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# Restless Legs Syndrome is associated with major comorbidities in a population of Danish blood donors

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## Abstract

**Background:** Restless Legs Syndrome (RLS) is characterized by uncomfortable nocturnal sensations in the legs making sedentary activities and sleep difficult, and thus is linked with psychosocial distress. Due to the symptomatology and neurobiology of RLS (disrupting brain iron and dopamine) it is likely that RLS associates with poorer health-related quality of life (HRQL) and depressive disorder. The objective of this study was to investigate the RLS-HRQL and the RLS-depressive disorder links in a generally healthy population that is not biased by medications.

**Methods:** Complete data, including the Cambridge-Hopkins RLS questionnaire, the 12-item short-form standardized health survey (SF-12), the Major Depression Inventory (MDI), body mass index, smoking status, alcohol consumption, and education were available for 24,707 participants enrolled in the Danish Blood Donor Study from May 1, 2015 to February 1, 2017. Information on quality of sleep was available for all RLS cases. T-tests and multivariable logistic regression models were applied to examine the associations of RLS and MDI scores, and the physical and mental component scores (PCS and MCS) of SF-12, respectively. Analyses were conducted separately for men and women.

**Results:** RLS associated with poorer MCS and poorer PCS. Moreover, Participants with RLS were more likely to classify with depressive disorder. Poor quality of sleep was associated with depressive disorder and poorer MCS among RLS cases, and with poorer PCS in female RLS cases.

**Conclusion:** Thus, we demonstrated that RLS is associated with a significantly lower HRQL and a higher prevalence of depressive disorder among otherwise healthy individuals.


***Key words: Restless legs syndrome; RLS; WED; Willis-Ekbom; quality of life; depressive disorder; sleep disturbance; comorbidities; the Danish blood donor study***

## Highlights

- Restless leg syndrome (RLS) is associated with increased depressive symptoms
- RLS is associated with reduced health related quality of life
- Poor quality of sleep increases the risk of these detrimental links among RLS cases

Restless Legs Syndrome (RLS) is a common neurological sensorimotor disorder with symptoms that include uncomfortable or painful sensations in the extremities, predominantly in the legs. Symptoms occur or become worse during rest and at night, therefore individuals with RLS often suffer from severe sleep disturbance [1, 2]. Previous studies have reported that RLS sufferers are more prone to experiencing inhibited cognitive functioning and psychosocial distress, including problems with social activities, family life, and occupational life due to fatigue following disruptions in sleep [3, 4]. Furthermore, associations between RLS and reduced health-related quality of life (HRQL) and increased depression, respectively, have been reported in several studies [1, 5-14]. The majority of these studies have included either a small population sample or participants with multiple medical problems plausibly linked with RLS, HRQL, or with depressive disorder. Thus, it is possible that the reported associations were biased by other diseases or medical treatments. For instance, it has been found that antidepressant medications exacerbate or provoke RLS symptoms [15]. However, due to the nature of the RLS symptomatology and neurobiology [14, 16] it is plausible that RLS and depression have a common etiology, as RLS has been linked with disruptions in major brain iron and therefore dysfunction in the dopaminergic system [17]. It may also be that the reduced HRQL and increased depression observed in RLS sufferers were caused by RLS-related sleep problems. Furthermore, HRQL can be divided into two components: mental HRQL (MCS) and physical HRQL (PCS), which are both likely to be negatively affected by RLS, but perhaps in different ways. It is well-known that the RLS disorder varies greatly between sufferers in intensity, herein both severity and frequency of the symptoms, and we have previously reported that the RLS prevalence in a Danish blood donor population was higher among women than among men (7.5% in women versus 4.5% in men), and that RLS-related risk factors differed according to sex [18]. Therefore, it is possible that RLS affects men and women differently and that the magnitude of consequences varies according to the intensity of the disorder. Hence, potential negative consequences of suffering from RLS need to be studied in a large population sample that can be classified as otherwise healthy. As blood donors are required to be generally healthy and not subjected to medical treatment to be eligible for donation [19], they are ideal for studying such consequences. Moreover, because it is well established that blood donors have an

increased risk of becoming iron deficient, which in turn may lead to RLS [20, 21], it is imperative that detrimental links to RLS in blood donors is clarified. However, it should be noted that we have previously shown that iron deficiency does not increase the risk of reduced HRQL among Danish blood donors [22].

The purpose of this study was therefore to extend current knowledge by examining potential negative consequences of RLS (reduced MCS, reduced PCS, and increased depression), stratified according to sex and symptom intensity, while taking relevant covariate factors into account, in a large population of otherwise healthy Danish blood donors.

# 1. Methods

### 1.1 Data

The present study is based on data from the Danish Blood Donor Study (DBDS) (www.dbds.dk), which is an ongoing national cohort of Danish blood donors with only 5% of the invited donors declining to participate. The cohort is described in detail elsewhere [23]. Briefly, the DBDS utilizes the infrastructure in the Danish blood banks to collect data for a range of specific and general health research purposes. Blood donors who donated two or more times in a Danish blood bank were asked to participate in the study. Upon inclusion into the study, each participant filled out a comprehensive health related electronic questionnaire. This questionnaire provided information on RLS, HRQL, depressive symptoms, and previous depression diagnoses. Moreover, the participants gave permission for their questionnaire data to be linked with data from the Danish population registers. Before donating blood, Danish blood donors are asked a number of screening questions to asses if they felt completely well, if they have taken any medications, or if they have been sick since their last donation. These screening questions cover both physical and mental health, which assures that the donors are generally healthy upon each donation they make, and therefore also upon inclusion into this study as well.

### *1.1.1 Ethics statement*

Oral and written informed consent was obtained from all participants. The study was approved by The Scientific Ethical Committee of Central Denmark (M-20090237). Additionally, the biobank and research database have been approved by the Danish Data Protection Agency (2007-58-0015).

