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# Structural Brain Lesions and Restless Legs Syndrome: A cross-sectional population-based study

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**Abstract**

**Objective:** To evaluate the association between white matter lesion volume, silent infarcts, and restless legs syndrome in a population-based study of elderly individuals.

**Design:** Cross-sectional study

**Setting:** Population-based Three-City study

**Participants:** 1268 individuals from the Dijon, France center of the Three-City study who had available information on volume of white matter lesions (WML) from MRI scans and who answered questions about the prevalence of restless legs syndrome

**Primary Outcome Measure:** Prevalence of restless legs syndrome

**Results:** White matter lesion volume was measured using an automated tissue segmentation method. Logistic regression was used to evaluate adjusted associations between tertiles of WML volume and restless legs syndrome and between silent infarcts and restless legs syndrome. 218 individuals (17.2%) were determined to have restless legs syndrome. Compared to those in the first tertile of WML volume, individuals in the second tertile (OR=0.95; 95% CI: 0.66, 1.37) or third tertile (OR=1.00; 95% CI: 0.69, 1.46) did not have an increased prevalence of restless legs syndrome. We also did not observe associations between the volume of deep or periventricular WML and restless legs syndrome; nor did we observe an association between silent brain infarcts and RLS (OR=0.61; 95% CI: 0.33, 1.11). These findings were not modified by age or gender.

**Conclusion:** Higher volume of WML and the presence of silent infarcts were not associated with an increased prevalence of restless legs syndrome in this population-based cohort of elderly individuals.

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## Article Summary

### Article focus

- The aim of this study is to evaluate the association between white matter lesion volume, silent infarcts, and restless legs syndrome (RLS) in a population-based study of elderly individuals.

### Key messages

- We found no cross-sectional association between WML volume or brain infarcts and RLS. The results of this study do not support an association between RLS and vascular brain lesions.

### Strengths and limitations of this study

- Strengths include the population-based setting with available brain imaging, the size of the cohort, standardized assessment of RLS using criteria from the International Restless Legs Study Group and use of an automated measurement procedure to quantify and localize WML.

- Limitations to this study include its cross-sectional design, lack of information on kidney disease or iron deficiency for participants, and self-reported information on RLS. Additionally, RLS status was not assessed at baseline and participants who were still in the study at restless legs assessment may be healthier than participants who died or dropped out prior to RLS assessment.

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## Introduction

Restless legs syndrome (RLS) is a neurological disorder that originates in the motoneurons of the brain and is characterized by an urge to move the legs and unpleasant leg sensations. Population based studies using the minimal diagnostic criteria from the International Restless Legs Syndrome Study Group[1 2] have found the prevalence of RLS ranges from 6% to 12% with women being affected twice as often as men.[3] In addition, the prevalence of RLS increases with age.[3] While the causes of RLS are unknown, some studies have suggested that dysfunction of the dopaminergic system may contribute to disease development. However, neuroimaging studies have not shown any primary neurodegeneration of dopaminergic neurons in the substantia nigra.[4] A large genetics consortium study has found some genetic variants that may be associated with the development of RLS.[5]

Several studies have found associations between cardiovascular risk factors including smoking[6-10], diabetes[7 9 11], hypercholerolemia[9 10], exercise[9], body mass index[6 9 12 13] and hypertension[7 14 15] and RLS. However, evidence for an association between RLS and incident cardiovascular disease is mixed.[6 9 11 15-20] One recent longitudinal study did not find evidence of an association between RLS and incident cardiovascular disease[21] while others have found an association between RLS and coronary heart disease[22 23] and mortality.[24] Several possible biological mechanisms have been proposed to explain why RLS may be associated with high blood pressure, heart disease, and stroke. RLS is associated with sympathetic hyperactivity which may lead to daytime hypertension which itself is a risk factor for heart disease and stroke. Alternatively, even if the sympathetic hyperactivity does not lead to daytime hypertension, it may impact atherosclerotic plaque formation and rupture which could lead to heart disease and stroke. Other comorbidities associated with RLS may also be risk

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factors for heart disease and stroke.[25] In addition to its association with cardiovascular risk factors, RLS is also associated with migraine in both men and women.[26 27]

Given the association of RLS with several vascular risk factors as well as with migraine, we were interested to know if white matter lesions (WML), which are also strongly related to vascular risk factors, including migraine, and cardiovascular disease, were associated with RLS. Using a population-based cohort of elderly individuals residing in France, we examined the cross-sectional association between white matter lesion volume and RLS prevalence.

