SLEEPJ, 2020, 1–6

doi: 10.1093/sleep/zsz265
Advance Access Publication Date: 30 October 2019
Original Article

ORIGINAL ARTICLE

Genetic and epidemiological characterization of restless legs syndrome in Québec

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Abstract

Currently, a total of 19 genetic loci are associated with the risk for developing RLS. This study aimed to assess these RLS predisposing genetic variants, as well as investigate the epidemiological profile and diagnostic features of individuals with RLS in the Québec population, using an interviewer–administered questionnaire. A total of 18 RLS-associated variants were genotyped in the Québec population-based CARTaGENE cohort. A case–control series consisting of 1,362 RLS cases and 1,379 age-matched unaffected controls was used to conduct a genetic and epidemiological association study that integrated the first four RLS diagnostic features of affected individuals, as well as additional RLS-related questions (e.g. frequency of the symptoms and number of total pregnancies in female). Five RLS-predisposing variants were significantly associated after Bonferroni correction and an additional five variants were nominally associated with RLS ($p < 0.05$). *BTBD9* was the strongest genetic risk factor in our cohort (rs9296249, OR = 1.71, $p = 9.57 \times 10^{-10}$). The patient group that met all four essential diagnostic criteria of RLS provided the most significant genetic findings. These results suggest that employing the questionnaire which included standard diagnostic criteria of RLS could improve the accuracy of the survey-based studies.

Statement of Significance

Over the past 20 years, several clinical and epidemiological studies were performed for restless legs syndrome (RLS) that identified the prevalence, clinical features, and comorbidities. Currently, a total of 19 genetic variants have been associated with RLS through GWAS. This is the first genetic study to examine the genetic association of those risk factors in a population-based samples using standardized questionnaires with detailed diagnostic criteria, as well as the impact of accurate diagnosis and diagnostic criteria.

Key words: restless legs syndrome; *BTBD9*; *MEIS1*; genetic risk factor; IRRLSSG; CARTaGENE; Québec

Submitted: 21 July, 2019; **Revised:** 16 October, 2019

Introduction

Restless legs syndrome (RLS), also known as Willis–Ekbom disease, is a sleep-related movement disorder that is characterized by abnormal sensations and an irresistible urge to move the legs, usually before falling asleep or during the night [1, 2]. Epidemiological studies have shown that 5%–15% of European and North American populations suffer from RLS [3]; in particular, an increased prevalence of up to 18% was reported in the Québec population [4]. In addition, pregnancy is frequently associated with an increased risk of developing RLS [5, 6].

In 2003, the International Restless Legs Syndrome Study Group (IRLSSG) established four criteria that are essential for the diagnosis of RLS: (1) an urge to move the legs usually but not always accompanied by or felt to be caused by uncomfortable and unpleasant sensations in the legs; (2) an urge to move the legs and any accompanying unpleasant sensations begin or worsen during periods of rest or inactivity such as lying down or sitting; (3) an urge to move the legs and any accompanying unpleasant sensations are partially or totally relieved by movement, such as walking or stretching, at least as long as the activity continue; and (4) an urge to move the legs and any accompanying unpleasant sensations during rest or inactivity only occur or are worse in the evening or night than during the day [1]. In 2014, a revised version of the diagnostic criteria was published, which provides a greater specificity with an additional criterion to exclude mimicking conditions [7].

Using a population-based survey of 272 twin pairs from Canada, it has been shown that the concordant rate of RLS is 53.7% in monozygotic twins and 19% in dizygotic twins, indicating a heritability estimate of 69% [8]. Over the past several years, genome-wide association studies (GWAS) led to the identification of six RLS-associated loci: *MEIS1*, *BTBD9*, *SKOR1*, *PTPRD*, *TOX3*, and rs6747972 [9, 10]. In the largest meta-analysis of GWAS, a total of 13 novel variants were identified in individuals of European ancestry, raising the total number of RLS predisposing loci to 19. However, these variants only account for 11.7% of the total SNP heritability [11].

