Association of proton pump inhibitor and histamine H₂-receptor antagonists with restless legs syndrome

Eric J. Earley¹,*, Maria Didriksen², Bryan R. Spencer³,⁴, Joseph E. Kiss⁵,⁶, Christian Erikstrup⁷,⁸, Ole B. Pedersen⁹, Erik Sørensen², Kristoffer S. Burgdorf², Steven H. Kleinman⁹, Alan E. Mast¹⁰,¹¹, Michael P. Busch¹²,¹³, Henrik Ullum² and Grier P. Page¹⁴ on behalf of the NHLBII Recipient Epidemiology Donor Evaluation Study (REDS)-III Program and the Danish Blood Donor Study

¹RTI International, Research Triangle Park, NC, ²Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark, ³Department of Laboratory Medicine, Yale University, New Haven, CT, ⁴American Red Cross Scientific Affairs, Boston, MA, ⁵Department of Medicine, University of Pittsburgh, PA, ⁶Vitalant Northeast Division, Pittsburgh, PA, ⁷Department of Clinical Immunology, Aarhus University Hospital, Skejby, Aarhus, Denmark, ⁸Department of Clinical Immunology, Naestved Hospital, Naestved, Denmark, ⁹Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada, ¹⁰Blood Research Institute, Versiti, Milwaukee, WI, ¹¹Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI, ¹²Department of Laboratory Medicine, University of California San Francisco, CA, ¹³Vitalant Research Institute, San Francisco, CA and ¹⁴RTI International, Atlanta, GA

*Corresponding author. Eric J Earley, RTI International, 3040 E. Cornwallis Rd., P.O. Box 12194, Research Triangle Park, NC 27709-2194, USA. Email: eearley@rti.org.

Abstract

Restless legs syndrome (RLS) is a common sensorimotor disorder, which can disrupt sleep and is thought to be caused in part by low cellular iron stores. Proton pump inhibitors (PPI) and histamine H₁-receptor antagonists (H₁A) are among the most commonly used drugs worldwide and show evidence of causing iron deficiency. We conducted a case/non-case observational study of blood donors in the United States (N = 13,403; REDS-III) and Denmark (N = 50,323; Danish Blood Donor Study, DBDS), both of which had complete blood count measures and a completed RLS assessment via the Cambridge–Hopkins RLS questionnaire. After adjusting for age, sex, race, BMI, blood donation frequency, smoking, hormone use, and iron supplement use, PPI/H₁A use was associated with RLS (odds ratio [OR] = 1.41; 95% confidence interval [CI], 1.13–1.76; p = 0.002) in REDS-III for both PPI (OR = 1.43; CI, 1.03–1.95; p = 0.03) and H₁A (OR = 1.56; CI, 1.10–2.16; p = 0.01). DBDS exhibited a similar association with PPIs/H₁As (OR = 1.29; CI, 1.20–1.40; p < 0.001), and for PPIs alone (OR = 1.27; CI, 1.17–1.38; p < 0.001), but not H₁As alone (OR = 1.18; CI, 0.92–1.53; p = 0.2). We found no evidence of blood iron stores mediating this association. The association of PPI, and possibly H₁A, consumption with RLS independent of blood iron status and other factors which contribute to RLS risk suggest the need to re-evaluate the use of antacids in those at particular risk for RLS.

Key words: restless legs syndrome; RLS; iron deficiency; blood donors; ferritin

Statement of Significance

Restless legs syndrome (RLS) afflicts up to 15% of adults in the Western Hemisphere and although its causes are not fully understood, low cellular iron stores appears to be a major contributor. Common antacid medications, proton pump inhibitors, and histamine H₁-receptor antagonists appear to cause iron deficiency and are among the most consumed drugs worldwide. To date, no link between consumption of these drugs and RLS has been found. In this study, we sought to measure the association between antacid use and the risk of having RLS. Evidence of these drugs contributing to RLS risk could suggest a need to re-evaluate the use of antacids in those at higher risk for developing RLS.
Introduction

Restless legs syndrome (RLS; Willis–Ekman disease) is a sensori-motor condition affecting between roughly 4% and 15% of adults in the Western Hemisphere [1]. It is characterized by a compulsion to move one’s legs during inactive periods due to discomfort in the legs that is relieved while moving. Symptoms occur predominantly at night and impact sleep and quality of life [2, 3]. RLS is linked to chronic conditions such as insomnia and depression [4, 5].