**Methods**

The Three-City (3C) is a longitudinal cohort study enrolling subjects living in three French cities (Bordeaux, Dijon, and Montpellier) designed to estimate the risk of dementia and cognitive impairment attributable to vascular risk factors.[28] The present analysis only uses data from subjects living in Dijon because data on RLS were only collected in that city. Each subject signed an informed consent statement and the Ethical Committee of the University Hospital of Kremlin-Bicêtre approved the methods and procedures of the 3C study.

To be eligible for the 3C study, the participant needs to live in Dijon, be registered on the electoral rolls in 1999, be 65 years of older, and not be institutionalized. A total of 4,931 individuals were recruited at the Dijon site between 1999 and 2001. For the MRI sub-study, all subjects recruited from the Dijon center who were <80 years of age and enrolled between June 1999 and September 2000 were eligible to participate. Of those eligible, 2,285 (82%) subjects agreed to participate in the MRI study, but only 1,924 scans could be performed at baseline due to financial constraints.

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The process of obtaining the MRI information has been described in detail elsewhere.[29 30] In brief, MRI scans were obtained using a 1.5T Magnetom (Siemens, Erlangen, Germany). A 3-dimensional (3D) high-resolution T1-weighted brain volume was obtained using a 3D inversion recovery fast spoiled-gradient echo sequence. T2- and proton density (PD)-weighted brain volumes were acquired using a 2-dimensional dual spin echo sequences with two echo times. Each subject dataset (T1, T2, PD) was reconstructed and visually checked for major artifacts before being stored.

A fully-automated image processing software was used to detect, measure, and localize WMLs. The process has been described in detail previously[29 30]. Based on the morphologic parameters (center of mass coordinates, Euclidian distance to the ventricular system, principal axes dimension), each WML was labeled as being either periventricular if the distance to the ventricular system was <10 mm or deep otherwise. Total volume of periventricular and deep WMLs were estimated by summing the volumes of all periventricular and deep lesions.

We log transformed the values of total WML, periventricular WML, deep WML, and total white matter volume as they were not normally distributed. We then divided the log transformed values into tertiles to allow for non-linear associations between WML volumes and RLS.

Infarcts were rated on T1, T2, and PD-weighted images and defined as focal lesions ≥3 mm in diameter with the same signal characteristic as cerebrospinal fluid on all sequences. They were discriminated from dilated Virchow-Robin spaces using multi-planar reformatting. Those with a typical vascular shape and following the orientation of perforating vessels were classified as dilated Virchow-Robin spaces and not as infarcts.[31]

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*Restless Legs Syndrome Assessment*

RLS was assessed at the fifth and sixth follow-up waves of the study. Participants were asked a series questions designed to address the four minimal diagnostic criteria of the International Restless Legs Study Group which has been established and validated in previous studies.[32 33] The participants were asked: "Have if ever felt unpleasant sensation in the legs (restlessness, tingling, tension, annoyance, annoyances, contractions, twitching, numbness, electricity, etc.) with the irresistible need or want to move?" Response options were yes or no. If the participant responded "yes", he or she was further asked: "Do these unpleasant sensations occur solely or mainly at rest (when you are sitting or lying down, without moving your legs) and do they improve with movement?" and "Are these unpleasant sensations more intense in the evening or at night than in the morning?" Response options for these questions were yes or no. If the participant responded yes to all three questions, he or she was defined as having RLS.

*Covariates*

Trained psychologists collected sociodemographic and medical data on participants during home visits. Participants were asked if there were treated for various co-morbidities at baseline and in the fifth and sixth wave of the study. We used all available information from baseline to RLS assessment to determine a subject's co-morbidity history. History of cardiovascular disease was defined as a history of myocardial infarction, stroke, angina, percutaneous transluminal coronary angioplasty, or coronary artery bypass surgery. History of diabetes was defined as glycemia ≥7 mmol/L or use of anti-diabetic treatment.[34] High blood pressure was defined as measured systolic blood pressure ≥140 mmHg or measured diastolic blood pressure ≥90 mmHg. High cholesterol was defined as lipid lowering treatment or

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cholesterol ≥6.2 mmol/L. Information was also collected on history of peripheral artery disease, history of leg operation, and history of edema/swelling of legs and ankles.