### *1.1.2 Identifying restless legs syndrome (RLS)*

RLS was assessed using the 10 item Cambridge-Hopkins RLS questionnaire (CH-RLSq), the only tool validated in a population of blood donors [24] (diagnostic sensitivity 87.2% and specificity 94%) [25], as well as recommended as one of the most accurate scales [26]. When the CH-RLSq was translated from English to Danish it was done by three persons independently of each other, and then merged into a "consensus" translation by one of the translators. Subsequently, the Danish versions were translated back into English by three individuals who were not familiar with the original English version of the questionnaire. Again, the three translations were merged by one of the translators. The translated English versions were evaluated by two native English speaking experts in RLS. Both the translated English version and the reverse translated Danish version of the questionnaire were evaluated by a bilingual Danish expert in neurology and sleep medicine. Based on these evaluations, the final version was completed. Furthermore, participants with RLS symptoms rated their severity on a four point Likert scale, while they rated their frequency on a six point Likert scale ranging from "not uncomfortable" to "extremely uncomfortable" and from "one day a month" to "every day", respectively. Further, participants experiencing RLS symptoms were asked about their quality of sleep (four point Likert scale) and experience of involuntary jerking of the legs during sleep (binary measure).

### *1.1.3 Self-rated health-related quality of life (HRQL)*

HRQL was measured using the validated 12-item short form health survey (SF-12) [27], an abbreviated version of the 36-item short form standardized health survey (SF-36), which has been validated in the Danish language [27]. SF-36 was developed for population survey and is the most commonly used measure of HRQL [28]. SF-12 measures a mental and physical component score (MCS and PCS). Each

score comprises four subscales. MCS comprises of vitality, social functioning, role-emotional, and mental health. PCS comprises of physical functioning, role-physical, bodily pain, and general health. The SF-12 responses were scored assigning weights to each item (ranging from -16.15395 to +4.61446) and adding the sum of the items to a constant (57.65693 and 60.58857) for PCS and MCS, respectively as recommended by Quality Metric Inc. [28]. The lower the MCS and PCS scores, the worse the components of the HRQL are experienced by the respondent. MCS and PCS have both been normalized in a Danish background population to a mean MCS of 52.8 (SD, 8.3) and a mean PCS of 51.0 (SD, 8.1) [27].

#### *1.1.4 Depressive symptoms*

Information on current depressive symptoms was collected as self-report using the Major Depression Inventory (MDI), which has been validated in the Danish language [29-31]. The MDI covers both the ICD-10 and the DSM-IV symptoms of depression [32]. The MDI was used to appraise the presence of the ten depressive symptoms within the last two weeks prior to inclusion into the study with depressive disorder classified as an MDI score of more than 20 [29]. As supplementary analyses the participants were asked if they had ever been diagnosed with depressive disorder by a medical doctor. This question was used to assess whether previous clinical depression at some point in the participants' lives was associated with current RLS.

#### *1.1.5 Covariates*

The questionnaire provided information on body mass index (BMI), smoking status and alcohol consumption. The National Education Register of Denmark provided data on the participants' highest achieved educational level. Information on donation frequency for the past three years was collected from blood bank registers across Denmark. Linking of individual level data from the register with data from the questionnaire had high validity, due to the use of unique Danish Civil Registration Numbers

[33].

### 1.2 Statistical analyses

Statistical analyses were conducted using Stata/SE 14.0, StataCorp, College Station, TX. Normally distributed data were described with means and standard deviations (SD). MCS and PCS were dichotomized using the $10^{th}$ and the $25^{th}$ percentile scores as cutoffs to define participants with low or reduced HRQL, respectively. Low MCS and Low PCS were defined as the lowest $10^{th}$ percentile of scores because this definition has been used previously when examining the probability of low HRQL in the DBDS population [22]. The MDI score was dichotomized using a score of 20 or above to define participants with depressive disorder. Low educational level was defined as high school/vocational course or lower. Frequent alcohol consumption was defined as reports of alcohol consumption "several times a week" or "every day". Distributions of all binary variables among the participants were described in percentages and statistically significant differences were investigated by t-tests for normally distributed data and by $chi^2$ tests for dichotomous data. In order to obtain normal distributions of the scales before examining statistically significant differences in scores, a power transformation of five was applied to MCS and PCS, and the MDI scale was log-transformed. Difference in answers to specific questions on the SF-12 and the MDI scale between RLS cases and non-RLS cases was examined using t-tests. Multivariable logistic regression analyses were applied to assess the probability of scoring low and reduced PCS and MCS, classifying with depressive disorder, and having a previous diagnosis of depression among participants with RLS compared to participants without. Age, BMI, smoking, frequent alcohol consumption, low educational level, and number of whole blood donations three years prior to inclusion into this study were all considered possible covariates of the associations [21, 34, 35] and were entered in all multivariable regression analyses as binary variables, except for donation frequency, age, and BMI, which were entered as continuous variables. Analyses assessing the relationship between RLS and a previous depressive disorder diagnosis, and reduced MCS/PCS are presented as supplementary in the Appendix. Additionally, multivariable logistic regression models were applied among RLS cases to examine the effect of quality of sleep (poor vs. good) and involuntary

leg movements during sleep (ILM) (yes vs. no) on HRQL and depressive disorder. These analyses on the effect of sleep only comprised RLS cases. Thus, to maintain the statistical power they were adjusted for sex, but not stratified.

## 2. Results

### *2.1 Characteristics of the study population*

Complete data were available for 13,419 men and 11,288 women, of whom 1,298 (5.3%) were classified as suffering from RLS (4.0% men and 6.7% women). 46.5% of the RLS cases characterized their symptoms as moderately to extremely uncomfortable (severe) and 30.7% experienced the symptoms more than two times a week (frequent). Overall, there were 221 participants (0.9%) who had RLS symptoms that were characterized as both severe and frequent, while 1,077 (4.4%) presented with mild or infrequent RLS symptoms.