In the previous RLS GWAS [9–12], unrelated probands which were mostly familial RLS cases, and matched controls, were included. To further evaluate the contribution of RLS risk variants in the general Québec population, we conducted an association study using the CARTaGENE cohort, a population-based ascertainment and sample collection [13], in which the CARTaGENE participants completed a previously validated questionnaire addressing the four core IRLSSG criteria. This allowed us to unbiasedly estimate the contribution of RLS risk variants in the Québec population.

Methods

Study population and clinical assessment

CARTaGENE is a large-scale population-based biobank containing comprehensive information such as disease history, lifestyle, and various environmental factors, along with biospecimens of over 40,000 individuals from the province of Québec (Canada). For a detailed demographic composition of CARTaGENE, please refer to Awadalla et al. (2013) [13]. As part of the study design, the participants were invited to answer a set of RLS diagnosis and related questions in the CARTaGENE survey, such as the four diagnostic features of RLS, as well as number of pregnancies, side and frequency of discomforts were also taken into account (Table 1). DNA was available for 1,362 RLS cases (mean age = 55.1 ± 8.02) and 1,379 age-matched controls (mean age = 55.2 ± 7.99). The female to male ratio is 1.78 for cases and 1.76 for controls (Table 2).

Genotyping and statistical analyses

A single OpenArray of 18 RLS-associated variants (or variants that are in high linkage disequilibrium with them) from the last meta-analysis of GWAS were designed; and genotyping was performed using a custom designed TaqMan OpenArray Genotyping platform (Thermo Fisher Scientific, Carlsbad, CA) and analyzed using the QuantStudio 12K Flex v1.2.2 and TaqMan Genotyper v1.4 (Table 3). For the proxy SNPs, the pairwise linkage disequilibrium with the original SNPs was calculated in European population using LDpair [14]. Two RLS-associated variants (rs111652004, *SEMA6D*; rs12450895, *HOXB* cluster) were in a repeat region and failed the genotyping. The rs4776976 variant was genotyped at a later stage using a separate single TaqMan Genotyping assay following the standard protocols.

Quality control and statistical analyses were performed using PLINK version 1.90 [15]. Samples with a per-sample and per-SNP call rate >0.90 were included. A logistic regression test was applied to assess the relationship between the number of pregnancies and RLS in females. For genetic association tests, a step-by-step clustering of patients was done using the RLS diagnostic criteria, and additional RLS-related questions in the CARTaGENE survey (Figure 1, Table 1). A logistic regression adjusted with age and sex as covariates was used to test for association of the selected genetic variants. Additionally, a linear regression was employed to test the association of the variants with age at onset and frequency of symptoms in 851 cases (Supplementary Table S1–3). Association was considered significant below a Bonferroni multiple testing threshold of $p < 0.05/18$ (2.78×10^{-3}). A power calculation was performed using the gnpwr package in R (https://CRAN.R-project.org/package=genpwr) with an additive genetic model for a case-control study, a range of OR (1.3, 1.5, 1.7, 1.9) and MAF (0.05–0.4). The significance level (alpha) was set at Bonferroni multiple testing threshold, 2.78×10^{-3} (Supplementary Table S4). Furthermore, a weighted genetic risk score (GRS) for each individual was calculated in PLINK using the significantly associated variants. The (pseudo-) R^2 measure of the regression analyses was done using rms package in R (http://biostat.mc.vanderbilt.edu/rms) (Supplementary Table S5).