For the following covariates, we used values from the follow-up waves during which RLS was assessed: body mass index, smoking status, alcohol consumption and physical activity. If this value was missing, we used values from baseline. Height and weight were used to calculate body mass index. Smoking status was reported as never, past, or current, and alcohol consumption was measured in grams per day. Due to changes in the questionnaires on physical activity over time, we classified participants as active versus non-active.

*Statistical Analysis*

Of the 1924 individuals in the MRI sub-study, we excluded 581 participants who did not answer any of the questions about RLS and the 75 participants who did not have data on total WML volume, leaving a total of 1268 participants eligible for our analyses of which 218 (17.2%) reported RLS.

We performed a cross-sectional analysis using logistic regression to calculate odds ratios and 95% confidence intervals of reporting RLS for each tertile of the WML volumes using the lowest tertile as the reference group. Analyses examining the association between tertiles of total WML volume and RLS adjusted for tertiles of total white matter as the likelihood of WML correlates with the size of the total white matter. Analyses examining the association between tertiles of periventricular or deep WML and RLS adjusted for tertiles of WML. We also used logistic regression to examine the association between any brain silent infarct and RLS. For the infarct analyses, we excluded participants with a brain tumor detected at MRI.

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We performed age- and sex-adjusted analyses and multivariable-adjusted analyses. Our multivariable analyses adjusted for age (continuous), sex, smoking status (never, past, or current smoker), alcohol consumption (0, 0 to ≤12, 12 to ≤24, and >24 grams/day), physical activity (active versus not active), body mass index (<25 kg/m$^2$, 25 to <30 kg/m$^2$, and ≥30 kg/m$^2$), history of hypertension (yes/no), history of diabetes (yes/no), history of cardiovascular disease (yes/no), history of peripheral artery disease (yes/no), history of leg operation (yes/no), and history of edema/swelling of legs and ankles (yes/no). Further adjustment for measures of sleep quality, difficulty sleeping and taking sleep medications did not affect our results (results not shown). Less than 14 people were missing information on any covariate and were assigned to the most frequent value of that covariate. We also performed separate age-adjusted analyses stratified by sex or mean age (72 years).

We considered a two-tailed p value of <0.05 as statistically significant and used SAS 9.1.3 as statistical software (Cary, NC).

**Results**

The characteristics of the participants by RLS status can be seen in Table 1. Those who reported RLS were more likely to be female, never smokers, non-drinkers, and were less physically active than those who did not report RLS.

We did not observe an association between tertiles of WML and RLS (Table 2). Compared to those in the lowest tertile of WML, the multivariable-adjusted odds ratio of reporting RLS was 0.95 (95% CI: 0.66, 1.37) for those in the second tertile and 1.00 (95% CI: 0.69, 1.46) for those in the top tertile. We also did not observe an association between tertiles of deep or periventicular WML and RLS (Table 2).

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We also performed age-adjusted analyses stratified by sex or mean age (72 years). Among men, compared to those in the lowest tertile of WML, the age-adjusted odds ratio of reporting RLS was 0.73 (95% CI: 0.36, 1.52) for those in the second tertile and 0.78 (95% CI: 0.38, 1.56) for those in the top tertile. Among women, compared to those in the lowest tertile of WML, the age-adjusted odds ratio of reporting RLS was 1.02 (95% CI: 0.67, 1.55) for those in the second tertile and 1.07 (95% CI: 0.69, 1.66) for those in the top tertile. For those under 72 years of age, the age-adjusted odds ratio of reporting RLS was 0.88 (95% CI: 0.55, 1.42) for those in the second tertile and 0.99 (95% CI: 0.59, 1.66) for those in the top tertile compared to those in the lowest tertile of WML. For those 72 years of age or older, the age-adjusted odds ratio of reporting RLS was 1.02 (95% CI: 0.58, 1.80) for those in the second tertile and 1.05 (95% CI: 0.61, 1.78) for those in the top tertile compared to those in the lowest tertile of WML.

We also explored whether there was an association between infarcts and RLS. Of the 1330 people with information on brain infarcts, 131 had a brain infarct and 225 reported RLS. The age- and sex-adjusted odds ratio between infarcts and RLS was 0.59 (95% CI: 0.32, 1.07). The multivariable-adjusted odds ratios between infarcts and RLS was 0.61 (95% CI: 0.33, 1.11).