Evidence suggests a link between iron deficiency in the brain and RLS [6, 7]. Multiple genome-wide association studies found associations between variants in the genes BTBD9 and MEIS1 and RLS [8–10], which both appear to be involved in iron homeostasis [11, 12], as well as dopamine regulation and lower limb development [13–15]. Supplemental iron has been an effective treatment for some forms of RLS in clinical trials [16–19], and RLS is also seen more often in scenarios where iron deficiency is common, particularly in pregnant women [20], older people [21], and frequent blood donors [22]. However, the etiology of RLS is multifactorial and association with low peripheral iron stores is absent in some populations [23–25]. Interestingly, some medications have been linked to RLS including antidepressants [26, 27] and dopamine antagonists [28].

A growing body of evidence has shown a link between consumption of proton pump inhibitors (PPI) and H₂-receptor antagonists (H₂A) and reduced iron [29–33]. These drugs enzymatically block gastric hydrochloric acid production, and the subsequent increase in gut pH appears to reduce absorption of non-heme dietary iron [34]. At a population level, PPI/H₂A use is linked to an increased risk of iron deficiency [31, 32]. These drugs are some of the most widely used in the United States [35, 36], with use at roughly 8% among the general population and 22% among those older than 65 years [35]. Widespread use of these drugs may be contributing to the prevalence of RLS.

Given the potential connection through body iron stores, the aim of this study was to investigate the association between PPI/H₂A medication use and RLS risk in two groups of blood donors, one from the United States and another from Denmark.

Methods

Study populations

The National Heart Lung and Blood Institute’s Recipient Epidemiology Donors Study-III (REDS-III) RBC-Omics study [37] enrolled participants from four blood centers: American Red Cross (Farmington, CT), Institute for Transfusion Medicine (Pittsburgh, PA), BloodCenter of Wisconsin (Milwaukee, WI), and the Blood Centers of the Pacific (San Francisco, CA). Self-reported race, gender, and age, along with other data, were collected by self-administered questionnaire [38] which included questions on use of supplemental iron, PPI/H₂A medications, supplemental hormones, menstrual status, and pregnancy history. Participants also completed the Cambridge–Hopkins RLS questionnaire (CH-RLSq). Other demographic information including the prior 2 years donation history was linked from blood centers’ databases.

Parallel analysis (n = 50,232) was performed on a subset of participants from the Danish Blood Donor Study (DBDS) who had completed the CH-RLSq. The DBDS is an ongoing national cohort study comprising more than 115,000 Danish blood donors. Details of this cohort have been described elsewhere [39, 40]. Briefly, blood donors were asked to participate if they had previously donated at least twice in a Danish blood bank and upon inclusion participants completed a comprehensive health questionnaire and provided a whole blood sample for testing. Participants also provided consent for researchers to link their unique civil registration number to information in health-related registries [41].

Serum ferritin and complete blood counts were collected in both cohorts, including hemoglobin, red blood cell (RBC) count, hematocrit, and mean corpuscular volume (MCV).

Ethics statement

Written informed consent was obtained from all participants before enrollment. REDS-III RBC-Omics recruitment materials and protocols were approved by each participating site’s Institutional Review Board (IRB). The DBDS was approved by The Scientific Ethical Committee of the Central Denmark Region (M-20090237). The research database was approved by the Danish Data Protection Agency (2007-58-0015).

RLS diagnosis

RLS was diagnosed using the CH-RLSq. This tool has been validated as an effective means of RLS diagnosis (diagnostic sensitivity 87.2% and specificity 94%) [42, 43], and it includes questions on the four essential characteristics of RLS (uncomfortable feelings in the legs causing an urge to move them, symptoms are worse at night, symptoms begin at rest, and symptoms are relieved with movement) as well as questions designed to rule out non-RLS mimics. The CH-RLSq survey was translated from English to Danish using the back-translation method [3].

Because RLS patients experience a range of discomfort preceding the compulsion to move their legs often described as “an urge” or “irritating” instead of “uncomfortable” [44], we classified participants with RLS if they answered “yes” to either the first question (“…recurrent uncomfortable feelings…”) or the second question (“…recurrent need or urge to move your legs…”) and met the remainder of the CH-RLSq criteria. The Supplementary Appendix contains a detailed description of diagnosis criteria.