Results

During the study period of recruitment for the CARTaGENE biobank, a total of 3,230 individuals among 20,505 participants who answered "yes" to the first question "Do you have restless legs syndrome?" in the survey; therefore considered as potentially affected (15.8%) had RLS based on the first IRLSSG criteria, with a mean age at onset of 38 ± 14.6 y.o. The subjects who answered "no" to the first question were instructed to skip other RLS-related questions, and age-matched controls were selected among these individuals. DNA was available for 1,362 RLS cases (mean age at onset = 39.7 ± 14.90) and 1,379 age-matched controls from the CARTaGENE biobank. Among these, 851 individuals

Table 1. Questions used to diagnose and characterize the RLS symptoms in CARTaGENE cohort

Do you have restless legs syndrome?/Ressentez-vous des impatiences musculaires? (in overall CARTaGENE cohort)	
Yes/Oui	(14%) 3,230
No/Non	(75%) 17,275
Prefer not to answer/Préfère ne pas répondre	(0.05) 1,117
Do not know/Ne sait pas	(0.06) 1,430
Have you already been diagnosed with RLS?/Avez-vous été diagnostiqué comme étant atteint du syndrome d'impatiences musculaires?	
Yes/Oui	(8.6%) 117
No/Non	(89.6%) 1,220
Prefer not to answer/Préfère ne pas répondre	(1.8%) 25
Do not know/Ne sait pas	0
Generally, your discomforts are worse… / En général, vos impatiences musculaires sont plus marquées…	
At rest/Au repos	(91.5%) 1,246
During activity/À l'activité	(2.5%) 34
No difference/Pas de différence	(5.1%) 70
Prefer not to answer/Préfère ne pas répondre	(0.1%) 1
Do not know/Ne sait pas	(0.8%) 11
Generally, your discomforts are relieved by… / En général, vos impatiences musculaires sont soulagées par…	
Walking or movement/La marche ou le mouvement	(79%) 1,071
Immobility or relaxation/L'immobilité ou la relaxation	(12%) 169
Prefer not to answer/Préfère ne pas répondre	(0.6) 8
Do not know/Ne sait pas	(8.4%) 114
Generally, your discomforts occur… / En général, vous avez un inconfort lié au syndrome des impatiences musculaires…	
Less than once a week/Moins d'une fois par semaine	(49.7%) 677
1 to 3 times a week/1 à 3 fois par semaine	(23.7%) 323
More than 3 times a week/Plus de trois fois par semaine	(23.9%) 325
Prefer not to answer/Préfère ne pas répondre	(0.4%) 5
Do not know/Ne sait pas	(2.3%) 32
Generally, your discomforts are worse… / En général, vos impatiences musculaires sont plus marquées…	
In the morning/Le matin	(2.1%) 29
In the afternoon/L'après-midi	(2.2%) 30
Evening, bedtime, night/La nuit	(82.2) 1,120
No difference/Pas de différence	(11.2%) 152
Prefer not to answer/Préfère ne pas répondre	(0.2%) 2
Do not know/Ne sait pas	(2.1%) 29
Since they appeared your discomforts… / Depuis son apparition, votre inconfort lié au syndrome des impatiences musculaires…	
Are stable/Est stable	(58.5%) 797
Have increased/A diminué	(23.5%) 320
Have decreased/A augmenté	(14.5%) 198
Prefer not to answer/Préfère ne pas répondre	(0.2%) 3
Do not know/Ne sait pas	(3.2%) 44
Generally, your discomforts occur on what side of you body? / Généralement, votre inconfort lié au syndrome des impatiences musculaires apparait de quel côté de votre corps?	
On the left side/Du côté droit	(15%) 204
On the right side/Du côté gauche	(11.5%) 157
On one side but not always the same/D'un seul côté mais jamais le meme	(8.5%) 116
Both sides at the same time/Des deux côtés	(59.5%) 811
Prefer not to answer/Préfère ne pas répondre	(0.2%) 3
Do not know/Ne sait pas	(5.2%) 71
How many times have you been pregnant, including live births, stillbirth, spontaneous miscarriage or abortions?/Combien de fois avez-vous été enceinte, y compris les naissances vivantes, les mortinaissances, les fausses couches et les avortements?	
Male	(–) 771
0	(13.6%) 199
1	(13.1%) 191
2	(32.6%) 476
≥3	(40.5%) 591
Prefer not to answer/Préfère ne pas répondre	(0.2%) 3

*Restless legs syndrome (RLS) is characterized by discomforts in the limbs with an irresistible desire to move./Le syndrome des impatiences musculaires sont des inconforts au niveau des membres (bras, jambes) qui s'accompagnent d'un besoin irrésistible de bouger.