**Discussion**

In this large, population-based study of elderly individuals, we found no cross-sectional association between WML volume or brain infarcts and RLS. The results of this study do not support an association between RLS and vascular brain lesions.

Previous research on the association between WML volume and RLS is limited. A small study of 45 patients found that white matter hyperintensities were correlated with total limb movements per hour of sleep after adjusting for hypertension (r=0.66, p=0.01).[35] The authors

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suggest that leg movements may be associated with poor quality sleep which may contribute to episodes of nocturnal hypertension. Although nocturnal hypertension has been associated with the development of white matter hyperintensities even among those with daytime hypertension[36], this study did not present results on the association between RLS and white matter hyperintensities. Additionally, it is unclear if the authors adjusted for other potential confounders including age and sex.

Another study using data from the Memory and Morbidity in Augsburg Elderly Study (MEMO) examined the association between RLS and brain lesions detected using MRI. They found a non-significant increase risk of silent infarction (OR=2.11, 95% CI: 0.71-6.27) and subcortical brain lesions greater than or equal to 10mm (OR=1.35, 95% CI: 0.56-3.22) in those who reported RLS compared to those without RLS.[25] The small size of this study (26 RLS cases and 241 controls) and limited power to control for confounding by cardiovascular risk factors may explain some of the differences between the results of the MEMO study and our study.

While evidence for a direct association between WML and RLS is limited, some cross-sectional studies have suggested that RLS may be associated with hypertension[14], stroke[9 37], and migraine[26 27], which are associated with WML. However, a recent longitudinal study found no association between RLS and incident cardiovascular disease [21] and not all cross-sectional studies have found associations between hypertension and RLS.[9 11] Our study did not find an association between RLS and structural brain lesions which are strongly related to vascular risk factors and cardiovascular disease. This observation aligns with the previous study that did not find an association between RLS and incident cardiovascular disease.

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This study has several strengths including the population-based setting with available brain imaging, the size of the cohort, and standardized assessment of RLS using criteria from the International Restless Legs Study Group.[1 2] We also used an automated measurement procedure to quantify and localize WML. Compared to visual scale, automated procedures are not subject to a ceiling effect, permit better discrimination of lesion volume, and are more sensitive in detecting small group differences.[38]

Limitations to this study include its cross-sectional design which prevents us from determining the temporal ordering of RLS and WML or examining how RLS may impact WML progression over time. RLS was first assessed in the fifth and sixth waves of the study. Participants who were still in the study then may be healthier than participants who died or dropped out prior to RLS assessment. We did not have information on kidney disease or iron deficiency for participants, which may be related to RLS. Information on RLS was self-reported and potential misclassification is possible. However, we used the best available questionnaire for population-level assessment of RLS and this questionnaire has been validated in previous cohorts.[32 33]

While our data do not support a strong association between structural brain lesions and RLS, further targeted research is warranted to evaluate whether subgroups of patients with RLS exist who are at increased risk for structural brain lesions.

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**Authorship Contributions**

Pamela M. Rist: drafting/revising the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data

Christophe Tzourio: obtaining funding, interpretation of data, revising the manuscript for content, and supervision

Alexis Elbaz: interpretation of data and revising the manuscript for content

Aicha Soumare: interpretation of data and revising the manuscript for content

Carole Dufouil: interpretation of data and revising the manuscript for content

Bernard Mazoyer: interpretation of data and revising the manuscript for content

Tobias Kurth: Conception and design, interpretation of data, revising the manuscript for content, and supervision

All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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**Full Author Disclosures**:

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Tobias Kurth has received within the last 2 years investigator-initiated research funding from the French National Research Agency, and the US National Institutes of Health. Further, he has received honoraria from the American Academy of Neurology for educational lectures and from the *BMJ* and *Cephalalgia* for editorial services.

Alexis Elbaz has received within the last 2 years investigator-initiated research funding unrelated to this project from the French National Research Agency, EU Joint Programme –

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Bernard Mazoyer has no disclosures.

