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Original Article

Genetic Association Studies in Restless Legs Syndrome: Risk Variants & Ethnic Differences

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ABSTRACT: ***Background:*** Genetic association studies have not produced consistent results in restless legs syndrome (RLS). ***Objectives:*** To conduct a systematic review on genetic association studies in RLS to highlight the common gene variants and ethnic differences. ***Methodology:*** We conducted Pubmed, Embase, and Cochrane search using terms "Genetic association studies" and "restless legs syndrome" for candidate gene-based studies. Out of the initial 43 studies, 18 case control studies (from 2012 to 2022) were included. Thirteen studies including 10794 Caucasian subjects (4984 RLS cases and 5810 controls) and five studies involving 2009 Asian subjects (796 RLS cases and 1213 controls) were tabulated and analyzed. In addition, three Genome-Wide Association Studies (GWAS) in Asians and Europeans/Caucasians were included for comparisons. ***Results:*** In the Asian population, gene variants in BST1, SNCA Rep1, IL1B, BTBD9, and MAP2K5/SKOR1 increased the risk of RLS (odds ratio range 1.2–2.8). In Caucasian populations, examples of variants that were associated with an increased risk of RLS (odds ratio range 1.1–1.9) include those in GABRR3 TOX3, ADH1B, HMOX1, GLO1, DCDC2C, BTBD9, SKOR1, and SETBP1. Based on the meta-analysis of GWAS studies, the rs9390170 variant in UTRN gene was identified to be a novel genetic marker for RLS in Asian cohorts, whereas rs113851554 in MEIS1 gene was a strong genetic factor among the >20 identified gene variants for RLS in Caucasian populations. ***Conclusion:*** Our systemic review demonstrates that multiple genetic variants modulate risk of RLS in Caucasians (such as MEIS1 BTBD9, MAP2K5) and in Asians (such as BTBD9, MAP2K5, and UTRN).

RÉSUMÉ : Études d'associations génétiques dans le cadre du syndrome des jambes sans repos : variants à risque et différences ethniques. ***Contexte :*** À ce jour, les études d'associations génétiques n'ont pas permis d'obtenir des résultats cohérents en ce qui regarde le syndrome des jambes sans repos (SJSR). ***Objectifs :*** Réaliser une analyse systématique des études d'associations génétiques liées au SJSR afin de mettre en évidence des variants génétiques communs ainsi que des différences ethniques. ***Méthodologie :*** Nous avons donc effectué une recherche sur PubMed, Embase et Cochrane en utilisant les termes « études d'associations génétiques » et « syndrome des jambes sans repos » pour identifier des études basées sur des gènes candidats. Sur 43 études initialement identifiées, 18 études cas témoins menées de 2012 à 2022 ont été incluses à des fins de compilation et d'analyse ; de ce nombre, 13 études incluaient 10 794 sujets caucasiens (4984 cas de SJSR et 5810 témoins) et 5 études incluaient 2009 sujets asiatiques (796 cas de SJSR et 1213 témoins). En outre, trois études d'associations pangénomiques chez des sujets d'origine asiatique et européenne (ou caucasienne) ont été incluses à des fins de comparaison. ***Résultats :*** Dans la population asiatique, les variants des gènes BST1, SNCA Rep1, IL1B, BTBD9 et MAP2K5/SKOR1 augmentent le risque de SJSR (rapport de cotes de 1,2 à 2,8). Dans les populations caucasiennes, les variants associés à un risque accru de SJSR (rapport de cotes de 1,1 à 1,9) comprennent les gènes GABRR3 TOX3, ADH1B, HMOX1, GLO1, DCDC2C, BTBD9, SKOR1 et SETBP1. Sur la base d'une méta-analyse des études d'associations pangénomiques, le variant rs9390170 du gène UTRN a été identifié comme un nouveau marqueur génétique du SJSR au sein des cohortes asiatiques, tandis que le variant rs113851554 du gène MEIS1 s'est avéré un facteur génétique important parmi les >20 variants génétiques identifiés pour le SJSR au sein des populations caucasiennes. ***Conclusion :*** Notre analyse systémique démontre en somme que de multiples variants génétiques modulent le risque de SJSR chez des sujets d'origine caucasienne (comme MEIS1, BTBD9, MAP2K5) et chez des sujets d'origine asiatique (comme BTBD9, MAP2K5 et UTRN).

Keywords: Restless legs syndrome; Race; Ethnic; Variants

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Introduction

Restless legs syndrome (RLS) is characterized by an overwhelming urge to move the legs, especially at night and associated with unpleasant sensations in the legs that begin or worsen during inactivity or rest.[1,2] These unpleasant sensations can be partially or totally relieved by movement. RLS-related sleep disturbances can cause significant impact on patients' mood, energy, behavior, and cognition.[1,2]

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The prevalence of RLS varies (3%–14%) across different populations, although it appears to have a lower prevalence (<2%) in some Asian populations.[3,4] This discrepancy in the prevalence among Caucasians and Asians populations maybe due to differences in genetic susceptibility, lifestyle or environmental factors, or underdiagnosis in Asian populations. A positive family history of RLS is present in some patients and may be inherited in an autosomal dominant or recessive pattern. RLS is a complex genetic disorder in which environmental factors and genetic predisposition contribute to the phenotype. Current genetic association studies have identified numerous gene variants to be associated with RLS.[5] First- and second-degree relatives of patients with RLS had a significantly greater risk of RLS than similar relatives of controls.[6] Secondary RLS can be caused by a variety of conditions such as iron deficiency, pregnancy, and end-stage renal disease. Several medications like antidopaminergic medications may also exacerbate the symptoms of RLS.[1–4]

In the first genome-wide association study (GWAS) of RLS in 2007, Winkelmann et al. identified several genetic variants that have significant associations with RLS. The genes which were identified to be associated with RLS were MEIS1, BTBD9, and MAP2K5. The combined allelic variants for those genes conferred more than half the risk for RLS. However, after correcting for multiple testing, only MEIS1 rs2300478 was found to reach genome-wide significance ($OR = 1.74$).[7]

Since then, there have been numerous studies (mostly based on a candidate gene approach) examining the genetic risk factors of RLS. For example, in a cohort of Chinese, Li G et al. reported that the BTBD9 allelic variants rs9296249 and rs9357271 show higher frequency among RLS patients than controls ($OR = 1.44$ and $OR = 1.73$, respectively). MAP2K5/SKOR1 rs11635424 allelic variant G also shows higher frequency among RLS patients than controls ($OR = 1.49$).[8] In a cohort in Québec population, rs9296249 in BTBD9 ($OR = 1.71$), rs10494048 in PRMT6 ($OR = 0.80$), rs4776976 in SKOR1 ($OR = 1.34$), rs3104767 in TOX 3 ($OR = 1.28$), and rs12962305 in SETP1 ($OR = 1.26$) modulate RLS risk.[9]

Despite several genetic association studies over the past decades, there are still several unanswered questions. First, it is not clear if there are common gene variants linked to RLS risk that can be consistently replicated in independent studies. Second, if there is a publication bias between Caucasians and Asians. Third, if there are differences in genetic susceptibility among different ethnic races. Fourth, if identified genetic variants have potential functional relevance in RLS.

To address some of these gaps in knowledge, we conducted a systemic review of genetic association studies in RLS to summarize the common genetic variants that have been associated with sporadic RLS and highlight the limitations and challenges of genetic association studies in RLS.

Search Strategy

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We screened related studies and articles on Embase, Cochrane, and PubMED between 2012 and 2022. The search terms used in our search strategy included "restless legs syndrome" and "genetic association studies". The search strategy included free-text terms and any appropriate subject indexing (eg. MeSH). Figure 1 shows the PRISMA flow chart of our search strategy. The search results were then screened to remove duplicates.