(581 female, 270 male) answered 'yes' to all four questions based on the four IRLSSG criteria (Table 1, Figure 1). In women, having two and more pregnancies is associated with a higher risk of RLS (two pregnancies, OR = 1.28, 95% confidence interval = 1.01–1.62, $p = 0.042$; three or more pregnancies, OR = 1.29, 95% confidence interval = 1.03–1.61, $p = 0.025$).

Sample selection and grouping was based on the RLS-related questions in the CARTaGENE survey as shown in the flowchart

(Figure 1). After variant quality control and filtering steps, a total of 1,256 controls and 1,354 (group A); 1,207 (group B); 974 (group C); 851 (group D) cases were included in a series of regression analyses (Table 3). Five genomic risk loci were replicated in the Québec population, at a Bonferroni-corrected level of p-value less than 2.78×10^{-3}. The *BTBD9* variant was the most significantly associated genetic risk factor for RLS (OR = 1.71, $p = 9.57 \times 10^{-10}$). Other significantly associated loci were *PRMT6* (OR = 0.80, $p = 8.95 \times 10^{-4}$), *SKOR1* (OR = 1.34, $p = 2.81 \times 10^{-4}$), *TOX3* (OR = 1.28, $p = 1.90 \times 10^{-4}$) and *SETBP1* (OR = 1.26, $p = 1.39 \times 10^{-3}$). Allele frequencies of the selected variants are presented in Supplementary Table S1.

The overall strength of association increased as more diagnostic criteria were added to filter the individuals (Table 3). GRSs for all groups were associated with RLS ($p < 0.05$) (Supplementary Table S5). The logistic regression of group D had a lower p-value (1.1×10^{-4}) comparing to the other groups. The increased (pseudo-) R^2 for the association of group D indicated a small improvement prediction accuracy. Furthermore, group D had an increased percentage of participants with a diagnosis (10.2%) compared to group A (8.6%). Using a total of 178 cases with symptoms more than three times a week and 1256 controls, variants in *BTBD9* (OR = 1.79, $p = 3.8 \times 10^{-4}$) and *SETBP1* (OR = 1.48, $p = 1.84 \times 10^{-3}$) were found to be associated with more frequent symptoms (Supplementary Table S2). The linear regression test revealed no association of variants with age at onset in RLS (Supplementary Table S3).

Table 2. Demographic characteristics of CARTaGENE participants

	Cases (n = 1,362)	Controls (n = 1,379)
Male/female (%)	35.9/64.1	36.2/63.8
Mean age	55.1 ± 8.02	55.2 ± 7.99
Mean age at onset	38 ± 14.6	–
Self-reported ethnicity (%) (European/other/do not know)	96.5, 2.5, 1	94.1, 4.8, 1.1

Table 3. Association results for RLS-risk variants in the CARTaGENE cohort. Sample filtering was done based on the four diagnostic features for groups A, B, C, D; respectively, as represented in the flowchart in Figure 1