**Data Sharing Statement**

No additional data available

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## References

1. Allen RP, Picchietti D, Hening WA, et al. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep medicine 2003;**4**(2):101-19
2. Walters AS. Toward a better definition of the restless legs syndrome. The International Restless Legs Syndrome Study Group. Movement disorders : official journal of the Movement Disorder Society 1995;**10**(5):634-42 doi: 10.1002/mds.870100517[published Online First: Epub Date]|.
3. Berger K, Kurth T. RLS epidemiology--frequencies, risk factors and methods in population studies. Movement disorders : official journal of the Movement Disorder Society 2007;**22 Suppl 18**:S420-3 doi: 10.1002/mds.21589[published Online First: Epub Date]|.
4. Paulus W, Dowling P, Rijsman R, Stiasny-Kolster K, Trenkwalder C, de Weerd A. Pathophysiological concepts of restless legs syndrome. Movement disorders : official journal of the Movement Disorder Society 2007;**22**(10):1451-6 doi: 10.1002/mds.21533[published Online First: Epub Date]|.
5. Winkelmann J, Schormair B, Lichtner P, et al. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Nature genetics 2007;**39**(8):1000-6 doi: 10.1038/ng2099[published Online First: Epub Date]|.
6. Ohayon MM, Roth T. Prevalence of restless legs syndrome and periodic limb movement disorder in the general population. Journal of psychosomatic research 2002;**53**(1):547-54
7. Phillips B, Young T, Finn L, Asher K, Hening WA, Purvis C. Epidemiology of restless legs symptoms in adults. Archives of internal medicine 2000;**160**(14):2137-41
8. Sevim S, Dogu O, Camdeviren H, et al. Unexpectedly low prevalence and unusual characteristics of RLS in Mersin, Turkey. Neurology 2003;**61**(11):1562-9
9. Winter AC, Schurks M, Glynn RJ, et al. Vascular risk factors, cardiovascular disease, and restless legs syndrome in women. The American journal of medicine 2013;**126**(3):220-7, 27 e1-2 doi: 10.1016/j.amjmed.2012.06.040[published Online First: Epub Date]|.
10. Schlesinger I, Erikh I, Avizohar O, Sprecher E, Yarnitsky D. Cardiovascular risk factors in restless legs syndrome. Movement disorders : official journal of the Movement Disorder Society 2009;**24**(11):1587-92 doi: 10.1002/mds.22486[published Online First: Epub Date]|.
11. Winter AC, Berger K, Glynn RJ, et al. Vascular risk factors, cardiovascular disease, and restless legs syndrome in men. The American journal of medicine 2013;**126**(3):228-35, 35 e1-2 doi: 10.1016/j.amjmed.2012.06.039[published Online First: Epub Date]|.
12. Kim J, Choi C, Shin K, et al. Prevalence of restless legs syndrome and associated factors in the Korean adult population: the Korean Health and Genome Study. Psychiatry and clinical neurosciences 2005;**59**(3):350-3 doi: 10.1111/j.1440-1819.2005.01381.x[published Online First: Epub Date]|.
13. Gao X, Schwarzschild MA, Wang H, Ascherio A. Obesity and restless legs syndrome in men and women. Neurology 2009;**72**(14):1255-61 doi: 10.1212/01.wnl.0000345673.35676.1c[published Online First: Epub Date]|.
14. Batool-Anwar S, Malhotra A, Forman J, Winkelman J, Li Y, Gao X. Restless legs syndrome and hypertension in middle-aged women. Hypertension 2011;**58**(5):791-6 doi: 10.1161/HYPERTENSIONAHA.111.174037[published Online First: Epub Date]|.
15. Ulfberg J, Nystrom B, Carter N, Edling C. Prevalence of restless legs syndrome among men aged 18 to 64 years: an association with somatic disease and neuropsychiatric symptoms. Movement disorders : official journal of the Movement Disorder Society 2001;**16**(6):1159-63
16. Allen R, Chen C, Soaita A, et al. A randomized, double-blind, 6-week, dose-ranging study of pregabalin in patients with restless legs syndrome. Sleep medicine 2010;**11**(6):512-9 doi: 10.1016/j.sleep.2010.03.003[published Online First: Epub Date]|.