Study Selection

The studies were reviewed independently by at least two authors and any discrepancies were resolved through discussion. We included any case-control study that investigated the genetic associations between specific gene variants and the risk of RLS, regardless of ethnicity of study participants. Exclusion criteria included: a. studies on secondary RLS, b. studies without frequencies of individual SNPs among case and controls, c. studies in languages other than English. Only full journal articles were included, and conference abstracts, commentaries and editorials were excluded. After thorough screening, we managed to identify 18 case control candidate gene-based studies. Separately there were three studies using a GWAS approach.

Results

A total of 18 candidate gene-based case control studies[8–25] were examined using a systematic review. Out of these 18 studies, 13 were conducted in Caucasian populations (America and Europe).[9,14–25] A total of 10,794 Caucasian subjects, comprising 4984 RLS cases and 5810 controls were studied. The other five studies were conducted in Asian populations (Chinese and Korea),[8,10–13] involving a total of 2009 Asian subjects comprising 796 RLS cases and 1213 controls. Many gene loci were analyzed for associations with RLS, further details on the gene loci studied for each study are summarized in Tables 1 and 2.

Common gene loci studied in different studies were analyzed regarding their associations with RLS. Out of the 18 studies, only some gene variants were commonly studied, examples include HMOX1, HMOX2, ADH1B, GABRR3, GABRA4, and BTBD9.[8–25] Each of these gene loci were evaluated by two different studies, one conducted among Caucasian populations and one conducted among Asian populations. There were few consistent findings across the two major ethnic populations[8–25] [Table 1]. HMOX2 rs1051308 and GABRA4 rs2229940 were found to have no significant associations with RLS by the studies that analyzed these two gene loci. In the Asian population, the gene variants in BST1, SNCA Rep1, IL1B, BTBD9, and MAP2K5/SKOR1 were associated with risk of RLS (odds ratio range 1.2–2.8). Gene variants in GABRR3 TOX3, ADH1B, HMOX1, GLO1, DCDC2C, BTBD9, SKOR1, and SETBP1 were associated with an increased risk of RLS (odds ratio range 1.1–1.9) in Caucasian populations[8–25] (Tables 1, 2).

In addition, three recent large GWAS studies in Asian and Caucasian populations have identified other risk loci for RLS.[26–28] In the Korean GWAS study, 325 RLS patients and 2603 non-RLS subjects are investigated in initial analysis, followed by a replication study with 227 RLS and 229 control subjects.[26] Based on the results from the initial GWAS and replication meta-analysis, rs9390170 in UTRN gene, was identified to be a novel genetic variant to be associated with RLS.[26] There was a borderline association with rs3923809 and rs9296249 in BTBD9 in the replication cohort. The detailed GWAS results are illustrated in Table 3. In the GWAS study using three GWAS datasets (EU-RIS GENE, INTERVAL, and 23andMe) with diagnosis data collected from European cohorts from 2003 to 2017, 13 new risk loci for RLS were identified and replicated. Identified genes and pathways are associated with neurodevelopment, axon guidance, synapse formation, and neuronal specification.[27] Among these, rs113851554 in MEIS1

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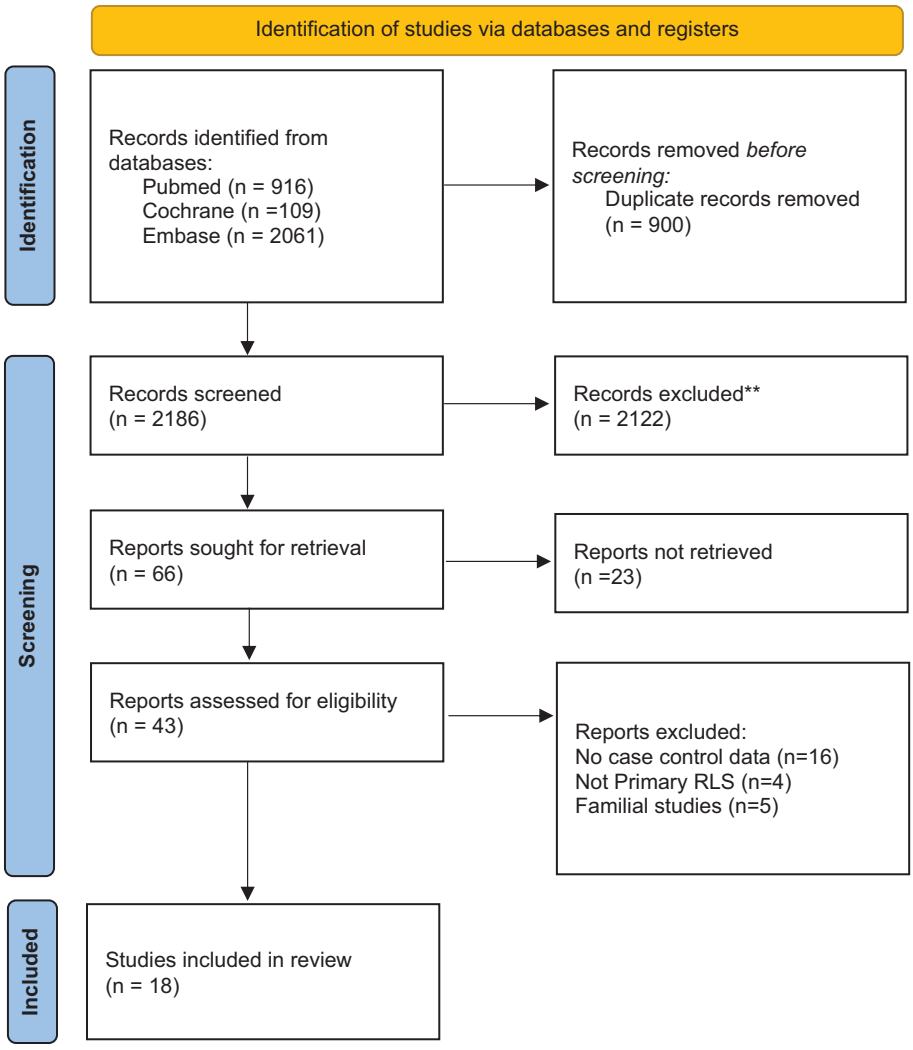

Figure 1: PRISMA flowchart.

gene was found to be the strongest genetic factor for RLS.[27] Others include variants in BTBD9, MAP2K5, TOX3, and novel variants in MYT1, DCDC2C, etc. The detailed GWAS data were summarized in Table 4. In 2020, a new GWAS analysis was conducted based on data from more than 500,000 Caucasian subjects.[28] Besides 20 previously reported RLS sequence variants, three novel RLS associated gene variants (rs112716420-G, rs10068599-T, and rs10769894-A) were identified.[28] Variants in MEIS1 and BTBD9 have the strongest association with RLS.[28] The detailed GWAS results are summarized and highlighted in Table 5.

Discussion

Based on the studies using a candidate gene approach, we highlight variants of several genes (GABA receptor, ADH1B, TOX3, BST1, HMOX1, alpha synuclein Rep1, and MAP2K5/SKOR1) that have been found to be significantly associated with RLS. Gabaminergic dysregulation has been implicated in RLS, such as the association with deficient Gamma-aminobutyric acid (GABA)-mediated inhibitory control.[29] GABA levels have also been found to be negatively correlated with severity of RLS in the cerebellum and positively correlated in the thalamus of RLS patients.[30] Furthermore, drugs that target GABA receptors have been used as a form of treatment for some RLS patients such as benzodiazepines.[31] In a Spanish study, the frequency of GABRR3 allelic variant rs832032T was higher in RLS patients than controls ($p = 0.028$, $OR = 1.66$).[14] GABRA4 rs2229940TT genotype has also been found to be associated with earlier age of onset of RLS. Separately the association between the GABRR3 rs832032T and RLS was also higher among RLS patients compared to controls in the Chinese population, though with borderline significance ($p = 0.137$, $OR = 3.42$).[12]

The link between alcohol consumption and risk of RLS has been reported by several authors. In an observation study, Mackie et al. reported the presence of RLS symptoms in 21.7% of primary alcohol use disorder subjects in the first few days following alcohol withdrawal.[32] A lower risk of RLS among subjects who had some consumption of alcohol has been highlighted.[33] These correlations suggest the possibility of alcohol metabolic genes modulating RLS risk.