PPI/H₂A medication usage assessment

REDS-III participants were asked about regular or occasional use of 10 commercial medication products comprising 9 unique compounds spanning PPIs (Pantoprazole, Omeprazole, Esomeprazole, Rabeprazole, and Lansoprazole) and H₂As (Cimetidine, Nizatidine, Ranitidine, and Famotidine) classes. Prevalence of use was measured as the number of compounds used in the prior 2 years divided by use of 10 commercial medication products comprising 9 unique compounds spanning PPIs (Pantoprazole, Omeprazole, Esomeprazole, Rabeprazole, and Lansoprazole) and H₂As (Cimetidine, Nizatidine, Ranitidine, and Famotidine) classes. Serum ferritin and complete blood counts were collected in both cohorts, including hemoglobin, red blood cell (RBC) count, hematocrit, and mean corpuscular volume (MCV).
**Statistical analysis**

In the REDS-III and DBDS cohorts, multivariate logistic regression was used to assess the association of RLS risk with antacid use as a binary (Y/N) variable. In REDS-III models were adjusted for sex (M/F), age, race (white/not-white), supplemental iron use (Y/N previous 30 days), hormone use (Y/N, 30 days), donation frequency in previous 2 years, BMI, and smoking status (Y/N, 30 days). DBDS used similar models but did not adjust for supplemental iron use nor hormone use.

The relationship between RLS and antacid use modeled the exposure in several ways: (1) Any PPI or H2A drug use, (2) Exclusive use of PPI or H2A classes, (3) Compound-specific testing (omitting nizatidine and rabeprazole due to low power, \( N = 30 \)). DBDS analyses were performed in Stata/SE v15 (Stata Corp., College Station, TX).

**Results**

**Study populations**

The REDS-III study consented 13,770 participants, and 367 were excluded due to informed consent issues, duplicate enrollment, failure to obtain sample for analyses, non-sufficient donation quantity, diversion to double RBC donation, or positive test for infectious disease marker. This resulted in 13,403 participants ages 18 and older (Supplementary Figure S1) with 6,745 women and 6,658 men. There were 558 cases (4.2%) of RLS (Table 1).

The DBDS cohort contains 115,000 blood donors, and 53,175 were excluded for not completing the entire CH-RLSq, and 227 more were excluded due to missing BMI or smoking status, resulting in a final total of 50,323 participants ≥18 (Supplementary Figure S1) with 24,441 women and 25,791 men. There were 3,540 cases (7.1%) of RLS (Table 1). A subset of 17,865 who had completed the RLS assessment also had CBC measures.

**Table 1. Characteristics of RBC-Omics and DBDS cohorts**

|                | REDS-III                      | DBDS              |
|----------------|-------------------------------|-------------------|
|                | RLS, \( N = 558 \)           | No RLS, \( N = 12,845 \) | RLS, \( N = 3,540 \) | No RLS, \( N = 46,692 \) |
| Age (mean ± SD) | 48.0 (±15.2)                  | 45.2 (±16.6)      | 0.90                    | 42.4 (±12.4)                  | 40.0 (±13.1) | <0.001 |
| Women—no. (%)   | 373 (67)                      | 6,372 (50)        | <0.001                  | 2,132 (60.2)                  | 22,309 (47.8) | <0.001 |
| Race—no. (%)    |                               |                   |                         |                               |              |        |
| White           | 472 (85)                      | 8,687 (68)        | <0.001                  | 3,487 (98.5)                  | 45,751 (98.0) | 0.020  |
| Black           | 32 (5.7)                      | 1,639 (13)        |                         | –                            | –             |        |
| Asian           | 22 (3.9)                      | 1,653 (13)        |                         | –                            | –             |        |
| Native American | 0                             | 32 (0.25)         |                         | –                            | –             |        |
| Hawaiian Pacific| 0                             | 20 (0.16)         |                         | –                            | –             |        |
| Multiple        | 4 (0.7)                       | 133 (1.0)         |                         | –                            | –             |        |
| Other           | 18 (3.0)                      | 480 (4.0)         |                         | –                            | –             |        |
| Danish immigrants| –                            | –                  |                         | 9 (0.25)                     | 280 (0.60)    |        |
| Descendants of Danish immigrants| – | –                  |                         | 44 (1.24)                    | 658 (1.43)    | 0.019  |
| Smoking—no. (%) | 49 (10.1)                     | 1,051 (9.2)       | 0.31                    | 512 (14.5)                    | 6,107 (13.1)  | 0.019  |
| Suppl. iron—no. (%)| 256 (46.4)                 | 4,691 (37.2)      | 0.09                    | –                            | –             |        |
| Hormones—no. (%)| 60 (10.8)                     | 1,247 (9.9)       | 0.42                    | –                            | –             |        |
| Donation frequency (mean ± SD) | 4.2 (3.9)                  | 3.6 (3.8)         | 0.05                    | 4.9 (2.6)                     | 5.0 (2.6)     | 0.829  |
| PPI—no. (%)     | 62 (12.5)                     | 893 (7.5%)        | 0.03                    | 954 (26.95)                   | 9,561 (20.48) | <0.001 |
| H2A—no. (%)     | 49 (10.1)                     | 702 (6.0)         | 0.01                    | 189 (5.34)                    | 1,677 (3.59)  | <0.001 |
| GERD drugs—no. (%)| 124 (22.2)                  | 1,729 (13.6)      | 0.002                   | 1,029 (29.1)                  | 10,344 (22.2) | <0.001 |

