>
<td>12q24.2-q24.31</td>
<td>163731</td>
<td>Winkelmann et al., 2008(^\text{a})</td>
<td>rs9777109</td>
<td>918 (NAD)</td>
<td>918 (NAD)</td>
<td>0.76 (0.64–0.90)</td>
<td>NAD</td>
</tr>
<tr>
<td>DRD</td>
<td>DRD1 (1812)</td>
<td>5q31.1</td>
<td>126449</td>
<td>Desautels et al., 2001(^\text{a})</td>
<td>-48A/G</td>
<td>92 (0.403)</td>
<td>182 (0.381)</td>
<td>1.10 (0.64–1.90)</td>
<td>0.712</td>
</tr>
<tr>
<td></td>
<td>DRD2 (1813)</td>
<td>11q23</td>
<td>126450</td>
<td>Desautels et al., 2001(^\text{a})</td>
<td>Ser311Cys</td>
<td>92 (0.007)</td>
<td>182 (0.033)</td>
<td>0.32 (0.01–2.76)</td>
<td>0.274</td>
</tr>
<tr>
<td></td>
<td>DRD3 (1814)</td>
<td>3q13.3</td>
<td>126451</td>
<td>Desautels et al., 2001(^\text{a})</td>
<td>DRD3Gly</td>
<td>298 (0.319)</td>
<td>506 (0.328)</td>
<td>0.96 (0.77–1.20)</td>
<td>0.723</td>
</tr>
<tr>
<td></td>
<td>DRD4 (1815)</td>
<td>11p15.5</td>
<td>126452</td>
<td>Desautels et al., 2001(^\text{a})</td>
<td>-521C/T</td>
<td>92 (0.553)</td>
<td>182 (0.560)</td>
<td>0.98 (0.57–1.67)</td>
<td>0.924</td>
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<tr>
<td></td>
<td>DRD5 (1816)</td>
<td>4p16.1</td>
<td>126453</td>
<td>Desautels et al., 2001(^\text{a})</td>
<td>Pro326Pro</td>
<td>92 (0.377)</td>
<td>182 (0.304)</td>
<td>1.42 (0.81–2.48)</td>
<td>0.194</td>
</tr>
<tr>
<td>Dopamine transporter</td>
<td>SLC6A3, DAT or DAT1 (6531)</td>
<td>5p15.3</td>
<td>126455</td>
<td>Desautels et al., 2001(^\text{a})</td>
<td>VNTR400bp</td>
<td>92 (0.219)</td>
<td>182 (0.266)</td>
<td>0.78 (0.41–1.46)</td>
<td>0.402</td>
</tr>
<tr>
<td>Tyrosine-hydroxylase</td>
<td>TH, DYT14 or DYT5b (7054)</td>
<td>11p15.5</td>
<td>191290</td>
<td>Desautels et al., 2001(^\text{a})</td>
<td>Val81Met</td>
<td>92 (0.364)</td>
<td>182 (0.342)</td>
<td>1.08 (0.62–1.89)</td>
<td>0.767</td>
</tr>
<tr>
<td>Dopamine-beta-hydroxylase</td>
<td>DBH (1621)</td>
<td>9q34</td>
<td>609312</td>
<td>Desautels et al., 2001(^\text{a})</td>
<td>44G/GA</td>
<td>92 (0.511)</td>
<td>182 (0.517)</td>
<td>0.98 (0.57–1.67)</td>
<td>0.930</td>
</tr>
<tr>
<td>MAO</td>
<td>MAOA (4129)</td>
<td>Xp11.3</td>
<td>309850</td>
<td>Desautels et al., 2001(^\text{a})</td>
<td>VNTR*</td>
<td>96 (0.176)</td>
<td>200 (0.265)</td>
<td>0.60 (0.31–1.14)</td>
<td>0.096</td>
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<tr>
<td></td>
<td>MAOB (6531)</td>
<td>Xp11.1-23</td>
<td>309660</td>
<td>Desautels et al., 2001(^\text{a})</td>
<td>-96G (GT)**</td>
<td>96 (0.149)</td>
<td>200 (0.172)</td>
<td>0.83 (0.40–1.72)</td>
<td>0.598</td>
</tr>
<tr>
<td>COMT</td>
<td>COMT (1312)</td>
<td>22q11.21</td>
<td>116790</td>
<td>Mihlus et al., 2010(^\text{a})</td>
<td>Val158Met</td>
<td>298 (0.471)</td>
<td>135 (0.509)</td>
<td>0.89 (0.58–1.36)</td>
<td>0.560</td>
</tr>
<tr>
<td>MAPT</td>
<td>MAPT (4137)</td>
<td>17q21.1</td>
<td>157140</td>
<td>Roco et al., 2013(^\text{a})</td>
<td>rs1052553G</td>
<td>205 (0.268)</td>
<td>324 (0.272)</td>
<td>0.98 (0.74–1.31)</td>
<td>0.906</td>
</tr>
<tr>
<td>Glutamate related genes</td>
<td>Solute carrier family 1-gal affinity glutamate transporter-member 2 (SCL1A2, EAT2 or GLT1) (6506)</td>
<td>11p13-p12</td>
<td>600330</td>
<td>Jiménez-Jiménez et al., 2013(^\text{a})</td>
<td>rs3794087T</td>
<td>205 (0.251)</td>
<td>324 (0.272)</td>
<td>1.00 (0.74–1.34)</td>
<td>0.991</td>
</tr>
</tbody>
</table>