A study among Caucasians reported an increased risk of RLS in carriers of rs1229984T ($p = 0.001$, $OR = 1.88$).[16] This allele codes for the most active form of the alcohol dehydrogenase 1B (ADH1B) enzyme, which correlates with higher rates of

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Table 1: Genetic association studies using candidate gene approach[8–25]

Name	Country	Year	Cases	Controls	Sample size	Race	Gene	SNP	Allele	Frequency of variants in		*p* value	Odds Ratio	Significance
										Cases	Controls			
Huang et al.[10]	China	2021	102	189	102 (RLS); 189 (Controls)	Asian, Shanghai	GBA	rs421016	G	0.5 (1)	1.3	0.373	0.39	N
							IL 19	rs79798148	A	8.7 (18)	6.9	0.419	1.29	N
							MTHFR	rs1801133	A	42.2	43.8	0.83	0.94	N
							NOS1AP	rs77878167	G	11.5	12.8	0.284	0.88	N
							RAB29/RAB7L1	rs823144	C	39.7	46.8	0.174	0.75	N
							TMEM163	rs139488590	A	2.5	3.7	0.432	0.67	N
							MCCC1	rs12637471	G	34.7	37	0.588	0.90	N
							BST1	rs4698412	A	29	40.7	0.027	0.60	Y
								rs4273468	G	20	8.1	<0.001	2.85	Y
							MMRN1	rs6532194	C	40.1	41.2	0.953	0.95	N
							SNCA/LOC105377329	rs356182	A	21.4	19.8	0.711	1.10	N
								rs356219	A	48.3	41.5	0.206	1.31	N
							ARSB	rs1071598	T	0.5	1.9	0.183	0.27	N
							KIAA1217	rs74340187	T	16.2	17	0.34	0.94	N
							SFXN2	rs149029896	A	1.6	0.3	0.076	6.05	N
							LRRK2	rs34778348	A	3	2.7	0.787	1.15	N
							IGHM	rs1136534	A	49	44.4	0.233	1.20	N
							MAPT	rs242562	G	45.9	38.8	0.276	1.34	N
							POLG2/MILR1	rs1427463	T	2.6	3.4	0.57	0.74	N
							UPK1A	rs2267582	A	30.6	26.7	0.224	1.21	N
							TCN2	rs75680863	T	5.6	6.1	0.802	0.91	N
Seo et al.[13]	Republic of Korea	2021	227	229	227 (RLS); 229 (Controls)	Asian	CLOCK	rs1801260	A	88.99 (404)	85.15 (390)	0.085	1.05	N
									G	11.01 (50)	14.85 (68)		0.74	N
								rs2412646	C	73.57 (334)	72.05 (330)	0.607	1.02	N
									T	26.43 (120)	27.95 (128)		0.95	N
							NPAS2	rs6725296	A	16.52 (75)	17.90 (82)	0.58	0.92	N
									G	83.48 (379)	82.10 (376)		1.02	N
								rs2305160	A	19.82 (90)	23.19 (109)	0.146	0.85	N
									G	80.18 (364)	74.25 (349)		1.08	N
Zhu et al.[11]	China	2020	215	369	215 (RLS); 369 (controls)	Asian	SNCA Rep1	SNCA Rep1	267bp	31.4 (135)	41.3 (305)	0.001	0.76	Y
									269bp	37.4 (161)	31.0 (229)	0.025	1.20	Y
									271bp	29.5 (127)	26.3 (194)	0.23	1.12	N
									Others	1.6 (7)	1.4 (10)	0.707	1.14	N

(*Continued*)

Table 1: (*Continued*)

Name	Country	Year	Cases	Controls	Sample size	Race	Gene	SNP	Allele	Frequency of variants in Cases	Frequency of variants in Controls	*p* value	Odds Ratio	Significance
Chen et al.[12]	China	2019	136	226	136 (RLS); 226 (controls)	Asian	HMOX1	rs2071746	A	46 (125)	46 (208)	0.93	0.99	N
							HMOX2	rs4786504	T	40 (109)	40 (181)	0.97	1.01	N
								rs1051308	G	37 (101)	37 (167)	0.943	1.01	N
							VCR	rs731236	C	8 (22)	5 (23)	0.077	1.74	N
							IL17A	rs8193036	T	28 (76)	30 (136)	0.679	0.93	N
							IL1B	rs1143634	T	5 (14)	2 (9)	0.023	2.49	Y
							NOS1	rs693534	A	25 (68)	27 (122)	0.524	0.89	N
								rs7977109	G	22 (60)	23 (104)	0.78	0.95	N
							ADH1B	rs1229984	G	29 (79)	32 (145)	0.503	0.90	N
							GABRR3	rs832032	T	2 (5)	1 (5)	0.137	3.42	N
							GABRA4	rs2229940	A	42 (114)	38 (172)	0.244	1.20	N
Li et al.[8]	China	2017	116	200	116 (RLS); 200 (controls)	Chinese	MEIS1	rs2300478	G	29 (67)	28 (112)	0.698	1.07	N
								rs4544423	G	16 (37)	18 (72)	0.475	1.17	N
								rs12469063	G	33 (77)	29 (116)	0.345	0.85	N
								rs6710341	G	31 (72)	29 (116)	0.562	0.90	N
							BTBD9	rs3923809	A	43 (100)	40 (160)	0.47	1.13	N
								rs9296249	T	42 (97)	52 (208)	0.026	1.44	Y
								rs9357271	T	17 (39)	11 (44)	0.02	1.73	Y
							PTPRD	rs1975197	T	33 (77)	33 (132)	0.799	0.96	N
								rs4626664	A	44 (102)	47 (188)	0.335	0.85	N
								rs10977209	C	16 (37)	13 (52)	0.24	1.31	N
							TOX3	rs3104788	T	19 (44)	17 (68)	0.426	0.85	N
							MAP2K5/SKOR1	rs1026732	G	28 (65)	29 (116)	0.714	1.07	N
								rs2241420	G	41 (95)	40 (160)	0.791	0.98	N
								rs6494696	G	28 (65)	28 (112)	0.945	1.01	N
								rs3784709	C	31 (72)	29 (116)	0.56	1.11	N
								rs4489954	G	27 (63)	27 (108)	0.882	1.03	N
								rs11635424	G	40 (93)	31 (124)	0.022	1.49	Y
								rs12593813	G	41 (95)	32 (128)	0.2	1.50	N
							Intergenic region of chromosome 2p14	rs6747972	A	41 (95)	38 (152)	0.458	1.13	N
Jiménez-Jiménez et al.[25]	Spain	2022	285	350	285 (RLS) 350 (controls)	Caucasian Spanish	LAG3/CD4	rs1922452	A	41.9 (239)	40.4 (283)	0.589	1.06	N
								rs951818	C	40.0 (228)	39.6 (277)	0.877	1.02	N
								rs870849	T	35.4 (202)	36.6 (256)	0.676	0.95	N