**PPI/H2A medication use was associated with RLS**

Medication use was associated with RLS in multivariate logistic models in the REDS-III cohort after adjusting for sex, age, race, supplemental iron use, hormone use, donation frequency in previous 2 years, BMI, and smoking status (odds ratio [OR] = 1.41; CI, 1.13–1.76, \( p = 0.002 \)), and this association was seen for both classes independently (PPI: OR = 1.43; CI, 1.03–1.95, \( p = 0.03 \); H2A: OR = 1.56, CI, 1.10–2.16, \( p = 0.01 \)). This association was also seen in DBDS after adjusting for sex, age, ethnicity, donation frequency in previous 2 years, BMI, and smoking status (OR = 1.29, CI, 1.20–1.40, \( p < 0.001 \)). The association of PPIs was also observed in DBDS (OR = 1.27, CI, 1.17–1.38, \( p < 0.001 \)). We did not find association with H2As for all DBDS participants ≥18 y (OR = 1.18; CI, 0.92–1.53; \( p = 0.2 \)); however, there was evidence of association for DBDS participants <40 y (OR = 1.66; CI, 1.06–2.62; \( p = 0.03 \); Figure 1).

REDS-III participants using multiple medications exhibited increased rates of RLS compared with participants using one \( (p < 0.001) \). From a baseline of 3.8% (zero; \( n = 11,447 \)), RLS increased to 6.4% when using one compound \( (n = 1,623) \), and 8.7% for two or more different compounds of either class \( (n = 230) \). A similar trend was observed in the DBDS cohort \( (p < 0.001) \): 6.5% RLS rate for zero drugs \((n = 38,859)\), 8.6% for one \( (n = 7,965) \) and 10.2% for two or more compounds of either class \((n = 3,408) \). This trend was also observed both for those taking PPIs exclusively and taking H2As exclusively \((PPI, p < 0.001; H2A, p = 0.014; Figure 2)\).
Pantoprazole was associated with higher RLS rates in both REDS-III (OR = 2.40; CI, 1.15–4.49; p = 0.01) and DBDS (OR = 1.17; CI, 1.05–1.30; p = 0.004). Famotidine was associated in REDS-III (OR = 1.73; CI, 1.13–2.54; p = 0.008) but not DBDS. Omeprazole (OR = 1.20; CI, 1.06–1.36; p = 0.004), lansoprazole (OR = 1.17; CI, 1.04–1.32; p = 0.008), and rabeprazole (OR = 2.22; CI, 1.23–4.02; p = 0.009) were each associated with RLS in DBDS but not REDS-III (Figure 1).

Use of antidepressants (ATC: N06A), dopaminergic agents (N04B), and gabapentin (N03AX12) have also been linked to increased risk of RLS. We investigated whether previous use of these classes of drugs influenced the association between RLS and consumption of PPI/H₂A. Multivariate logistic models which adjusted for the common confounders as above (i.e. age, race, sex, BMI, smoking, and donation history) as well as previous use of antidepressants, dopaminergic agents, and gabapentin did not alter the observed association between RLS and use of PPI/H₂A (data not shown).

No evidence of blood iron measures mediating this association

In multivariate linear models with REDS-III participants, we observed reduced ferritin (−10.5%; CI, −6.1% to −14.7%; p < 0.001), reduced HGB (β = −0.09; CI, −0.15 to −0.04; p = 0.001), and reduced MCV (β = −0.35; CI, −0.62 to −0.08; p = 0.01) among consumers of PPI/H₂A but observed no appreciable difference in RBC count (p = 0.4). We found no evidence of reduced ferritin (p = 0.2), HGB (p = 0.4), MCV (p = 0.2), nor RBC (p = 0.1) in consumers of PPI/H₂A within the DBDS.