A total of 27,315 participants were recruited to this study from May 1 2015 to February 1 2017. Among these, 891 were missing at least one item of the CH-RLSq, 626 were missing information on covariates (education, lifestyle, and donation history), 791 and 605 were missing at least one item on the MDI and SF-12, respectively, and were therefore excluded from the study. Finally, 24 participants who would otherwise characterize with RLS were excluded because they answered "yes" or "don't know" to both of the following two questions from the CH-RLSq "*Are these feelings ever due to muscle cramps?*" and "*If so, are they always due to muscle cramps?*" and 6 RLS cases were excluded due to missing information on frequency and severity of RLS symptoms, quality of sleep, and ILM. Thus, analyses were conducted on 24,707 study participants with complete data available. We investigated interactions between covariates and found the effect of all covariates (except low education) on RLS to be significantly correlated with sex ($P$<0.001). The subsequent analyses were therefore stratified on sex. The prevalence of depressive disorder, previous depression diagnosis, and poorer MCS/PCS was higher among RLS cases compared to participants without RLS ($P$<0.001) (Table 1).

**Table 1. Demographic and clinical features of the study population**

| Characteristic[×] | RLS | No RLS symptoms | |
|---|---|---|---|
| | N=1,298 | N=23,409 | P * |
| Female sex (%) | 58.2 | 45.0 | <0.001 |
| Age (mean ±SD) | 42.2 (±12.2) | 41.4 (±12.7) | <0.01 |
| Low MCS (%) | 14.3 | 9.8 | <0.001 |
| Reduced MCS (%) | 32.4 | 24.5 | <0.001 |
| Low PCS (%) | 14.6 | 9.6 | <0.001 |
| Reduced PCS (%) | 33.3 | 24.5 | <0.001 |
| Depressive disorder (%) | 5.6 | 2.9 | <0.001 |
| Previous diagnosis with depressive disorder (%) | 12.2 | 7.5 | <0.001 |
| Body mass index (Mean ±SD) | 25.8 (4.3) | 25.7 (4.1) | 0.287 |
| Low education (%) | 56.9 | 54.3 | 0.073 |
| Smoker (%) | 15.3 | 12.8 | <0.01 |
| Frequent alcohol consumption (%) | 38.1 | 35.5 | 0.065 |
| Donation frequency for the past 3 years (mean, SD) | 5.2 (2.5) | 5.4 (2.6) | <0.001 |

[×]Low mental health-related quality of life (MCS) and low physical health related quality of life (PCS) were defined using the 10$^{th}$ percentile as cutoff and reduced MCS and PCS were defined using the 25$^{th}$ percentile as cutoff. Depressive disorder was defined as a Major depression inventory score (MDI) > 20 and previous diagnosis with depressive disorder was defined as a diagnosis given by a medical doctor at some point in the participants' lives.

*Age and MDI were log-transformed and a power five transformation was applied to MCS and PCS in order to obtain normal distribution before examining significant differences between RLS and non-RLS sufferers.

*2.2 Differences in scores between individuals with and without restless legs syndrome (RLS)*

The median MCS score was 56.9 (25$^{th}$ and 75$^{th}$ percentiles: 52.9, 58.9) for women and 57.5 (53.9, 58.9) for men. The median PCS score was 56.6 (25$^{th}$ and 75$^{th}$ percentiles: 54.5, 57.9) for women and 56.6 (54.4, 57.7) for men. The median MDI score was 4 (25$^{th}$ and 75$^{th}$ percentiles: 2, 7) for women and 4 (1, 7) for men. We found lower median scores of the MCS and PCS, respectively, among RLS cases with all levels of symptom intensity among both men and women; they were however, statistically insignificant for PCS score among male RLS cases with mild or infrequent symptoms. The lowest median MCS score was observed among participants who reported frequent experience of RLS symptoms among both men and women. Men with severe RLS symptoms and women with frequent symptoms had the lowest median of PCS scores (Table 2). Not one specific question on the SF-12 caused the lower MCS and PCS scores in RLS cases. Finally, we found statistically significant higher

mean MDI scores among both female and male RLS cases with all levels of symptoms severity (Table 2). No specific questions on the MDI scale caused this.

**Table 2. Differences in mental- and physical health-related quality of life scores (MCS and PCS) and major depression inventory score (MDI) according to Restless Legs Syndrome (RLS) status**

| **T-tests*** | | Women (n controls = 10,532) | | | | Men (n controls = 12,877) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Cases** | **Non-cases** | | | **Cases** | **Non-Cases** | | |
| Score | Symptom severity | Median ($25^{th}$;$75^{th}$ percentiles) | | P | n (cases) | Median ($25^{th}$;$75^{th}$ percentiles) | | P | n (cases) |
| MCS | *All cases of RLS* | 56.2 (50.9;57.8) | 56.9 (52.9;58.9) | <0.001 | 756 | 56.5 (51.7;57.9) | 57.6 (54.0;58.9) | <0.001 | 542 |
| | *Frequent*[××] | 55.3 (47.1;58.6) | | <0.001 | 225 | 55.4 (49.5;57.7) | | <0.001 | 174 |
| | *Severe* | 56.7 (51.2;58.6) | | 0.011 | 392 | 56.2 (50.9;58.6) | | <0.001 | 212 |
| | *Infrequent or mild* | 56.3 (51.3;57.7) | | <0.001 | 609 | 56.7 (52.4;58.2) | | <0.001 | 468 |
| PCS | *All cases of RLS* | 56.2 (53.2;59.3) | 56.6 (54.6;57.9) | <0.01 | 756 | 56.1 (53.2;57.5) | 56.6 (54.5;57.7) | <0.001 | 542 |
| | *Frequent*[××] | 55.4 (50.5;57.0) | | <0.01 | 225 | 55.6 (52.1;57.6) | | <0.001 | 174 |
| | *Severe* | 55.9 (52.8;57.5) | | <0.001 | 392 | 55.4 (51.8;57.0) | | <0.001 | 212 |
| | *Infrequent or mild* | 56.4 (53.5;57.6) | | <0.01 | 609 | 56.4 (53.8;57.6) | | 0.084 | 468 |
| MDI | *All cases of RLS* | 5 (3;8) | 4 (2;7) | <0.01 | 756 | 5 (2;8) | 4 (1;7) | <0.01 | 542 |
| | *Frequent*[××] | 6 (3;10) | | <0.01 | 225 | 6 (3;11) | | <0.01 | 174 |
| | *Severe* | 5 (3;8) | | <0.01 | 392 | 5 (2;10) | | <0.01 | 212 |
| | *Infrequent or mild* | 5 (3;8) | | 0.01 | 609 | 5 (2;8) | | <0.01 | 468 |