Jiménez-Jiménez et al.[19]	Spain	2021	273	325	273 (RLS); 325 (Controls)	Caucasian, Spanish	NOS3	rs1799983	T	36.6 (200)	37.4 (243)	0.97	0.97	N
									G	63.4 (346)	62.6 (407)	1.03	1.03	N
								rs79467411	G	76.9 (420)	77.5 (504)	0.97	0.97	N
									A	23.1 (126)	22.5 (146)	1.04	1.04	N
								rs2070744	T	52.6 (287)	55.5 (361)	0.89	0.89	N
									C	47.4 (259)	44.5 (289)	1.13	1.13	N
Jiménez-Jiménez et al.[14]	Spain	2018	205	230	205 (RLS); 230 (controls)	Caucasian Spanish	GABRR1	rs12200969	T	69.3 (284)	71.5 (329)	0.59	0.90	N
									C	30.7 (126)	28.5 (131)	0.59	1.11	N
								rs1186902	T	96.8 (397)	95.2 (438)	0.539	1.53	N
									C	3.2 (13)	4.8 (22)	0.539	0.65	N
							GABRR2	rs282129	G	77.8 (319)	81.1 (373)	0.539	0.82	N
									A	22.2 (91)	18.9 (87)	0.539	1.22	N
							GABRR3	rs832032	A	77.1 (316)	84.8 (390)	0.028	0.60	Y
									T	22.9 (94)	15.2 (70)	0.028	1.66	Y
							GABRA4	rs2229940	G	62.7 (257)	61.5 (283)	0.725	1.05	N
									T	37.3 (153)	38.5 (177)	0.725	0.95	N
							GABRE	rs1139916	C	62.9 (258)	60.0 (276)	0.59	1.13	N
									A	37.1 (152)	40.0 (184)	0.59	0.88	N
							GABRQ	rs3810651	T	65.9 (270)	63.7 (293)	0.59	1.10	N
									A	34.1 (140)	36.3 (167)	0.59	0.91	N
Mohtashami et al.[15]	Canada, France	2018	424	325	424 (RLS); 325 (controls)	Caucasian European	TOX3	rs3104767	G	65.2 (553)	56.6 (368)	0.000702	1.15	Y
									T	34.8 (295)	43.4 (282)		0.80	Y
								rs4784226	C	79.6 (675)	75.5 (491)	0.60745	1.05	N
									T	20.4 (173)	24.5 (159)		0.83	N
Jiménez-Jiménez et al.[16]	Spain	2017	205	505	205 (RLS); 505 (controls)	Caucasian European	ADH1B	rs1229984	C	87.6 (359)	93.0 (939)	0.001	0.53	Y
									T	12.4 (51)	7.0 (71)	0.001	1.88	Y
								rs6413413	T	99.0 (406)	98.4 (994)	0.378	1.63	N
									A	1.0 (4)	1.6 (16)	0.378	0.61	N
Jiménez-Jiménez et al.[20]	Spain	2017	205	410	205 (RLS); 410 (controls)	Caucasian European	HNMT	rs11558538	C	90.7 (372)	88.0 (722)	0.157	1.33	N
									T	9.3 (38)	12.0 (98)	0.157	0.75	N

(*Continued*)

Table 1: (*Continued*)

Name	Country	Year	Cases	Controls	Sample size	Race	Gene	SNP	Allele	Frequency of variants in Cases	Frequency of variants in Controls	*p* value	Odds Ratio	Significance
Gan Or et al.[21]	USA, Canada	2016	227	217	227 RLS, 217 controls	USA	PTPRD	rs10977171	G	96 (437)	96 (417)	ref	ref	N
									C	4 (17)	1 (17)	0.39	0.97	N
								rs72694737	A	97 (442)	98 (423)	ref	ref	N
									G	3 (12)	2 (11)	0.26	1.02	N
								rs35929428	G	93 (420)	90 (389)	ref	ref	N
									A	7 (34)	10 (45)	0.79	0.71	N
								rs2381970	T	95 (433)	94 (407)	ref	ref	N
									C	5 (21)	6 (26)	0.71	0.75	N
			350	238	350 RLS, 238 controls	French Canadian		rs10977171	G	93 (652)	94 (448)	ref	ref	N
									C	7 (48)	6 (28)	0.93	1.28	N
								rs72694737	A	98 (683)	97 (462)	ref	ref	N
									G	2 (17)	3 (14)	0.96	0.59	N
								rs35929428	G	91 (639)	92 (436)	ref	ref	N
									A	9 (61)	8 (40)	0.15	1.07	N
								rs2381970	T	94 (660)	94 (449)	ref	ref	N
									C	6 (40)	6 (27)	0.35	0.90	N
García-Martín et al.[18]	Spain	2015	205	445	205 RLS and 445 gender-matched controls	Caucasian Spanish	HMOX1	rs2071746	A	59.8 (245)	52.1 (463)	0.01	1.37	Y
									T	40.2 (165)	47.9 (425)	0.01	0.73	Y
								rs2071747	G	96.8 (397)	95.5 (848)	0.259	1.44	N
									C	3.2 (13)	4.5 (40)	0.259	0.69	N
									Null	0	0.2 (2)	0.336	0.00	N
							HMOX2	rs2270363	G	71.2 (292)	69.0 (613)	0.425	1.11	N
									A	28.8 (118)	31.0 (275)	0.425	0.90	N
								rs1051308	A	67.8 (278)	64.6 (574)	0.265	1.15	N
									G	32.2 (132)	35.4 (314)	0.265	0.87	N
									Null	0	0.2 (2)	0.336	0.00	N
Gan Or et al.[17]	Canada	2015	627	410	627 RLS and 410 controls	French Canadians and USA	GLO1	rs4746 419	A	0.48	0.42	0.009	1.28	Y
								rs1049346	C-7T	0.46	0.48	0.26	0.91	N
Jiménez-Jiménez et al.[22]	Spain	2014	205	328	205 RLS, 328 controls	White hispanic	SLC1A2	rs3794087	G	74.9 (307)	74.8 (491)	0.991	1.00	N
									T	25.1 (103)	25.2 (165)	0.991	1.00	N
Roco et al.[23]	Spain	2013	205	324	205 RLS and 324 controls	Caucasian Spanish	MAPT1	rs1052553	A	73.2 (300)	72.8 (472)	0.906	1.02	N
									G	26.8 (110)	27.2 (176)	0.906	0.98	N
Jiménez-Jiménez et al.[24]	Spain	2013	206	324	206 RLS and 324 controls	Caucasian Spanish	DRD3	rs6280	Gly9	33.0 (136)	34.1 (221)	0.723	0.96	N
									Ser9	67.0 (276)	65.9 (427)	0.723	1.04	N

Table 2: Case-control study using candidate gene approach[9]

Author	Country	Year	Cases	Controls	Sample size	Race	Gene	SNP	Allele	Frequency of cases	Variants in controls	*P* value	Odds ratio	Significance (after correction)
Akcimen et al.[9]	Canada	2020	1354	1256	1354 RLS and 1256 controls	Québec Population Caucasian	PRMT6	rs12046503	G	0.56	0.61	0.00264	0.84	Y
							DCDC2C	rs10208712	G	0.33	0.37	0.00189	1.21	Y
							MEIS1	rs113851554	T	0.14	0.13	0.0328	1.22	N
							intergenic	rs6747972	A	0.43	0.4	0.0153	1.15	N
							CCDC148	rs80319144	T	0.23	0.24	0.0837	0.89	N
							CRBN	rs1848460	T	0.31	0.28	0.00812	1.18	N
							ATP2C1	rs35987657	G	0.32	0.32	0.707	1.02	N
							CCDC167	rs17636328	G	0.19	0.20	0.349	0.94	N
							BTBD9	rs61192259	T	0.77	0.83	<0.001	1.46	Y
							ZNF804B	rs10952927	G	0.13	0.12	0.0606	1.18	N
							PTPRD	rs1836229	C	0.47	0.5	0.0259	0.88	N
							PTPRD	rs4626664	A	0.13	0.13	0.244	1.11	N
							DACH1	rs340561	T	0.23	0.2	0.0357	1.16	N
							DPH6	rs996064	G	0.06	0.05	0.111	1.22	N
							SKOR1	rs4776976	C	0.79	0.76	<0.001	1.23	Y
							TOX3	rs45544231	G	0.41	0.45	0.00217	1.19	Y
							SETBP1	rs12962305	T	0.27	0.24	0.0445	1.14	N
							MYT1	rs365032	G	0.28	0.27	0.21	1.08	N
			1207	1256			PRMT6	rs12046503	G	0.56	0.61	0.00163	0.83	Y
							DCDC2C	rs10208712	G	0.33	0.36	0.00904	1.18	N
							MEIS1	rs113851554	T	0.14	0.13	0.0101	1.28	N
							intergenic	rs6747972	A	0.43	0.4	0.0392	1.13	N
							CCDC148	rs80319144	T	0.23	0.24	0.0985	0.89	N
							CRBN	rs1848460	T	0.31	0.28	0.0095	1.18	N
							ATP2C1	rs35987657	G	0.32	0.32	0.94	1	N
							CCDC167	rs17636328	G	0.19	0.20	0.293	0.92	N
							BTBD9	rs61192259	T	0.77	0.84	<0.001	1.54	Y
							ZNF804B	rs10952927	G	0.13	0.12	0.0233	1.22	N
							PTPRD	rs1836229	C	0.47	0.5	0.026	0.88	N
							PTPRD	rs4626664	A	0.13	0.13	0.245	1.11	N
							DACH1	rs340561	T	0.24	0.2	0.0298	1.17	N
							DPH6	rs996064	G	0.06	0.05	0.1140	1.23	N
							SKOR1	rs4776976	C	0.80	0.75	<0.001	1.29	Y
							TOX3	rs45544231	G	0.41	0.45	0.00129	1.21	Y
							SETBP1	rs12962305	T	0.28	0.24	0.0111	1.18	N
							MYT1	rs365032	G	0.28	0.27	0.36	1.08	N