Multivariate logistic regression showed that RLS was not associated with reduced ferritin, HGB, MCV, nor RBC in either REDS-III or DBDS. Mediation analysis also found no evidence of blood iron mediating the association between PPI/H₂A and RLS status in either cohort (Supplemental Table S1).

Discussion

PPI/H₂A medication use was associated with increased prevalence of RLS and this appeared not to be mediated by serum
ferritin levels. Blood donors who reported regularly or occasionally using two or more different gastric acid reducing drugs were 1.6–2.2 times more likely to have RLS compared with people who took none. This association was observed in two large blood donor cohorts: REDS-III in the United States representing multiple racial groups, and independently for PPI in DBDS, a predominantly white cohort from Denmark.

Participants in this study were otherwise healthy adults who successfully donated a whole blood unit and reported no history of diabetes or other conditions which would have excluded them from donation. Previous work has shown that blood donation is associated with reduced serum ferritin [46], and repeated donations are associated with iron deficiency [38]. RLS has been associated with iron deficiency anemia [47], and oral or intravenous iron is effective at treating RLS in some cases [48]. However, within blood donor populations the association between levels of serum ferritin and RLS is either weak [24, 49] or non-existent [23].

We hypothesized that iron level mediated the observed association between PPI/H2A use and RLS. While REDS-III respondents exhibited reduced ferritin and HGB among consumers of PPI/H2A, we observed no evidence of either ferritin nor HGB mediating the association between PPI/H2A use and RLS. No reduction in ferritin nor HGB was observed in DBDS participants who consumed PPI/H2A medications.

This association of gastric acid suppression with RLS independent of peripheral iron was surprising and suggests some possibilities. The lack of observed peripheral iron depletion as measured by serum ferritin may not necessarily correspond to a similar state in the brain. For example, serum ferritin does not appear to correlate with iron transport across models of the blood–brain barrier [50], and serum ferritin can be an imperfect measure of body iron stores especially in those suffering from inflammatory diseases [51]. On the other hand, the underlying cause for PPI/H2A use may itself be the contributing factor to RLS risk. For example, GERD or other gastric pathologies unmeasured by either cohort could result in chronic bleeding resulting in subclinical iron deficiency. This study is limited in a few ways. First, since RLS status was measured at a single time point in both REDS-III and DBDS, we were not able to determine whether RLS developed after exposure or was concurrent. Second, exposure in REDS-III was determined by questionnaire which captured both prescription and OTC use and could be affected by recall bias, whereas in DBDS, exposure was determined from filled prescriptions only and could reflect a population that was suffering more from GERD. The lack of association between H2A drugs and RLS in the DBDS cohort may be due to these medications being available over the counter (OTC) in Denmark, but PPI not being OTC, and thus H2A use may be undercounted. Third, we do not have indications for PPI/H2A use and an underlying condition could be promoting RLS risk independently of medication use. Finally, cohorts of blood donors have lower iron stores on average compared with the general population. On the other hand, blood donors are generally healthier than non-donors, and this “healthy donor effect” [49, 52] could skew our estimates of iron levels, RLS risk, or both. However, as RLS was assessed when participants were eligible to donate blood, case status at time of enrollment was likely not affected by other medications or known related diagnoses needing chronic medical treatment, which is a strength of the present study. Finally, as RLS severity is not assessed in the CH-RLSq, it is not clear whether PPI/H2A use influences the strength or frequency of RLS.

The results from this study show a strong replicated association between PPI use and RLS risk even after controlling for many factors which influence RLS risk and blood iron levels. There is a possible association of H2A with RLS. Future studies will need to untangle the causality of this association applying longitudinal studies tracking exposure and RLS emergence over time.

Supplementary material
Supplementary material is available at SLEEP online.

Acknowledgments
The authors would like to express gratitude to the REDS-III group members and research staff at participating blood centers and testing laboratories for their invaluable contributions to this study. Additionally, the authors are grateful to N. Whitehead, A. Moore, and S. Erickson for their thoughts on initial drafts of the manuscript. We thank personnel employed in all blood banks across Denmark for making DBDS inclusion a part of their work routine. Finally, we thank the anonymous reviewers for their constructive feedback. Red Blood Cell (RBC)-Omics Study Group Members.