**Mental health-related quality of life score (MCS) and Physical health related quality of life score (PCS) were transformed using a power transformation of five, while the Major Depression Inventory Score (MDI) was log-transformed to obtain a normal distribution before conduction T-tests

[××] Frequent RLS symptoms were defined as symptoms occurring two to three times a week or more

*2.3 Restless legs syndrome (RLS) and the mental component score of health related quality of life (MCS)*

Participants suffering from RLS were more likely to have a low MCS (women: OR=1.61 *P*<0.001; men: OR=1.79 *P*=<0.001) (Figures 1 and 2). Moreover, the probability of low MCS was highest among participants with frequent RLS symptoms (women: OR=2.54 *P*<0.001; men: OR=2.51 *P*<0.001). Even RLS sufferers with mild or infrequent symptoms also had an increased probability of low MCS

(women: OR=1.46 *P*<0.001; men: OR=1.52 *P*<0.001). Finally, participants with RLS were more likely to have a reduced PCS (Appendix, Table 5).

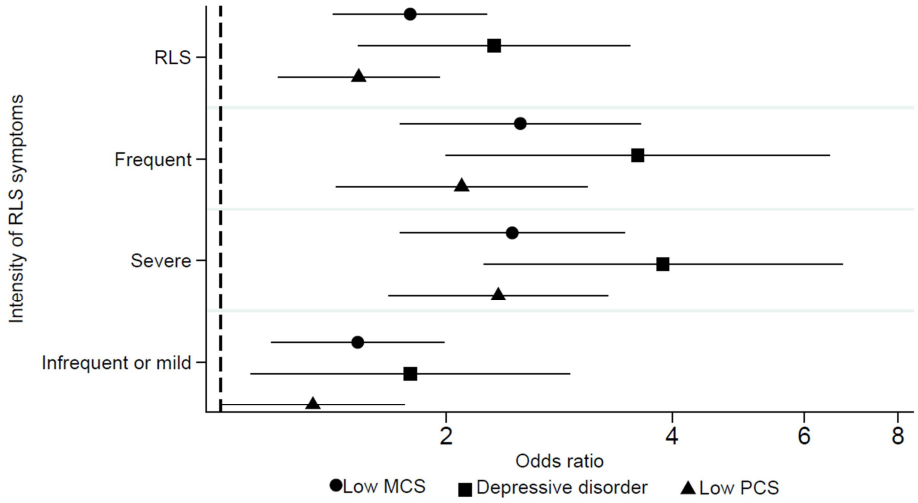
*2.4 Restless legs syndrome (RLS) and the physical component score of health related quality of life (PCS)*

Both men and women suffering from RLS were more likely to have a low PCS (women: OR=1.49 *P*<0.001; men: OR=1.53 *P*<0.001) (Figure 1 and 2). Severe RLS symptoms were associated with a more than doubled prevalence of low PCS among both men (OR=2.35 *P*<0.001) and (OR=2.75 *P*<0.001). Moreover, men with mild or infrequent RLS had an increased probability of having a low PCS (OR=1.33 *P*<0.048) (Figure 1). We found no association between infrequent or mild RLS and low PCS among women (Figure 2). Furthermore, participants with RLS were more likely to have a reduced PCS regardless of symptom intensity (Appendix, Table 5).

*2.5 Restless legs syndrome (RLS) and depressive disorder*

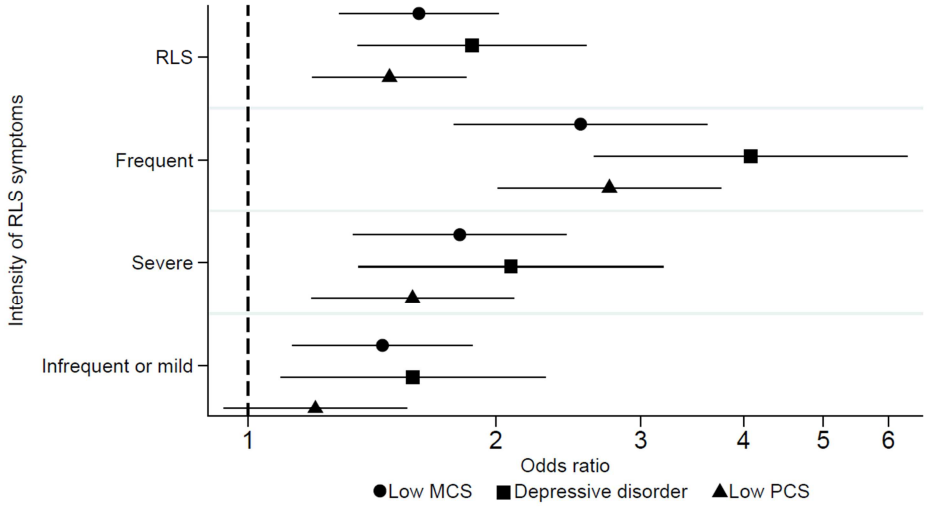
We found that men and women with severe RLS symptoms had 3.60 and 4.08 fold higher odds for being classified with depressive disorder compared to non-RLS sufferers, respectively (both sexes, *P*<0.001) (Figure 1 and 2). Furthermore, participants with mild or infrequent RLS symptoms had a more than 50% increased probability of classifying with depressive disorder compared to non-RLS sufferers (women: OR=1.59 *P*<0.015; men: OR=1.79 *P*<0.020) (Figure 1). We found that 8.2% of the study participants were diagnosed with a depressive disorder by a medical doctor. Our analyses showed an increase in RLS occurrence among men and women with a previous diagnosis. However, there was no association between having a previous diagnosis and currently suffering from RLS with mild or infrequent symptoms among men (Appendix, Table 6).