(*Continued*)

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Table 2: (*Continued*)

Author	Country	Year	Cases	Controls	Sample size	Race	Gene	SNP	Allele	Frequency of cases	Variants in controls	*P* value	Odds ratio	Significance (after correction)
			974	1256			PRMT6	rs12046503	G	0.56	0.61	0.00205	0.82	Y
							DCDC2C	rs10208712	G	0.34	0.36	0.0246	1.15	N
							MEIS1	rs113851554	T	0.14	0.13	0.0182	1.27	N
							intergenic	rs6747972	A	0.43	0.4	0.062	1.12	N
							CCDC148	rs80319144	T	0.23	0.24	0.0785	0.88	N
							CRBN	rs1848460	T	0.31	0.28	0.00846	1.19	
							ATP2C1	rs35987657	G	0.32	0.32	0.957	1	
							CCDC167	rs17636328	G	0.19	0.20	0.128	0.89	
							BTBD9	rs61192259	T	0.77	0.84	<0.001	1.51	Y
							ZNF804B	rs10952927	G	0.13	0.12	0.0315	1.22	
							PTPRD	rs1836229	C	0.47	0.5	0.0348	0.88	
							PTPRD	rs4626664	A	0.14	0.13	0.26	1.11	
							DACH1	rs340561	T	0.24	0.2	0.00907	1.21	
							DPH6	rs996064	G	0.06	0.05	0.21	1.18	
							SKOR1	rs4776976	C	0.80	0.75	<0.001	1.28	Y
							TOX3	rs45544231	G	0.40	0.45	0.00102	1.22	Y
							SETBP1	rs12962305	T	0.28	0.24	0.00767	1.2	
							MYT1	rs365032	G	0.28	0.27	0.495	1.05	
			851	1256			PRMT6	rs12046503	G	0.56	0.61	<0.001	0.80	Y
							DCDC2C	rs10208712	G	0.34	0.36	0.0358	1.15	
							MEIS1	rs113851554	T	0.14	0.13	0.0837	1.21	
							intergenic	rs6747972	A	0.43	0.4	0.0435	1.14	
							CCDC148	rs80319144	T	0.23	0.24	0.139	0.89	
							CRBN	rs1848460	T	0.31	0.28	0.00384	1.23	
							ATP2C1	rs35987657	G	0.32	0.32	0.357	0.94	
							CCDC167	rs17636328	G	0.19	0.20	0.0695	0.86	
							BTBD9	rs61192259	T	0.77	0.84	<0.001	1.71	Y
							ZNF804B	rs10952927	G	0.13	0.12	0.0849	1.19	
							PTPRD	rs1836229	C	0.47	0.5	0.0324	0.87	
							PTPRD	rs4626664	A	0.14	0.13	0.155	1.15	
							DACH1	rs340561	T	0.24	0.2	0.0216	1.2	
							DPH6	rs996064	G	0.06	0.05	0.337	1.15	
							SKOR1	rs4776976	C	0.80	0.75	<0.001	1.34	Y
							TOX3	rs45544231	G	0.40	0.45	0.00019	1.28	Y
							SETBP1	rs12962305	T	0.28	0.24	0.00139	1.26	Y
							MYT1	rs365032	G	0.28	0.27	0.364	1.07	

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Table 3: Gene variants associated with RLS in GWAS analysis in Korean cohorts[26]

s/n	SNP	Study model	Position	Genes	Gene functions	RLS patient numbers	Healthy subject numbers	Allele 1	Allele 2	*P* value	*OR*	Significance
1	Rs11645604	Initial GWAS	16q23.3 chr16:82152967	MPHOSPH6	RNA binding protein	325	2603	A	G	1.18×10^{-6}	1.531	Y
2	Rs11645604	Replication GWAS	chr16:82152967	MPHOSPH6	RNA binding protein	227	229	A	G	0.6161	0.8734	N
3	Rs9390170	Initial GWAS	chr6:144530548	UTRN	Neurodevelopment	325	2603	C	G	7.67×10^{-6}	0.6778	Y
4	Rs9390170	Replication GWAS	chr6:144530548	UTRN	Neurodevelopment	227	229	C	G	0.036	0.6778	Y
5	RS1918752	Initial GWAS	chr6:144587941	UTRN	Neurodevelopment	325	2603	A	T	1.93×10^{-6}	0.6582	Y
6	Rs3923809	Replication validation	chr6:38473194	BTBD9	Protein-protein interaction	227	229	G	A	0.045	1.3119	Y
7	Rs9296249	Replication validation	chr6:38398065	BTBD9	Protein-protein interaction	227	229	C	T	0.046	1.3028	Y
8	Rs9357271	Replication validation	chr6:38398097	BTBD9	Protein-protein interaction	227	229	T	C	0.8448	0.9669	N
9	Rs1918752	Replication validation	chr6:144587941	UTRN	Neurodevelopment	227	229	T	A	0.6154	1.1205	N
10	Rs6710341	Replication validation	chr2:66531290	MEIS1	DNA binding protein	227	229	G	A	0.8326	1.0818	N
11	Rs2300478	Replication validation	chr2:66554321	MEIS1	DNA binding protein	227	229	G	T	0.5814	0.8087	N
12	Rs1975197	Replication validation	chr9:8846955	PTPRD	A protein tyrosine phosphatase	227	229	A	G	0.666	1.1195	N
13	Rs4626664	Replication validation	chr9:9261737	PTPRD	A protein tyrosine phosphatase	227	229	A	G	0.1567	0.6855	N

Table 4: Gene variants associated with RLS in GWAS analysis in European cohorts[27]