The NHLBI REDS-III, Red Blood Cell (RBC)-Omics Study, is the responsibility of the following people:

- **Hubs:** A.E. Mast, J.L. Gottschall, W. Bialkowski, L. Anderson, J. Miller, A. Hall, Z. Udee, and V. Johnson, BloodCenter of Wisconsin, Milwaukee, WI; D.J. Triulzi, J.E. Kiss, and P.A. D’Andrea, Vitalant Northeast Division, Pittsburgh, PA; E.L. Murphy and A.M. Guiltinan, University of California, San Francisco, San Francisco, CA; R.G. Cable, B.R. Spencer, and S.T. Johnson, American Red Cross Blood Services, Farmington, CT.
- **Data coordinating center:** D.J. Brambilla, M.T. Sullivan, S.M. Endres, G.P. Page, Y. Guo, N. Haywood, D. Ringer, and B.C. Siege, RTI International, Rockville, MD; Central and testing laboratories: M.P. Busch, M.C. Lanteri, M. Stone, and S. Keating, Blood Systems Research Institute, San Francisco, CA; T. Kanias and M. Gladwin, Pittsburgh Heart, Lung, Blood, and Vascular Medicine Institute, Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA.
- **Steering committee chairman:** S.H. Kleinman, University of British Columbia, Victoria, BC, Canada.
- **NHLBI, National Institutes of Health:** S.A. Glynn, K.B. Malkin, and A.M. Cristman.

**Danish Blood Donors Study consortium members:** Mie T. Bruun, Henrik Hjalgrim, Karina Banasik, Kaspar Nielsen, Mikkel Petersen, Lise Wegner Thørner, and Thomas F. Hansen.

**Funding**
The National Heart, Lung, and Blood Institute (NHLBI) REDS-III was supported by NHLBI contracts NHLBI HHSN2682011-000011, 000021, 000031, 000041, 000051, 000061, 000071, 000081, and 000091. DBDS was supported by The Novo Nordisk Foundation NNF17OC0027594, Danish Regions, Rigshospitalet’s Research Group Members.

**Supplementary material**
Supplementary material is available at SLEEP online.

Acknowledgments
The authors would like to express gratitude to the REDS-III group members and research staff at participating blood centers and testing laboratories for their invaluable contributions to this study. Additionally, the authors are grateful to N. Whitehead, A. Moore, and S. Erickson for their thoughts on initial drafts of the manuscript. We thank personnel employed in all blood banks across Denmark for making DBDS inclusion a part of their work routine. Finally, we thank the anonymous reviewers for their constructive feedback. Red Blood Cell (RBC)-Omics Study Group Members.

The NHLBI REDS-III, Red Blood Cell (RBC)-Omics Study, is the responsibility of the following people:

- **Hubs:** A.E. Mast, J.L. Gottschall, W. Bialkowski, L. Anderson, J. Miller, A. Hall, Z. Udee, and V. Johnson, BloodCenter of Wisconsin, Milwaukee, WI; D.J. Triulzi, J.E. Kiss, and P.A. D’Andrea, Vitalant Northeast Division, Pittsburgh, PA; E.L. Murphy and A.M. Guiltinan, University of California, San Francisco, San Francisco, CA; R.G. Cable, B.R. Spencer, and S.T. Johnson, American Red Cross Blood Services, Farmington, CT.
- **Data coordinating center:** D.J. Brambilla, M.T. Sullivan, S.M. Endres, G.P. Page, Y. Guo, N. Haywood, D. Ringer, and B.C. Siege, RTI International, Rockville, MD; Central and testing laboratories: M.P. Busch, M.C. Lanteri, M. Stone, and S. Keating, Blood Systems Research Institute, San Francisco, CA; T. Kanias and M. Gladwin, Pittsburgh Heart, Lung, Blood, and Vascular Medicine Institute, Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA.
- **Steering committee chairman:** S.H. Kleinman, University of British Columbia, Victoria, BC, Canada.
- **NHLBI, National Institutes of Health:** S.A. Glynn, K.B. Malkin, and A.M. Cristman.

**Danish Blood Donors Study consortium members:** Mie T. Bruun, Henrik Hjalgrim, Karina Banasik, Kaspar Nielsen, Mikkel Petersen, Lise Wegner Thørner, and Thomas F. Hansen.