**Figure 1. Association of restless legs syndrome (RLS) and depressive disorder and health related quality of life, respectively, among men**




Legend: Displaying odds ratios (OR) with 95% confidence intervals for depressive disorder, low mental component score (MCS), and low physical component score (PCS) (SF-12©) according to frequency and severity of RLS symptoms (Adjusted for age, body mass index, alcohol consumption, smoking status, educational level, and donation frequency for the past three years)

**Figure 2. Association of restless legs syndrome (RLS) and depressive disorder and health related quality of life, respectively, among women**




Legend: Displaying odds ratios (OR) with 95% confidence intervals for depressive disorder, low mental component score (MCS), and low physical component score (PCS) (SF-12©) according to frequency and severity of RLS symptoms (Adjusted for age, body mass index, alcohol consumption, smoking status, educational level, and donation frequency over the past three years)

*2.6 Quality of sleep and involuntary leg movements during sleep*

Poor quality of sleep and ILM were reported by 22.6% and 22.1% of RLS cases, respectively. We found that poor quality of sleep was associated with an increased risk of depressive disorder and low MCS among participants with RLS ($P$<0.001), and with an increased risk of low PCS among women with RLS (Table 3). The risk estimates changed marginally when adjusted for ILM. Furthermore, we found that RLS cases reporting frequent and severe symptoms were more likely to have depressive disorder ($P$<0.01), low MCS ($P$<0.001), and low PCS ($P$<0.01) compared to cases reporting infrequent or mild symptoms. Quality of sleep and ILM were included in these regression models, ORs attenuated and P values increased (Table 4). Moreover, we found that poor quality of sleep was associated with reduced MCS and reduced PCS among participants with RLS ($P$<0.01) (Appendix, Tables 7 and 8). Furthermore, the MDI scale comprises a question asking; "Did you have difficulties sleeping at night for the past two weeks", which is classified as a *accompanying symptom* in depressive disorder. For an accompanying symptom to be considered present the participant must answer that they experience the symptom "more than half the time" or even more frequently. This specific symptom was present in

49.5% of participants that classified with depression, and it was not more prominent in RLS cases with depression compared to non-RLS cases with depression. However, overall the prevalence of the symptom was more than double among RLS cases (9.6%) compared to non-RLS cases (4.5%), regardless of whether they were classified with a depression or not ($P$<0.001). Similarly, the symptom was more prevalent among RLS cases reporting severe symptoms (18.6%) compared to RLS cases reporting mild symptoms (7.7%) ($P$<0.001).

**Table 3. Multivariable logistic regression analyses comprising all donors suffering from restless legs syndrome (RLS)**

| | Men (n=542) | | | | Women (n=756) | | | |
|---|---|---|---|---|---|---|---|---|
| | Model 1* | | Model 2** | | Model 1* | | Model 2** | |
| **Logistic regression** | OR | P | OR | P value | OR | P | OR | P |
| Comparison groups: poor quality of sleep vs. good quality of sleep | | | | | | | | |
| Low MCS[×] | 3.74 | <0.001 | 3.71 | <0.001 | 3.75 | <0.001 | 3.72 | <0.001 |
| Low PCS[×] | 1.70 | 0.060 | 1.63 | 0.083 | 2.60 | <0.001 | 2.57 | <0.001 |
| depressive disorder[×] | 4.40 | <0.001 | 4.19 | <0.01 | 9.22 | <0.001 | 9.20 | <0.001 |

[×]Low mental health-related quality of life (MCS) and physical health related quality of life (PCS) were defined using the 10$^{th}$ percentile as cutoff. Depressive disorder was defined as a Major Depression Inventory Score (MDI) score > 20.

*Adjusted for age, body mass index, alcohol consumption, smoking status, educational level, and donation frequency for the past three years

**Adjusted for age, body mass index, alcohol consumption, smoking status, educational level, donation frequency for the past three years, and involuntary leg movements during sleep

**Table 4. The probability of low MCS, PCS and depression, respectively, among participants with restless legs syndrome (RLS)**

| **N=1,298 RLS cases** | **Model 1*** | | **Model 2**** | |
|---|---|---|---|---|
| Characteristic (%) | OR (95 % CI) | P | OR (95 % CI) | P |
| Comparison groups: Frequent and severe RLS vs. infrequent or mild RLS | | | | |
| Low MCS[×] | 2.05 (1.39-3.05) | <0.001 | 1.71 (1.14-2590) | 0.010 |
| Low PCS[×] | 2.08 (1.45-2.99) | <0.01 | 1.76 (1.21-2.57) | <0.01 |
| Depression[×] | 2.57 (1.47-4.51) | <0.01 | 1.99 (1.11-3.60) | 0.023 |

[×]Low mental health-related quality of life (MCS) and physical health related quality of life (PCS) were defined using the 10$^{th}$ percentile as cutoff. Depression was defined as a Major Depression Inventory score (MDI) > 20.