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

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.\(^5\)

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,\(^5\) 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 colleagues\(^5\) described association between the rs932889A 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 BTBD9 gene impaired function. The loss of the drosophila homologue CG1826 (dBTBD9) disrupts sleep with concomitant increases in waking and motor activity. 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.56

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.57

Winkelmann and colleagues58 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 colleagues59 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 and OR of 1.47, both in familial cases or in the combined sample of familial and sporadic cases.

Vilariño-Güell and colleagues60 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 colleagues61 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 rs3000478, 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 colleagues62 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 colleagues63 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 colleagues64 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/SKOr family transcriptional corepressor 1 (MAP2K5/SKOR1, chromosome 15q23, OMIM 602520/611273) gene and the risk of RLS.65–67 SNPs in the in the gene MAP2K5/SKOR1 have not been found associated with the risk of RLS in end-stage renal disease.68 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/MEXK3, 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 colleagues69 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 5,935 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 (rs6749772, OR=1.23) and a locus on 16q12.1 (rs5104767, 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 (PCDHAS3, 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.44

**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 colleagues\(^4^4\) 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.\(^6^5\) Kang and colleagues\(^4^5\) 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 colleagues\(^4^4\) 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.\(^6^6\) Kang and colleagues\(^4^5\) 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 colleagues\(^4^5\) found no association between catechol-O-methyltransferase (COMT) Val158Met polymorphism and RLS in a study involving 298 RLS patients and 135 controls.

### 2 – Other Candidate Genes

Li and colleagues\(^4^5\) 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).\(^1^3\)\(^2^4\) Desautels and colleagues\(^4^4\) analysed CAG repeat expansions at the SCA3 locus in 125 RLS patients and 188 controls, and Konieczny and colleagues\(^4^5\) analysed the frequency of CAG repeat expansions SCA1, SCA2, SCA3, SCA4, 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,\(^4^6\) while the DM1T1 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.\(^4^7\)

Because a recent study with proton magnetic resonance spectroscopy (1HMR) described a significant increase of thalamic glutamate concentrations in RLS patients compared with controls,\(^6^8\) suggesting a possible role of the glutamatergic system in the pathophysiology of RLS, the solute carrier family 1, member 1 (SLC1A2, also known as EAAT2 or GLT-1; chromosome 11p31-p132; 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.\(^4^9\)

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 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.\(^4^5\)
Movement Disorders

Restless Legs Syndrome


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