**Funding**
The National Heart, Lung, and Blood Institute (NHLBI) REDS-III was supported by NHLBI contracts NHLBI HHSN2682011-000011, 000021, 000031, 000041, 000051, 000061, 000071, 000081, and 000091. DBDS was supported by The Novo Nordisk Foundation NNF17OC0027594, Danish Regions, Rigshospitalet’s Research Group Members.
References

1. Innes KE, et al. Prevalence of restless legs syndrome in North American and Western European populations: a systematic review. Sleep Med. 2011;12(7):623–634.
2. Allen RP, et al. Restless legs syndrome prevalence and impact: REST general population study. Arch Intern Med. 2005;165(11):1286–1292.
3. Didriksen M, et al. Restless legs syndrome is associated with major comorbidities in a population of Danish blood donors. Sleep Med. 2018;45:124–131.
4. Earley CJ, et al. Restless legs syndrome: understanding its consequences and the need for better treatment. Sleep Med. 2010;11(9):807–815.
5. Innes KE, et al. Restless legs syndrome and conditions associated with metabolic dysregulation, sympathoadrenal dysfunction, and cardiovascular disease risk: a systematic review. Sleep Med Rev. 2012;16(4):309–339.
6. Connor JR, et al. Profile of altered brain iron acquisition in restless legs syndrome. Brain. 2011;134(Pt 4):959–968.
7. Allen RP, Earley CJ. The role of iron in restless legs syndrome. Mov Disord. 2007;22(Suppl 15):S440–S448.
8. Winkelmann J, et al. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Nat Genet. 2007;39(8):1000–1006.
9. Vilarino-Guell C, et al. A genetic risk factor for periodic limb movements in sleep. N Engl J Med. 2008;358(4):425–427.
10. Schormair B, et al. Identification of novel risk loci for restless legs syndrome in genome-wide association studies in individuals of European ancestry: a meta-analysis. Lancet Neurol. 2017;16(11):898–907.
11. Stefansson H, et al. A genetic risk factor for periodic limb movements in sleep. N Engl J Med. 2007;357(7):639–647.
12. Catoire H, et al. Restless legs syndrome-associated MEIS1 risk variant influences iron homeostasis. Ann Neurol. 2011;70(1):170–175.
13. Sarayloo F, et al. MEIS1 and restless legs syndrome: a comprehensive review. Front Neurol. 2019;10:935.
14. Lyu S, et al. BTBD9 and dopaminergic dysfunction in the pathogenesis of restless legs syndrome. Brain Struct Funct. 2020;225(6):1743–1760.
15. Freeman A, et al. Sleep fragmentation and motor restlessness in a Drosophila model of Restless Legs Syndrome. Curr Biol. 2012;22(12):1142–1148.
16. Wang J, et al. Efficacy of oral iron in patients with restless legs syndrome and a low-normal ferritin: a randomized, double-blind, placebo-controlled study. Sleep Med. 2009;10(9):973–975.
17. Macher S, et al. The effect of parenteral or oral iron supplementation on fatigue, sleep, quality of life and restless legs syndrome in iron-deficient blood donors: a secondary analysis of the ironwoman RCT. Nutrients. 2020;12(5):1313–1328.
18. Allen RP, et al. Clinical efficacy and safety of IV ferric carboxymaltose (FCM) treatment of RLS: a multi-centred, placebo-controlled preliminary clinical trial. Sleep Med. 2011;12(9):906–913.
19. Cho YW, et al. Clinical efficacy of ferric carboxymaltose treatment in patients with restless legs syndrome. Sleep Med. 2016;25:16–23.
20. Gupta R, et al. Restless legs syndrome and pregnancy: prevalence, possible pathophysiological mechanisms and treatment. Acta Neurol Scand. 2016;133(5):320–329.
21. Manconi M, et al. When gender matters: restless legs syndrome. Report of the “RLS and woman” workshop endorsed by the European RLS Study Group. Sleep Med Rev. 2012;16(4):297–307.
22. Di Angelantonio E, et al. Efficiency and safety of varying the frequency of whole blood donation (INTERVAL): a randomised trial of 45 000 donors. Lancet. 2017;390(10110):2360–2371.
23. Spencer BR, et al. Restless legs syndrome, pica, and iron status in blood donors. Transfusion. 2013;53(8):1645–1652.
24. Bryant BJ, et al. Ascertainment of iron deficiency and depletion in blood donors through screening questions for pica and restless legs syndrome. Transfusion. 2013;53(8):1637–1644.
25. Trotti LM, et al. Iron for restless legs syndrome. Cochrane Database Syst Rev. 2012;5:CD007834.