*Adjusted for sex, age, body mass index, alcohol consumption, smoking status, educational level, and donation frequency for the past three years
** Adjusted for sex, age, body mass index, alcohol consumption, smoking status, educational level, and donation frequency for the past three years, and quality of sleep and involuntary leg movements during sleep

## 3. Discussion

The present findings showed that both men and women with RLS had higher odds for experiencing low MCS, low PCS, and depressive disorder in a large population that was not affected by other diagnoses or medical treatments. Our findings also indicated that higher severity and frequency of RLS symptoms increases the odds for detrimental links to the disorder, and that this effect varied between sexes. Thus, it is evident that RLS is a troublesome disorder with severe consequences. Furthermore, we observed that RLS sufferers who reported poor quality of sleep had higher odds for depressive disorder and low MCS compared to RLS sufferers who reported good quality of sleep. Among women with RLS reporting poor quality of sleep we also observed higher odds for low PCS. This could mean that quality of sleep plays an important role in the observed associations, and maybe also that this role differs between sexes.

The present findings together with our previous report of sex-related differences in RLS-associated demographic and lifestyle factors [18] indicate that the detrimental links to RLS vary between sexes. Findings from other previous studies support this suggestion. RLS has proved to be an independent predictor of HRQL in several studies [1, 6, 7, 9, 11-13, 36, 37]. A Swedish study, including a random sample of 5,000 women (aged 25-64) also using the SF-12 reported that RLS affected MCS more negatively than PCS [13]. This is in line with our findings in both men and women. Specifically, we observed no association between RLS and low PCS among women with infrequent or mild symptoms. In contrast, a study from the USA discovered that the burden of RLS was more significant on PCS than on MCS [9], which included a significantly smaller sample size than the present study (n=158) and included a nationwide random sample of US adults.

RLS has also been found to be associated with psychiatric and psychological disorders at long term [38, 39], and to further emphasize the serious nature of our findings, it should be noted that previous studies

have reported that HRQL scores in RLS sufferers resemble HRQL scores among patients with other chronic disorders, such as type 2 diabetes, osteoarthritis, Parkinson's disease, narcolepsy, multiple sclerosis, and stroke [7, 37]. Happe et al. (2009) additionally underlined the severity of the RLS disorder, by stating that the impact that RLS has on HRQL is equal to or even worse than the impact of other chronic neurological disorders [7]. Moreover, Allen et al. (2005) found that RLS was associated with poorer SF-36 score and reported that the reduction in score was similar to the one seen in clinical depression cases [8, 37]. Our findings support this comparison as we found that RLS cases were more likely to be classified with depressive disorder, which is in line with other reports of high prevalence of depressive disorder among RLS sufferers [5, 8, 15, 40]. We observed that RLS sufferers with severe and infrequent symptoms had higher odds for depression compared to RLS sufferers reporting mild or infrequent symptoms. Castillo et al. (2014) reported a similar increased risk of depressive disorder among individuals with severe RLS symptoms compared to individuals with mild to moderate symptoms [40]. Moreover, participants with mild or infrequent RLS symptoms were more likely to be classified with depressive disorder than participants without RLS. This suggests that all RLS cases, regardless of symptom severity and frequency, warrant medical attention. This has practical significance for the management of the RLS disorder.

The pathogenesis of the associations reported is still unknown and plausibly multifactorial. Quality of sleep is an important aspect of individuals' general health that has continuously been reported as the most troublesome morbidity of RLS [8, 12, 14]. We found that RLS sufferers reporting poor quality of sleep were more likely to have a low or reduced MCS and classify with depressive disorder, compared to RLS sufferers with good quality of sleep. Similarly, women with RLS reporting poor quality of sleep were more likely to have low PCS compared to female RLS cases reporting good quality of sleep. These findings were robust to adjustment for ILM. Furthermore, RLS cases with frequent and severe symptoms had statistically insignificant higher odds for low MCS, low PCS, and increased depressive compared to participants with infrequent or mild symptoms. However, the ORs attenuated and the P values increased when quality of sleep and ILM were entered into the logistic regression model. This is in line with reports by Koo et al. (2016), who found that insomnia was most prevalent among

individuals with severe RLS symptoms [8]. As we did not have information on quality of sleep or ILM for non-RLS sufferers it was not possible to assess whether these factors were actual mediators of the associations found in this study. However, interpreting our findings according to previous reports of effect mediation and moderation of the RLS-depressive disorder relationship [8], it seems that quality of sleep plays an important role in the associations shown in this study, at least to some extent. Another explanation for the observed association between RLS and depression could be the overlap of symptoms between the two disorders. Poor quality of sleep related to RLS may result in irritable mood, agitation, and problems with concentrating [39], which could make an individual with RLS classify with depressive disorder. Finally, it is plausible that RLS symptoms, including uncomfortable or painful sensations combined with the constant urge to move caused the decreased PCS in RLS cases.

The main strength of the present study was the size of the cohort and the data available. To our knowledge, this study is the first to apply the CH-RLSq, SF-12 and MDI in a generally healthy cohort of this size. The tools applied in this study are widely used and validated scales [24, 27, 29, 32]. Using the CH-RLSq assured correct identification of RLS [26]. Moreover, it was reported that the MDI has high content validity, as the scale isolates the most applicable items for negative wellbeing [32], and there is evidence that the clinical validity of the MDI as a unidimensional severity depression scale is high [29]. Furthermore, SF-12 is a generic tool, meaning it can be used to measure HRQL in population groups regardless of their age, sex, or health status. However, a specific RLS-related quality of life questionnaire does exist, called the Restless Legs Syndrome Quality of Life Instrument [4]. This questionnaire includes items that are specific to RLS symptoms, primarily regarding sedentary activities and insomnia [41]. Our results indicate that poor sleep quality plays a role in the associations found in this study. Thus, the odds ratios reported here were possibly underestimations of the true association between RLS and reduced HRQL. The questions used in this study to assess intensity of the RLS disorder was imbedded in a larger health-related questionnaire administered to blood donors. In order to be considerate of the blood donors' time we had to limit the number of questions assessing the severity and frequency of symptoms among RLS cases to two questions. It would have provided a broader insight into the symptoms if we had applied the International RLS Severity Scale or the RLS-6 scale,

which assesses the severity of the disorder using ten and six questions, respectively. Moreover, MCS has previously been used as a screening tool to identify people with depressive disorder [28, 42]. This means, that the links we found between RLS and MCS and MDI score, respectively, are likely to be expressions of the same association.