For peer review only

17. Juuti AK, Laara E, Rajala U, et al. Prevalence and associated factors of restless legs in a 57-year-old urban population in northern Finland. Acta neurologica Scandinavica 2010;**122**(1):63-9 doi: 10.1111/j.1600-0404.2009.01262.x[published Online First: Epub Date]|.
18. Wesstrom J, Nilsson S, Sundstrom-Poromaa I, Ulfberg J. Restless legs syndrome among women: prevalence, co-morbidity and possible relationship to menopause. Climacteric : the journal of the International Menopause Society 2008;**11**(5):422-8 doi: 10.1080/13697130802359683[published Online First: Epub Date]|.
19. Winkelman JW, Finn L, Young T. Prevalence and correlates of restless legs syndrome symptoms in the Wisconsin Sleep Cohort. Sleep medicine 2006;**7**(7):545-52 doi: 10.1016/j.sleep.2006.01.004[published Online First: Epub Date]|.
20. Winkelman JW, Shahar E, Sharief I, Gottlieb DJ. Association of restless legs syndrome and cardiovascular disease in the Sleep Heart Health Study. Neurology 2008;**70**(1):35-42 doi: 10.1212/01.wnl.0000287072.93277.c9[published Online First: Epub Date]|.
21. Winter AC, Schurks M, Glynn RJ, et al. Restless legs syndrome and risk of incident cardiovascular disease in women and men: prospective cohort study. BMJ open 2012;**2**(2):e000866 doi: 10.1136/bmjopen-2012-000866[published Online First: Epub Date]|.
22. Dong M, Wu Z, Lu M, Wang Z, Li Z. Combining the physical adsorption approach and the covalent attachment method to prepare a bifunctional bioreactor. International journal of molecular sciences 2012;**13**(9):11443-54 doi: 10.3390/ijms130911443[published Online First: Epub Date]|.
23. Liu H, Han Y, Fu H, et al. Construction and expression of sTRAIL-melittin combining enhanced anticancer activity with antibacterial activity in Escherichia coli. Applied microbiology and biotechnology 2013;**97**(7):2877-84 doi: 10.1007/s00253-012-4541-y[published Online First: Epub Date]|.
24. Li Y, Wang W, Winkelman JW, Malhotra A, Ma J, Gao X. Prospective study of restless legs syndrome and mortality among men. Neurology 2013;**81**(1):52-9
25. Walters AS, Moussouttas M, Siddiqui F, et al. Prevalence of stroke in Restless Legs Syndrome: Initial Results Point to the Need for More Sophisticated Studies. The open neurology journal 2010;**4**:73-7 doi: 10.2174/1874205X01004010073[published Online First: Epub Date]|.
26. Schurks M, Winter AC, Berger K, Buring JE, Kurth T. Migraine and restless legs syndrome in women. Cephalalgia;**32**(5):382-9
27. Winter AC, Schurks M, Berger K, Buring JE, Gaziano JM, Kurth T. Migraine and restless legs syndrome in men. Cephalalgia;**33**(2):130-5
28. Group CS. Vascular factors and risk of dementia: design of the Three-City Study and baseline characteristics of the study population. Neuroepidemiology 2003;**22**(6):316-25
29. Maillard P, Delcroix N, Crivello F, et al. An automated procedure for the assessment of white matter hyperintensities by multispectral (T1, T2, PD) MRI and an evaluation of its between-centre reproducibility based on two large community databases. Neuroradiology 2008;**50**(1):31-42 doi: 10.1007/s00234-007-0312-3[published Online First: Epub Date]|.
30. Soumare A, Elbaz A, Zhu Y, et al. White matter lesions volume and motor performances in the elderly. Annals of neurology 2009;**65**(6):706-15 doi: 10.1002/ana.21674[published Online First: Epub Date]|.
31. Zhu YC, Dufouil C, Soumare A, Mazoyer B, Chabriat H, Tzourio C. High degree of dilated Virchow-Robin spaces on MRI is associated with increased risk of dementia. Journal of Alzheimer's disease : JAD 2010;**22**(2):663-72 doi: 10.3233/JAD-2010-100378[published Online First: Epub Date]|.
32. Berger K, von Eckardstein A, Trenkwalder C, Rothdach A, Junker R, Weiland SK. Iron metabolism and the risk of restless legs syndrome in an elderly general population--the MEMO-Study. Journal of neurology 2002;**249**(9):1195-9 doi: 10.1007/s00415-002-0805-2[published Online First: Epub Date]|.