s/n	SNP	Novelty	Position	Genes	Effect Allele	Other Allele	Effect-allele frequency in RLS	Effect-allele frequency in European	Discovery stage meta-analysis		Replication stage meta-analysis		Joint stage meta-analysis	
									P value	*OR* (95%CI)	*P* value	*OR* (95%CI)	*P* value	*OR* (95%CI)
1	rs113851554	Previously Reported	chr2:66523432	MEIS1	T	G	0.07	0.02399	1.1×10^{-180}	2.16 (2.04–2.29)	4.80×10^{-236}	1.82 (1.75–1.89)	2.00×10^{-280}	1.92 (1.85–1.99)
2	rs1820989	Previously Reported	chr2:67842758	MEIS1, C1D, APLF	C	A	0.4693	0.50762	1.23×10^{-20}	0.88 (0.86–0.90)	1.98×10^{-39}	0.89 (0.87–0.90)	1.39×10^{-58}	0.88 (0.87–0.90)
3	rs61192259	Previously Reported	chr6:38486186	BTBD9, GLO1	A	C	0.59	0.58113	1.36×10^{-78}	1.31 (1.28–1.34)	1.05×10^{-112}	1.22 (1.20–1.25)	3.58×10^{-202}	1.26 (1.25–1.28)
4	rs1836229	Previously Reported	chr9:8820573	PTPRD	G	A	0.48	0.45899	1.94×10^{-15}	0.90 (0.88–0.91)	1.57×10^{-29}	0.90 (0.89–0.92)	7.36×10^{-42}	0.90 (0.89–0.91)
5	rs62535767	Previously Reported	chr9:9290311	PTPRD	T	C	0.32	0.30911	3.13×10^{-10}	0.91 (0.88–0.95)	8.77×10^{-7}	0.95 (0.93–0.97)	3.23×10^{-9}	0.94 (0.93–0.96)
6	rs868036	Previously Reported	chr15:67762675	MAP2K5, SMAD3, SKOR1, CLN6	T	A	0.32	0.31476	1.09×10^{-48}	0.80 (0.77–0.83)	9.23×10^{-70}	0.85 (0.84–0.87)	5.48×10^{-69}	0.84 (0.83–0.86)
7	rs45544231	Previously Reported	chr16:52598818	CASC16, TOX3	G	C	0.42	0.5313	4.72×10^{-48}	0.81 (0.79–0.83)	4.36×10^{-87}	0.84 (0.83–0.86)	7.27×10^{-133}	0.83 (0.81–0.84)
8	rs12046503	Novel	chr1:106652717	PMRT6, NTNG1	T	C	0.59	0.57448	3.32×10^{-31}	0.85 (0.84–0.87)	2.03×10^{-29}	0.90 (0.89–0.92)	3.25×10^{-63}	0.88 (0.86–0.89)
9	rs10208712	Novel	chr2:3986856	DCDC2C	G	A	0.36	0.35966	3.78×10^{-15}	0.90 (0.88–0.91)	7.74×10^{-19}	0.92 (0.91–0.94)	1.41×10^{-34}	0.91 (0.90–0.92)
10	rs80319144	Novel	chr2:158343323	CCDC148, PKP4, TANC1	T	C	0.24	0.16998	3.18×10^{-14}	0.89 (0.85–0.92)	1.40×10^{-22}	0.90 (0.89–0.92)	2.55×10^{-26}	0.90 (0.88–0.92)
11	rs1848460	Novel	chr3:3406460	CNTN4, CRBN, LRRN1	T	A	0.26	0.24290	5.38×10^{-14}	1.13 (1.08–1.17)	1.93×10^{-9}	1.06 (1.04–1.08)	2.01×10^{-13}	1.07 (1.05–1.10)
12	rs35987657	Novel	chr3:130816723	ATP2C1, ASTE1,	G	A	0.33	0.33361	4.37×10^{-13}	0.90 (0.88–0.91)	3.34×10^{-23}	0.91 (0.90–0.93)	3.96×10^{-38}	0.90 (0.89–0.92)
13	rs17636328	Novel	chr6:37522755	RNF8, CCDC167, MDGA1, LINC02520	G	A	0.20	0.197532	6.43×10^{-11}	0.89 (0.85–0.92)	7.63×10^{-18}	0.90 (0.89–0.92)	2.55×10^{-26}	0.90 (0.88–0.92)
14	rs10952927	Novel	chr7:88729746	ADAM2, STEAP4, ZNF804B	G	A	0.13	0.12989	1.86×10^{-15}	1.17 (1.13–1.22)	5.01×10^{-17}	1.12 (1.09–1.14)	1.73×10^{-34}	1.13 (1.11–1.15)
15	rs340561	Novel	chr13:72274018	DACH1, DIS3	T	G	0.20	0.20309	3.93×10^{-8}	1.09 (1.05–1.14)	4.91×10^{-7}	1.05 (1.03–1.07)	3.23×10^{-9}	1.06 (1.04–1.08)
16	rs996064	Novel	chr15:35916797	DPH6, MEIS2	T	A	0.06	0.04472	2.96 × 10−9	1.21 (1.14–1.28)	5.45×10^{-21}	1.22 (1.17–1.27)	3.39×10^{-27}	1.22 (1.17–1.26)
17	rs111652004	Novel	chr15:47068169	SEMA6D	T	G	0.10	0.09877	1.05×10^{-10}	0.84 (0.80–0.89)	3.83×10^{-17}	0.87 (0.84–0.90)	2.69×10^{-16}	0.86 (0.83–0.89)
18	rs12450895	Novel	chr17:48695414	HOXB cluster, PRAC1, LINC02086	A	G	0.21	0.20783	4.87×10^{-8}	1.09 (1.05–1.14)	2.01×10^{-10}	1.07 (1.05–1.09)	4.27×10^{-14}	1.08 (1.06–1.10)
19	rs12962305	Novel	chr18:44290278	SETBP1	T	C	0.25	0.25512	1.37×10^{-10}	1.11 (1.06–1.15)	6.59×10^{-5}	1.04 (1.02–1.06)	1.11×10^{-7}	1.05 (1.03–1 07)
20	rs365032	Novel	chr20:64164052	MYT1	G	A	0.27	0.26691	3.36×10^{-14}	1.13 (1.08–1.17)	7.83×10^{-36}	1.13 (1.11–1.15)	1.73×10^{-34}	1.13 (1.11–1.15)

Table 5: Gene variants associated with RLS in GWAS analysis of Caucasian cohorts[28]