			A		B		C		D	
Variant (Variant in LD (D'/R^2))	Effect allele	Gene	P	Odds ratio (95% CI)	P	Odds ratio (95% CI)	P	Odds ratio (95% CI)	P	Odds ratio (95% CI)
rs10494048* (rs12046503 (1/0.99))	G	*PRMT6*	**2.64×10^{-3}**	0.84 (0.75–0.94)	**1.63×10^{-3}**	0.83 (0.74–0.93)	**2.05×10^{-3}**	0.82 (0.74–0.93)	**8.95×10^{-4}**	0.80 (0.71–0.92)
rs10208712	G	*DCDC2C*	**1.89×10^{-3}**	1.21 (1.07–1.36)	9.04×10^{-3}	1.18 (1.04–1.33)	2.46×10^{-2}	1.15 (1.02–1.31)	3.58×10^{-2}	1.15 (1.01–1.32)
rs113851554	T	*MEIS1*	3.28×10^{-2}	1.22 (1.02–1.47)	1.01×10^{-2}	1.28 (1.06–1.55)	1.82×10^{-2}	1.27 (1.04–1.54)	8.37×10^{-2}	1.21 (0.98–1.49)
rs6747972	A	*Intergenic*	1.53×10^{-2}	1.15 (1.03–1.28)	3.92×10^{-2}	1.13 (1.01–1.26)	6.20×10^{-2}	1.12 (0.99–1.26)	4.35×10^{-2}	1.14 (1.00–1.29)
rs80319144	T	*CCDC148*	8.37×10^{-2}	0.89 (0.78–1.02)	9.85×10^{-2}	0.89 (0.78–1.02)	7.85×10^{-2}	0.88 (0.77–1.01)	1.39×10^{-1}	0.89 (0.77–1.04)
rs1848460	T	*CRBN*	8.12×10^{-3}	1.18 (1.04–1.33)	9.50×10^{-3}	1.18 (1.04–1.34)	8.46×10^{-3}	1.19 (1.05–1.35)	3.84×10^{-3}	1.23 (1.07–1.41)
rs11713931* (rs35987657 (1/1))	G	*ATP2C1*	7.07×10^{-1}	1.02 (0.90–1.15)	9.40×10^{-1}	1.00 (0.88–1.13)	9.57×10^{-1}	1.00 (0.88–1.13)	3.57×10^{-1}	0.94 (0.82–1.08)
rs17636328	G	*CCDC167*	3.49×10^{-1}	0.94 (0.81–1.08)	2.93×10^{-1}	0.92 (0.79–1.06)	1.28×10^{-1}	0.89 (0.77–1.03)	6.95×10^{-2}	0.86 (0.73–1.01)
rs9296249* (rs61192259 (0.86/0.33)	T	*BTBD9*	**1.68×10^{-7}**	1.46 (1.27–1.68)	**1.40×10^{-8}**	1.54 (1.32–1.78)	**6.00×10^{-8}**	1.51 (1.31–1.78)	**9.57×10^{-10}**	1.71 (1.44–2.02)
rs10952927	G	*ZNF804B*	6.06×10^{-2}	1.18 (0.99–1.39)	2.33×10^{-2}	1.22 (1.03–1.46)	3.15×10^{-2}	1.22 (1.02–1.46)	8.49×10^{-2}	1.19 (0.98–1.44)
rs1434273* (rs1836229(0.99/0.97))	C	*PTPRD*	2.59×10^{-2}	0.88 (0.79–0.98)	2.60×10^{-2}	0.88 (0.78–0.98)	3.48×10^{-2}	0.88 (0.78–0.99)	3.24×10^{-2}	0.87 (0.77–0.99)
rs4626664	A	*PTPRD*	2.44×10^{-1}	1.11 (0.93–1.31)	2.45×10^{-1}	1.11 (0.93–1.32)	2.60×10^{-1}	1.11 (0.93–1.33)	1.55×10^{-1}	1.15 (0.95–1.40)
rs340542* (rs340561(0.97/0.94))	G	*DACH1*	3.57×10^{-2}	1.16 (1.01–1.32)	2.98×10^{-2}	1.17 (1.02–1.34)	9.07×10^{-3}	1.21 (1.05–1.40)	2.16×10^{-2}	1.20 (1.03–1.40)
rs996064	T	*DPH6*	1.11×10^{-1}	1.22 (0.96–1.56)	1.14×10^{-1}	1.23 (0.95–1.58)	2.10×10^{-1}	1.18 (0.91–1.54)	3.37×10^{-1}	1.15 (0.87–1.53)
rs4776976	C	*SKOR1*	**7.99×10^{-5}**	1.23 (1.08–1.41)	**3.49×10^{-5}**	1.29 (1.12–1.48)	**7.73×10^{-4}**	1.28 (1.11–1.48)	**2.81×10^{-4}**	1.34 (1.15–1.57)
rs3104767* (rs45544231 (1/0.99))	G	*TOX3*	**2.17×10^{-3}**	1.19 (1.07–1.36)	**1.29×10^{-3}**	1.21 (1.08–1.36)	**1.02×10^{-3}**	1.22 (1.08–1.38)	**1.90×10^{-4}**	1.28 (1.08–1.38)
rs12962305	T	*SETBP1*	4.45×10^{-2}	1.14 (1.00–1.29)	1.11×10^{-2}	1.18 (1.04–1.35)	7.67×10^{-3}	1.2 (1.05–1.37)	**1.39×10^{-3}**	1.26 (1.09–1.45)
rs365032	G	*MYT1*	2.12×10^{-1}	1.08 (0.96–1.27)	3.60×10^{-1}	1.06 (0.93–1.20)	4.95×10^{-1}	1.05 (0.92–1.20)	3.64×10^{-1}	1.07 (0.93–1.23)