26. Page RL, 2nd, et al. Restless legs syndrome induced by escitalopram: case report and review of the literature. Pharmacotherapy. 2008;28(2):271–280.
27. Rottach KG, et al. Restless legs syndrome as side effect of second generation antidepressants. J Psychiatr Res. 2008;43(1):70–75.
28. Cotter PE, et al. Restless legs syndrome: is it a real problem? Ther Clin Risk Manag. 2006;2(4):465–475.
29. Hutchinson C, et al. Proton pump inhibitors suppress absorption of dietary non-haem iron in hereditary haemochromatosis. Gut. 2007;56(9):1291–1295.
30. Sharma VR, et al. Effect of omeprazole on oral iron replacement in patients with iron deficiency anaemia. South Med J. 2004;97(9):887–889.
31. Tran-Duy A, et al. Use of proton pump inhibitors and risk of iron deficiency: a population-based case-control study. J Intern Med. 2019;285(2):205–214.
32. Lam JR, et al. Proton pump inhibitor and histamine-2 receptor antagonist use and iron deficiency. Gastroenterology. 2017;152(4):821–829.e1.
33. Bialkowski W, et al. Estimates of total body iron indicate 19 mg and 38 mg oral iron are equivalent for the mitigation of iron deficiency in individuals experiencing repeated phlebotomy. Am J Hematol. 2017;92(9):851–857.
34. Skikne BS, et al. Role of gastric acid in food iron absorption. Gastroenterology. 1981;81(6):1068–1071.
35. Kotzé SR, et al. Predictors of hemoglobin in Danish blood donors: results from the Danish Blood Donor Study. Transfusion. 2015;55(6):1303–1311.
36. Pottegård A, et al. Use of proton-pump inhibitors among adults: a Danish nationwide drug utilization study. Therap Adv Gastroenterol. 2016;9(5):671–678.
37. Endres-Dighe SM, et al. Blood, sweat, and tears: Red Blood Cell-omics study objectives, design, and recruitment activities. Transfusion. 2019;59(1):46–56.
38. Cable RG, et al. Iron deficiency in blood donors: analysis of enrollment data from the REDS-II Donor Iron Status Evaluation (RISE) study. Transfusion. 2011;51(3):511–522.
39. Pedersen OB, et al. The Danish Blood Donor Study: a large, prospective cohort and biobank for medical research. Vox Sang. 2012;102(3):271.
40. Burgdorf KS, et al. Digital questionnaire platform in the Danish Blood Donor Study. Comput Methods Programs Biomed. 2016;135:101–104.
41. Pedersen CB. The Danish civil registration system. Scand J Public Health. 2011;39(7 Suppl):22–25.
42. Allen RP, et al. Validation of the self-completed Cambridge-Hopkins questionnaire (CH-RLSq) for ascertainment of restless legs syndrome (RLS) in a population survey. Sleep Med. 2009;10(10):1097–1100.
43. Walters AS, et al. Review of diagnostic instruments for the restless legs syndrome/Willis-Ekbom Disease (RLS/ WED): critique and recommendations. J Clin Sleep Med. 2014;10(12):1343–1349.
44. Kerr S, et al. Descriptors of restless legs syndrome sensations. Sleep Med. 2012;13(4):409–413.
45. Kildemoes HW, et al. The Danish National Prescription Registry. Scand J Public Health. 2011;39(7 Suppl):38–41.
46. Finch CA, et al. Effect of blood donation on iron stores as evaluated by serum ferritin. Blood. 1977;50(3):441–447.
47. Allen RP, et al. The prevalence and impact of restless legs syndrome on patients with iron deficiency anemia. Am J Hematol. 2013;88(4):261–264.
48. Allen RP, et al. Evidence-based and consensus clinical practice guidelines for the iron treatment of restless legs syndrome/Willis-Ekbom disease in adults and children: an IRLSSG task force report. Sleep Med. 2018;41:27–44.
49. Didriksen M, et al. Prevalence of restless legs syndrome and associated factors in an otherwise healthy population: results from the Danish Blood Donor Study. Sleep Med. 2017;36:55–61.
50. Connor JR, et al. Evidence for communication of peripheral iron status to cerebrospinal fluid: clinical implications for therapeutic strategy. Fluids Barriers CNS. 2020;17(1):28.
51. Dignass A, et al. Limitations of serum ferritin in diagnosing iron deficiency in inflammatory conditions. Int J Chronic Dis. 2018;2018:9394060.
52. van den Hurk K, et al. Associations of health status with subsequent blood donor behavior—an alternative perspective on the Healthy Donor Effect from Donor InSight. PLoS One. 2017;12(10):e0186662.