s/n	SNP	Novelty	Position	Genes	Effect Allele	Other Allele	Effect-allele frequency in RLS	Effect-allele frequency in Caucasian cohort	Discovery analysis		Follow up analysis		Combined analysis	
									P value	*OR* (95%CI)	*P* value	*OR* (95%CI)	*P* value	*OR* (95%CI)
1	rs10188680	Novel	chr2:189584800	SLC40A1	T	A	0.41	0.40557	4.3×10^{-8}	1.09 (1.06–1.13)	0.13	1.04 (0.99–1.09)	5.4×10^{-8}	1.07 (1.05–1.11)
2	rs10068599	Novel	chr5:171001975	RANBP17	T	C	0.33	0.33669	4.3×10^{-8}	1.10 (1.06–1.13)	0.0031	1.07 (1.03–0.90)	6.9×10^{-10}	1.09 (1.06–1.12)
3	rs112716420	Novel	chr7:1343010	MICA112, UNCX	G	C	0.08	0.03898	4.9×10^{-14}	1.24 (1.18–1.30)	5.6×10^{-6}	1.22 (1.20–1.25)	1.5×10^{-18}	1.25 (1.19–1.31)
4	rs10769894	Novel	chr11:8313948	LMO1	A	G	0.45	0.5646	5.8×10^{-12}	0.89 (0.86–0.93)	0.0029	0.92 (0.87–0.97)	9.4×10^{-14}	0.90 (0.88–0.93)
5	rs58127855	Novel	chr18:59943413	PMAIP1	T	C	0.01	0.0011	5.1×10^{-9}	4.72 (4.20–5.24)	0.84	0.91 (-0.01–1.83)	6.3×10^{-7}	3.03 (2.01–4.97)
6	rs12046503	Previously Reported	chr1:106652717	PMRT6, NTNG1	C	T	0.41	0.42552	1.09×10^{-17}	1.15 (1.11–1.18)	3.32×10^{-32}	1.18 (1.15–1.20)	7.1×10^{-48}	1.16 (1.14–1.18)
7	rs10208712	Previously Reported	chr2:3986856	DCDC2C	G	A	0.36	0.35966	2.34×10^{-9}	0.91 (0.88–0.94)	3.78×10^{-15}	0.90 (0.87–0.93)	5.9×10^{-23}	0.90 (0.88–0.92)
8	rs113851554	Previously Reported	chr2:66523432	MEIS1	T	G	0.07	0.02399	4.5×10^{-100}	1.89 (1.83–1.94)	1.1×10^{-180}	2.16 (2.11–2.21)	3.3×10^{-276}	2.03 (1.99–2.07)
9	rs1820989	Previously Reported	chr2:67842758	MEIS1, C1D, APLF	A	C	0.47	0.5307	2.86×10^{-13}	1.12 (1.09–1.15)	1.23×10^{-20}	1.14 (1.11–1.16)	3.1×10^{-32}	1.13 (1.11–1.15)
10	rs80319144	Previously Reported	chr2:158343323	CCDC148, PKP4, TANC1	T	C	0.24	0.16998	2.11×10^{-7}	0.91 (0.88–0.95)	3.18×10^{-14}	0.89 (0.86–0.92)	5.5×10^{-20}	0.90 (0.88–0.92)
11	rs1848460	Previously Reported	chr3:3406460	CNTN4, CRBN, LRRN1	T	A	0.26	0.24290	7.3×10^{-5}	1.06 (1.03–1.08)	5.38×10^{-14}	1.13 (1.10–1.16)	3.0×10^{-15}	1.09 (1.07–1.11)
12	rs35987657	Previously Reported	chr3:130816723	ATP2C1, ASTE1,	G	A	0.33	0.33361	1.45×10^{-9}	0.90 (0.87–0.94)	4.37×10^{-13}	0.90 (0.87–0.93)	3.9×10^{-21}	0.90 (0.88–0.92)
13	rs17636328	Previously Reported	chr6:37522755	RNF8, CCDC167, MDGA1, LINC02520	G	A	0.20	0.197532	7.63×10^{-8}	0.90 (0.86–0.94)	6.43×10^{-11}	0.89 (0.86–0.92)	2.7×10^{-17}	0.89 (0.86–0.92)
14	rs61192259	Previously Reported	chr6:38486186	BTBD9, GLO1	C	A	0.41	0.41887	4.71×10^{-30}	0.83 (0.80–0.86)	1.36×10^{-78}	0.76 (0.730.79)	1.9×10^{-103}	0.79 (0.77–0.81)
15	rs10952927	Previously Reported	chr7:88729746	ADAM2, STEAP4, ZNF804B	G	A	0.13	0.12989	1.9×10^{-9}	1.13 (1.09–1.17)	1.86×10^{-15}	1.17 (1.13–1.21)	4.1×10^{-21}	1.15 (1.12–1.18)
16	rs1836229	Previously Reported	chr9:8820573	PTPRD	G	A	0.48	0.45899	3.68×10^{-8}	0.92 (0.89–0.95)	1.94×10^{-15}	0.91 (0.89–0.93)	6.2×10^{-22}	0.90 (0.89–0.91)
17	rs62535767	Previously Reported	chr9:9290311	PTPRD	T	C	0.32	0.30911	2.2×10^{-5}	0.93 (0.89–0.96)	3.13×10^{-10}	0.91 (0.88–0.94)	4.8×10^{-14}	0.92 (0.89–0.94)
18	rs340561	Previously Reported	chr13:72274018	DACH1, DIS3	T	G	0.20	0.20309	0.001	1.07 (1.03–1.10)	3.93×10^{-8}	1.09 (1.06–1.21)	2.5×10^{-10}	1.08 (1.06–1.10)
19	rs996064	Previously Reported	chr15:35916797	DPH6, MEIS2	T	A	0.06	0.04472	2.8×10^{-9}	1.21 (1.14–1.27)	2.96×10^{-9}	1.21 (1.15–1.27)	4.4×10^{-16}	1.21 (1.16–1.26)
20	rs111652004	Previously Reported	chr15:47068169	SEMA6D	T	G	0.10	0.09877	2.2×10^{-11}	0.83 (0.77–0.88)	1.05×10^{-10}	0.84 (0.79–0.89)	1.5×10^{-20}	0.83 (0.79–0.87)

(*Continued*)

Table 5: (*Continued*)

s/n	SNP	Novelty	Position	Genes	Effect Allele	Other Allele	Effect-allele frequency in RLS	Effect-allele frequency in Caucasian cohort	Discovery analysis		Follow up analysis		Combined analysis	
									P value	*OR* (95%CI)	*P* value	*OR* (95%CI)	*P* value	*OR* (95%CI)
21	rs868036	Previously Reported	chr15:67762675	MAP2K5, SMAD3, SKOR1, CLN6	T	A	0.32	0.31476	4.67×10^{-28}	0.83 (0.79–0.86)	1.09×10^{-48}	0.80 (0.77–0.83)	1.8×10^{-74}	0.81 (0.79–0 83)
22	rs45544231	Previously Reported	chr16:52598818	CASC16, TOX3	G	C	0.42	0.5313	5.71×10^{-34}	0.82 (0.79–0.85)	4.72×10^{-48}	0.81 (0.78–0.84)	3.9×10^{-80}	0.81 (0.79–0.83)
23	rs12450895	Previously Reported	chr17:48695414	HOXB cluster, PRAC1, LINC02086	A	G	0.21	0.20783	5.69×10^{-6}	1.09 (1.05–1.13)	4.87×10^{-8}	1.09 (1.06–1.12)	1.3×10^{-12}	1.09 (1.07–1.11)
24	rs12962305	Previously Reported	chr18:44290278	SETBP1	T	C	0.25	0.25512	0.0113	1.03 (1.01–1.05)	1.37×10^{-10}	1.11 (1.08–1.14)	4.5×10^{-9}	1.06 (1.04–1.08)
25	rs365032	Previously Reported	chr20:64164052	MYT1	G	A	0.27	0.26691	2.13×10^{-6}	1.09 (1.05–1.12)	3.36×10^{-14}	1.13 (1.10–1.16)	1.5×10^{-18}	1.11 (1.09–1.13)

metabolism of alcohol into acetaldehyde,[16] suggesting that alcohol has protective effects against developing RLS.

TOX high mobility group box family member 3 (TOX3) gene variants (rs3104767) have been found to be associated with painful RLS, which is a sub-phenotype of the condition.[34] Hence, there might be a possible correlation between TOX3 gene variants and risk of developing RLS. In a Caucasian population case-control study, rs3104767 minor allele ($p = 0.0007$, $OR = 0.80$) have been associated with reduced risk in RLS.[15]

In a Chinese study, the BST1 gene variant (rs4273468) has been associated with increased risk of RLS (p value= < 0.001, OR = 2.85).[10] BST1 has a role to play in the brain oxytocin system and is also found to be associated with Parkinson's disease (PD) in some populations.[35] The possible etiological similarities between PD and RLS point to underlying pathophysiologic links to dopaminergic disorders.[36] Similar risk genes such as BST1 raise the possibility of shared pathophysiology mechanisms in both conditions.

CLOCK genes are one of a few circadian genes that control our body's circadian rhythmicity, others include NPAS2 and BMAL1.[37] As RLS often worsens at night, there is a clear circadian rhythm to the condition,[38] suggesting a biological link between circadian genes and the development of primary RLS. A case-control study in Korea reported a lower frequency of the G allele of CLOCK rs1801260 ($p = 0.085$, $OR = 0.74$) among RLS patients. Though the association was borderline, it suggests potential protective effects of the allele on RLS risk.[13]

Heme oxygenase (HMOX) enzymes are involved in the initial steps of heme catabolism and they break down heme into carbon monoxide, iron and biliverdin. The HMOX1 and HMOX2 genes, respectively, code for the two isozymes which are an inducible HMOX-1 and constitutive HMOX-2.[39] HMOX is known to be protective against aging of the brain due to free radical oxidative stress.[40] It is also interesting to note that peripheral hypoxia has been associated with RLS symptoms, and dopaminergic therapy led to improvement of hypoxia and symptoms.[41] Since Iron deficiency anemia (IDA) is a well-studied cause of secondary RLS, genes involved in the iron metabolism pathways are hypothesized to play a role in primary RLS as well.[18] A case-control study in Chinese showed no significant association between HMOX genes and RLS[12] though a weak association was fund between HMOX1 rs2071746T allele ($p = 0.010$, $OR = 0.73$) and decreased risk of RLS.[18]

Alpha synuclein (SNCA) Rep 1 allele variants (265-, 269-, 271-bp alleles) have been associated with increased risk of developing PD,[42] probably through its effects on striatal dopaminergic pathways.[43] In contrast with PD, there was a decrease in Rep 1 271-bp allele frequency among RLS subjects in Caucasian populations.[43] However, a study in Chinese found an increase in the Rep1 269-bp allele frequency ($p = 0.025$, $OR = 0.650$) and decrease in Rep1 267-bp allele frequency ($p = 0.001$, $OR = 0.650$) among RLS patients.[11] Ethnicity differences may contribute to the variance in allele frequencies in the two studies.