*Variants in high linkage disequilibrium (LD) with the originally defined loci.

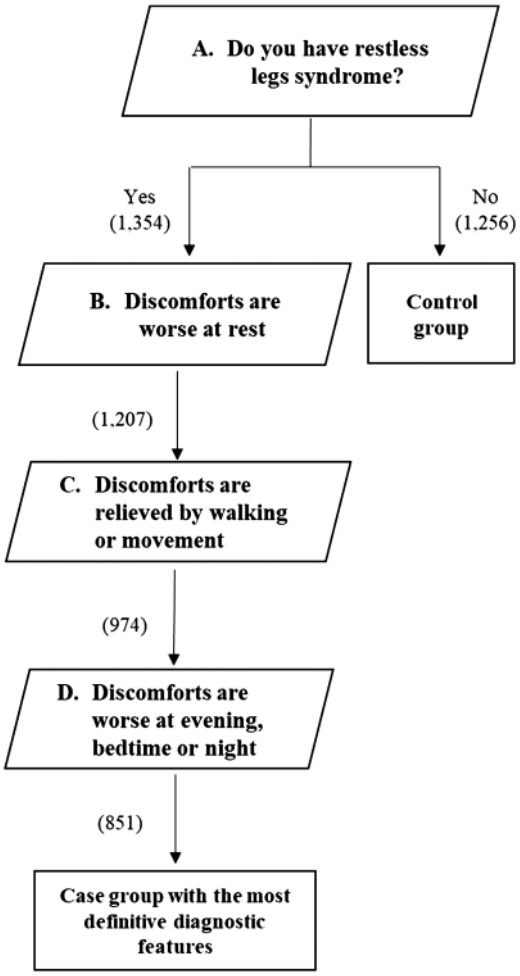

Figure 1. The flowchart of sample selection based on the RLS-related questions in the CARTaGENE survey.

For a minimum power of 80%, variants with an OR above 1.7 or a MAF higher than 0.3 would be required using the 851 cases and 1,256 controls. The available sample size could not provide the sufficient power to establish the associations for variants with an OR ≤ 1.3 (Supplementary Table S4).

Discussion

A search of the relevant literature suggests this study is the first to examine the RLS risk variants in a population-based ascertainment/diagnosis using a standardized RLS questionnaire. Using the answers to the questionnaire of the CARTaGENE biobank, we performed a step-by-step filtering, and observed the effect of each filter on the genetic association results. We confirmed the increased prevalence [4], the female preponderance and association of number of total pregnancies with an increased risk of RLS in the Québec population [16]. Variants in *MEIS1*, *BTBD9*, *SKOR1* [9], *PTPRD* [10], and *TOX3* [17] were previously associated with RLS in French-Canadian population. However, only *BTBD9*, *SKOR1*, and *TOX3* variants were replicated in the present study. Despite the significant decrease in sample size, with the addition of each criterion, an overall increase in the strength of genetic association was observed, supporting the important contribution of the diagnostic criteria in survey-based studies.