As previously mentioned, the associations found in this study cannot be attributed to differential morbidities, especially ones requiring medical treatment because the donors are required to be healthy. Notably, patients taking antidepressants are not eligible for donation, which is a significant methodological strength. Previous studies examining the RLS-depression link might be biased, because it is reported that antidepressants can provoke or exacerbate RLS symptoms [15]. Thus, the use of antidepressants is a potential confounding factor in other studies. Additionally, it is plausible that the most severe RLS cases do not become blood donors due to the selection of generally healthy individuals. Furthermore, it should also be noted that because data examined in this study were cross-sectional we were not able to make any conclusions on directionality or causality of the observed associations. Similarly we cannot conclude on long term health effects of RLS. In our analyses we adjusted for sociodemographic factors (age and sex), socioeconomics (education), and lifestyle factors (BMI, smoking, alcohol consumption, and number of whole blood donations for the past three years), which is a specific strength. Nonetheless, this study could be affected by "the internal healthy donor effect". The healthy donor effect describes the fact that blood donors represent a healthy subgroup of the general population, and that donors who donate frequently are generally healthier than infrequently donating donors [19]. This means that it is possible that donors suffering from severe RLS has either stopped donating or donated fewer times than donors without RLS or with a mild case of the disorder. Hence, the internal healthy donor effect may have masked any potential negative effects of donation-induced iron deficiency on both RLS and related consequences.

Thus, our study is susceptible to misclassification bias due to the self-report nature of the questionnaire data. This particularly applies to the assessment of ILM presence. It would have been more reliable had we used EMG or actigraphy measurements or asked about ILM during wakefulness instead of during sleep to exclude potential recall-bias. Overall, it is possible that participants misunderstood some of the

questions. Furthermore, is also possible that some participants might have under or over reported health related behaviors. However, because validated questions and scoring systems were used, we do believe that the potential misclassification would be random and not bias the results.

## 4. Conclusions

In conclusion, this study provides evidence that blood donors, who comprise a population with a high level of general health that is not biased by other diagnoses or by any medications, suffering from RLS, are more likely to experience poor HRQL and to be classified with depressive disorder compared to people without RLS. HRQL is a direct measure of individuals' subjective experience of their wellbeing, and it is reported to be a predictor of future morbidity and mortality in the general Danish population [43]. Thus, RLS appears to be a significantly troublesome disorder, even when the symptoms are categorized as either infrequent or mild. Even so, RLS is still thought to be underdiagnosed [44], probably due to lack of knowledge of its existence. The findings of this study suggest that the majority of RLS cases warrant medical attention and that practical management of RLS should include interventions that aim at increasing the HRQL and decreasing the occurrence of depressive symptoms among these patients.

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## 6. Author contributions

Henrik Ullum (HU), Erik Sørensen (ES), Christian Erikstrup (CE), Ole B. Pedersen (OP), Mie T. Bruun (MB), Kristoffer S. Burgdorf (KB), Richard Allen (RA), Brendan J. Burchell (BB), Poul J. Jennum

(PJ), and Thomas Werge (TW), Lise W. Thørner (LT), Kaspar Nielsen (KN): Collecting data. HU, ES, RA, Maria Didriksen (MD): Planning study design. MD and Andreas S. Rigas (AR): Statistical analyses. MD: Interpretation of results and completion of first manuscript draft. HU, ES, CE, OP, MB, RA, PJ, TW, LT, AR, Maria Haahr Nielsen, KB, and Emanuele Di Angelantonio: Interpretation of analyses and revision of first manuscript draft.

## 7. Conflict of interest

Henrik Ullum has previously received a grant from the pharmaceutical company Novartis ©. This grant was not associated with the activities related to the present study. The other authors have no conflict of interest to declare.