MAP2K5/SKOR1 gene variants have been implicated with RLS risk in genome wide association studies.[7] It is suggested that MAP2K pathway has an important role in the protection of dopaminergic neurons, which can contribute to dopaminergic disorders leading to RLS.[7] An earlier study in America showed an association of MAP2K5 rs1026732 with RLS.[44] Marginal associations with RLS in the Chinese cohort have been reported with MAP2K5/SKOR1 rs11635424 ($p = 0.022$, $OR = 1.49$) and

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rs12593813 ($p = 0.2$, $OR = 1.50$).[8] These observations suggest a possible role of these variants in RLS.

In addition to gene variants that have been found to modulate RLS risk, our systemic review also identified some differences in the findings between Asians and Caucasians. As an illustration, the gene variants that increased the risk of RLS in Asian populations include BST1 rs4273468, SNCA Rep1 269bp, IL1B rs1143634, BTBD9 rs9296249, BTBD9 rs9357271, and MAP2K5/SKOR1 rs11635424.[8,10–12] However, the gene variants associated with an increased risk of RLS in Caucasian populations include GABRR3 rs832032, TOX3 rs3104767, ADH1B rs1229984, HMOX1 rs2071746, and GLO1 rs4746419.[14–17] Some of the gene variants that are associated with a decreased risk of RLS in Asian populations include BST1 rs4698412 and SNCA Rep1 267bp,[10,11] whereas in Caucasians, the GABRR3 rs832032, TOX3 rs3104767, and ADH1B rs1229984, and HMOX1 rs2071746 are associated with reduced RLS risk.[14–18]

The apparent differences in the findings between Asians and Caucasians may be due to different ancestral origins, genetic drift (change in frequency of a gene variant due to random chance), or even natural selection. The allele frequency and linkage disequilibrium patterns of genetic loci across populations may vary. For a complex disease like RLS, there may be gene–gene and gene–environmental interactions and other factors that may account for unexplained differences. The differences may also be a result of false positive or negative findings due to the various inherent limitations of genetic association studies (refer to section on limitations below) especially when vast majority of the reported RLS studies have been in Caucasian populations, and it is also unclear if there are any mixed ethnicities in some of the study subjects. Furthermore, most of these candidate gene-based studies were carried out in small cohorts.

During the period of the systematic review, three GWAS studies (one in Asian and two in Caucasian populations) identified several new risk loci/variants for RLS,[26–28] details of which were summarized in Tables 3–5. The risk loci profile appears to be largely different between Asian and Caucasian populations. The rs9390170 variant in UTRN gene was identified to be a genetic marker for RLS in a Korean cohort, whereas rs113851554 in MEIS1 gene was suggested to be a strong genetic factor in Caucasian population.[26–28] BTBD9 and MAP2K5 are two examples of the genes implicated in both Asians and Caucasians when both candidate gene-based and GWAS studies were considered. RLS can be affected by unhealthy lifestyle, such as smoking, alcohol drinking and obesity. However, genetic factors affecting embryonic neurodevelopment, neurogenesis, axon guidance and synapse formation can be the risk factors for RLS.[26–28] Recently, Schormair *et al.*[45] was unable to confirm the significant single-variant associations from candidate gene studies conducted in European populations using the GWAS dataset of the International EU-RLS-GENE Consortium, suggesting that some of the candidate gene-based study findings may be false positive or there are other unknown confounding factors to account for the lack of replication. Interestingly, a recent transcriptome-wide association study involving 15,126 RLS cases and 95,725 controls identified 13 genetic associations (in eight independent loci) at the transcriptome-wide significant level.[46] Consistent with the previous GWAS studies, MEIS1, SKOR1, and MAP2K5 genes are associated with RLS reported in transcriptome-wide association study.[46] However, the transcriptome-wide association study identified six new genetic associations with RLS, including SKAP1, SLC36A1, CCDC57, FN3KRP, and NICOA6/TRPC4AP genes, which have not been identified in the previous GWAS studies.[46]

Limitations & Future Directions

The litmus test of any genetic association studies is the ability to replicate the positive or negative finding. In this regard, most of the reported studies using a candidate gene approach either did not have an independent replication cohort or the findings have not been consistently replicated. In addition, the small sample size has been a major limitation. This is further compounded by the low prevalence of RLS in Asian populations. The sample sizes may have limited the ability to uncover more modest genetic associations with RLS and small effect size differences will not be identified. In addition, publication bias towards positive studies and against negative studies will invariably limit the detection of multiples small gene effects of many variants. This is particularly so for complex disorders such as RLS.

Population stratification can also complicate analysis especially in small sample sizes[11] and frequently documentation of ethnicity has been based on self report which may not be accurate. The recruitment of RLS patients were frequently carried out in tertiary centers and the gender ratios between studies may differ. Inclusion of RLS patients with mild peripheral neuropathy into the case population may also be a confounder in some cases. Control subjects are usually not selected based on a thorough physical examination and detailed history taking and invariably not follow up longitudinally. It is possible that some of them may develop RLS symptoms subsequently. Some studies tried to minimize this by choosing control subjects with mean ages above the age of onset of RLS in case subjects. The definition of RLS is based on key clinical criteria which are primarily based on history taking. Without a clear biological diagnostic marker and gold standard diagnosis, there is a risk of selection of a non homogenous group of patients. Most published studies thus far have utilized a candidate gene approach, which may be biased in the selection of certain gene variants and missing out on testing a large portion of genomic variants.

Large scale multicenter genetic association studies with a standardized recruitment, diagnostic and evaluation protocol will be needed to address some of the major limitations of current studies. When there are sufficient independent studies, meta-analysis to increase the power of analysis will further help to identify more gene variants. Genome wide association approaches using large single nucleotide polymorphism arrays, and if cost not an issue, whole genomic analysis, are more likely to uncover novel variants.[47,48] Recent GWAS studies[26–28] (one study in Asian and two studies in Caucasians) with larger sample sizes and with validation cohorts have attempted to address some of the limitations and also managed to identify additional gene variants and provided useful functional insights into potential pathophysiology.[26–28] The use of contemporary bioinformatic tools to study population structure, ancestry, and significance of structural variants will be useful. It is important to determine if the association signals reflect variants and genes with direct biological relevance to disease. Determination of polygenic scores based on selected variants will add to the data for risk prediction and personalized medicine. For example, a RLS polygenic score has been shown to correlate negatively with duration of education and cognitive scores.[28]

The identification of specific biomarkers for diagnosis or disease progression will be particularly useful in risk stratification

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of patients or in subset analysis. Genotype and phenotype correlation studies can potentially provide clinical value as RLS is a common sleep-related disorder.[1,2,49]

Conclusions

Our systemic review demonstrates that multiple genetic variants modulate risk of RLS in Caucasians and in Asians. While there are a few common genetic loci, genetic susceptibility in sporadic RLS appears to be largely different between the two races, though this interpretation is potentially confounded by the limited studies in Asians. There is a need to expand RLS genetic association studies in multi-ancestry and admixed cohorts to identify potential shared or unique genetic factors. Current identified gene variants are linked to functions affecting embryonic neurodevelopment, neurogenesis, axon guidance, and synapse formation. Functional studies of identified gene variants in both in vitro and in vivo models will help shed further light and identify novel pathophysiologic clues that may lead to development of new therapeutic targets.

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B Tan, XL Pang, S Pang, ZD Zhou searched literature and extracted the data. All authors involved in the analysis and drafting of the manuscript and approved the final version. EK Tan supervised the study.

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