So far, the most significantly associated genetic variant identified in RLS is *MEIS1*. The *MEIS1* risk haplotype was reported to regulate ferritin expression suggesting a role in iron homeostasis, which is an important pathophysiological pathway in RLS [18, 19]. Furthermore, a key interaction between two RLS associated genes *MEIS1* and *SKOR1* was established, with the *MEIS1* risk haplotype having a positive effect on the expression of the *SKOR1* transcription factor. This interaction was observed in vitro and involved the direct binding of MEIS1 to specific regulatory sites in the upstream region of *SKOR1* [20]. This study also showed that the reduced *MEIS1* expression known to be associated with its risk haplotype leads to a reduced *SKOR1* expression only in the presence of the *SKOR1* risk allele (C) of the variant rs4776976 [20]. The association with the *MEIS1* variant was not replicated in our survey-based population; nevertheless, the association of *SKOR1* variant was confirmed. This may be due in part to a low sample size, absence of sufficient power for the variants with lower effect size, and/or reflect the difference between the kinds of subjects that are referred to a sleep clinic as opposed to a population based sample. For example, *MEIS1* variant may be more associated with severity of symptoms. Furthermore, our power calculation showed that due to low sample size, the sufficient power could not be provided for the variants with lower OR (Supplementary Table S4).

Hening et al. showed that symptoms and conditions not related to RLS sometimes satisfy all four characteristics of RLS. The first four diagnostic criteria of RLS were unable to exclude the confounding conditions [21]. In the updated version of IRLSSG diagnostic criteria, an additional criterion: "the occurrence of the above features are not solely accounted for as symptoms primary to another medical or a behavioral condition (e.g. myalgia, venous stasis, leg edema, arthritis, leg cramps, positional discomfort, habitual foot tapping)" helps to rule out individuals who are RLS mimics [7]. The IRLSSG rating scale (IRLS) is an objective way to measure the severity of the affected individuals disease, even in the context of a self-evaluation [22–24]. Future studies that would integrate the IRLS into a survey-based approaches like the one used here, as well as the availability of a larger cohort of affected individuals, might reveal a more accurate association.

Supplementary Material

Supplementary material is available at *SLEEP* online.

Table S1. Effect allele frequencies for the selected risk variants.

Table S2. Association results of RLS-risk variants using 178 cases with a frequency of symptoms more than three times a week and 1,256 controls.

Table S3. Association results of RLS-risk variants with age at onset.

Table S4. Power analysis using 851 cases and 1,256 controls. MAF = Minor Allele Frequency, OR = Odds Ratio.

Table S5. Association of GRSs with RLS. Groups A, B, C, D represent the original groups in Table 3 filtered by four diagnostic criteria. A′, B′, C′, D′ represent the groups reoriented based on the different combinations of filtering order.

Funding

We thank the CARTaGENE participants and team for data collection and assistance. This research was undertaken thanks

in part to funding from the Canada First Research Excellence Fund, awarded to McGill University for the Healthy Brains for Healthy Lives initiative. F.A. was funded by the Healthy Brains for Healthy Lives and the Fonds de Recherche du Québec–Santé. J.P.R. has received a Canadian Institutes of Health Research Frederick Banting & Charles Best Canada Graduate Scholarship (FRN 159279). Z.G.O. is supported by the Fonds de recherche du Québec - Santé (FRQS) Chercheurs-boursiers award, and by the Young Investigator Award by Parkinson's Canada. G.A.R. holds a Canada Research Chair in Genetics of the Nervous System and the Wilder Penfield Chair in Neurosciences.
Conflict of interest statement: None declared.

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