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# Supplemental material

**Table 5. Multiple logistic regression analyses with Restless Legs Syndrome (RLS) as the Independent variable**

| **Logistic regression** | | Men (n controls=12,877) | | | Women (n controls=10,532) | | |
|---|---|---|---|---|---|---|---|
| | | Multi adjusted* | | | Multi adjusted* | | |
| | Comparison groups | Odds ratio | P value | n(cases) | Odds ratio | P value | n(cases) |
| Low MCS[×] | *RLS: yes vs. no* | 1.79 | <0.001 | 542 | 1.61 | <0.001 | 756 |
| | *Frequent symptoms[××] vs. no RLS* | 2.52 | <0.001 | 174 | 2.54 | <0.001 | 225 |
| | *Severe RLS vs. no RLS* | 2.45 | <0.01 | 212 | 1.81 | <0.001 | 392 |
| | *Infrequent or mild RLS symptoms vs. no RLS* | 1.52 | <0.001 | 468 | 1.46 | <0.001 | 609 |
| Reduced MCS[×] | *RLS: yes. vs. no* | 1.61 | <0.001 | 542 | 1.41 | <0.001 | 756 |
| | *Frequent symptoms[××] vs. no RLS* | 2.23 | <0.001 | 174 | 2.10 | <0.001 | 225 |
| | *Severe RLS vs. no RLS* | 2.09 | <0.001 | 212 | 1.46 | <0.001 | 392 |
| | *Infrequent or mild RLS symptoms vs. no RLS* | 1.45 | <0.001 | 468 | 1.30 | <0.01 | 609 |
| Low PCS[×] | *RLS: yes. vs. no* | 1.53 | <0.001 | 542 | 1.49 | <0.001 | 756 |
| | *Frequent symptoms[××] vs. no RLS* | 2.10 | <0.001 | 174 | 2.75 | <0.001 | 225 |
| | *Severe RLS vs. no RLS* | 2.35 | <0.001 | 212 | 1.58 | <0.01 | 392 |
| | *Infrequent or mild RLS symptoms vs. no RLS* | 1.33 | 0.048 | 468 | 1.21 | 0.151 | 609 |
| Reduced PCS[×] | *RLS: yes. vs. no* | 1.47 | <0.001 | 542 | 1.50 | <0.001 | 756 |
| | *Frequent symptoms[××] vs. no RLS* | 1.75 | <0.001 | 174 | 2.03 | <0.001 | 225 |
| | *Severe RLS vs. no RLS* | 2.07 | <0.001 | 212 | 1.51 | <0.001 | 392 |
| | *Infrequent or mild RLS symptoms vs. no RLS* | 1.31 | <0.01 | 468 | 1.42 | <0.001 | 609 |
| Depression[×] | *RLS: yes. vs. no* | 2.32 | <0.001 | 542 | 1.87 | <0.001 | 756 |
| | *Frequent symptoms[××] vs. no RLS* | 3.60 | <0.001 | 174 | 4.08 | <0.001 | 225 |
| | *Severe RLS vs. no RLS* | 3.89 | <0.001 | 212 | 2.09 | <0.01 | 392 |
| | *Infrequent or mild RLS symptoms vs. no RLS* | 1.79 | 0.020 | 468 | 1.59 | 0.015 | 609 |

[×]Low mental health-related quality of life (MCS) and physical health-related quality of life (PCS) were defined using the 10$^{th}$ percentile as cutoff and reduced MCS and PCS were defined using the 25$^{th}$ percentile as cutoff. Depression was defined as a Major Depression Iventory Score>20.

[××] Frequent RLS symptoms was defined as symptoms occurring 2 – 3 times a week or more

*Adjusted for age, body mass index, alcohol consumption, smoking status, educational level, and donation frequency for the past three years

**Table 6. Multivariable linear and logistic regression analyses with clinical depression as the independent variable and Restless Legs Syndrome (RLS) as the dependent variable**

| | Men (n controls=10,532) | | | Women (n controls=12,877) | | |
|---|---|---|---|---|---|---|
| | Multi adjusted* | | | Multi adjusted* | | |
| **Logistic regression** | OR | P value | n (cases) | OR | P value | n (cases) |
| Previous depression-diagnosis[×] (yes vs. no) | | | 392 | | | 608 |
| RLS | 1.62 | <0.01 | 756 | 1.57 | <0.001 | 542 |
| Frequent RLS symptoms[××] | 2.05 | <0.01 | 225 | 2.00 | <0.001 | 174 |
| Severe RLS symptoms | 2.03 | <0.01 | 392 | 1.75 | <0.001 | 212 |
| Infrequent or mild RLS symptoms | 1.39 | 0.071 | 609 | 1.48 | <0.01 | 468 |

[×]Previous depression-diagnosis was defined as a depression-diagnosis given by a medical doctor at some point in the participants' lives.

[××] Frequent RLS symptoms was defined as symptoms occurring 2 – 3 times a week or more

*Adjusted for age, body mass index, alcohol consumption, smoking status, educational level, and donation frequency for the past three years

**Table 7. Multivariable logistic regression analyses comprising all donors suffering from Restless Legs Syndrome (RLS)**

| | | Men (n=542) | | | | Women (n=756) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Model 1* | | Model 2** | | Model 1* | | Model 2** | |
| **Logistic regression** | **Self-reported quality of sleep** | OR | P value | OR | P value | OR | P value | OR | P value |
| Reduced MCS[×] | *Poor vs. good* | 4.12 | <0.001 | 4.11 | <0.001 | 3.85 | <0.001 | 3.80 | <0.001 |
| Reduced PCS[×] | *Poor vs. good* | 1.75 | 0.012 | 1.72 | 0.014 | 1.15 | 0.440 | 1.16 | 0.427 |

[×]Reduced mental health-related quality of life (MCS) and physical health-related quality of life (PCS) were defined using the 25$^{th}$ percentile as cutoff.

*Adjusted for age, body mass index, alcohol consumption, smoking status, educational level, and donation frequency for the past three years

**Adjusted for age, body mass index, alcohol consumption, smoking status, educational level, donation frequency for the past three years, and periodic leg movements during sleep

**Table 8. Multivariable logistic regression analyses comprising all donors suffering from Restless Legs Syndrome (RLS)**

| **N=1,298 RLS cases** | **Model 1*** | | **Model 2**** | |
|---|---|---|---|---|
| Characteristic (%) | OR (95 % CI) | P value | OR (95 % CI) | P value |
| Comparison groups: Frequent and severe RLS vs. infrequent or mild RLS | | | | |
| Reduced MCS[×] | 1.60 (1.16-2.19) | <0.001 | 1.33 (0.95-1.87) | 0.092 |
| Reduced PCS[×] | 1.56 (1.15-2.11) | <0.01 | 1.42 (1.04-1.94) | 0.026 |

[×]Reduced mental health-related quality of life (MCS) and physical health-related quality of life (PCS) were defined using the 25$^{th}$ percentile as cutoff.

*Adjusted for sex, age, body mass index, alcohol consumption, smoking status, educational level, and donation frequency for the past three years

**Adjusted for sex, age, body mass index, educational level, smoking status, alcohol consumption, donation frequency for the past three years, and quality of sleep and involuntary leg movements during sleep