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33. Hogl B, Kiechl S, Willeit J, et al. Restless legs syndrome: a community-based study of prevalence, severity, and risk factors. Neurology 2005;**64**(11):1920-4 doi: 10.1212/01.WNL.0000163996.64461.A3[published Online First: Epub Date]|.
34. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes care 1997;**20**(7):1183-97
35. Nocturnal limb movements are correlated with cerebral white matter hyperintensities. American Academy of Neurology; 2012; New Orleans, LA, USA.
36. Henskens LH, Kroon AA, van Oostenbrugge RJ, et al. Associations of ambulatory blood pressure levels with white matter hyperintensity volumes in hypertensive patients. Journal of hypertension 2009;**27**(7):1446-52 doi: 10.1097/HJH.0b013e32832b5204[published Online First: Epub Date]|.
37. Elwood P, Hack M, Pickering J, Hughes J, Gallacher J. Sleep disturbance, stroke, and heart disease events: evidence from the Caerphilly cohort. Journal of epidemiology and community health 2006;**60**(1):69-73 doi: 10.1136/jech.2005.039057[published Online First: Epub Date]|.
38. van Straaten EC, Fazekas F, Rostrup E, et al. Impact of white matter hyperintensities scoring method on correlations with clinical data: the LADIS study. Stroke; a journal of cerebral circulation 2006;**37**(3):836-40 doi: 10.1161/01.STR.0000202585.26325.74[published Online First: Epub Date]|.

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Table 1. Characteristics of participants by RLS status.

| Characteristic (%) | No RLS (n=1050) | RLS (n=218) |
|---|---|---|
| Age (mean, SD) | 72.1 (4.1) | 71.6 (3.8) |
| Sex (% female) | 59.3 | 72.9 |
| Smoking status (%) | | |
| Never | 60.0 | 67.0 |
| Past | 35.2 | 29.4 |
| Current | 4.8 | 3.7 |
| Alcohol consumption (%) | | |
| Non-drinker | 33.6 | 39.9 |
| 0 to ≤12 g/day | 41.1 | 45.0 |
| 12 to ≤24 g/day | 15.8 | 11.0 |
| >24 g/day | 9.2 | 4.1 |
| Physically active (%) | 21.3 | 16.5 |
| Body mass index | | |
| <25 kg/m$^2$ | 45.3 | 44.5 |
| 25 to < 30 kg/m$^2$ | 40.2 | 40.8 |
| ≥30 kg/m$^2$ | 14.5 | 14.7 |
| History of high blood pressure (%) | 86.6 | 86.7 |
| History of high cholesterol (%) | 63.6 | 59.6 |
| History of diabetes (%) | 11.8 | 9.2 |

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| | | |
|---|---|---|
| History of cardiovascular disease (%) | 18.1 | 17.9 |
| History of peripheral artery disease (%) | 4.6 | 4.1 |
| History of leg operation (%) | 0.9 | 0.5 |
| History of edema/swelling of legs and ankles (%) | 29.0 | 36.7 |
| Difficulty sleeping | | |
| Never | 47.5 | 44.0 |
| Rarely | 30.2 | 20.2 |
| Regularly | 10.8 | 17.0 |
| Often | 10.8 | 18.8 |
| Quality of sleep | | |
| Good | 52.6 | 45.0 |
| Average | 31.8 | 33.5 |
| Mediocre or bad | 12.3 | 21.6 |
| Take medications for sleep | 35.2 | 45.0 |

*Numbers may not add to 100% due to missing data.

List of abbreviations: RLS=restless legs syndrome, SD=standard deviation

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Table 2. Cross-sectional age- and sex-adjusted and multivariable-adjusted* associations between RLS and brain white matter lesion volumes.

| | No restless legs syndrome (n=1050) | Restless legs syndrome (n=218) | | |
|---|---|---|---|---|
| Total WML volume in tertiles | N (%) | N (%) | Age and sex-adjusted odds ratio (95% CI) | Multivariable-adjusted odds ratio (95% CI) |
| Lowest Tertile | 343 (32.7) | 76 (34.9) | 1.00 | 1.00 |
| Middle Tertile | 348 (33.1) | 70 (32.1) | 0.95 (0.66, 1.36) | 0.95 (0.66, 1.37) |
| Highest Tertile | 359 (34.2) | 72 (33.0) | 1.00 (0.69, 1.45) | 1.00 (0.69, 1.46) |
| Periventricular WML volume in tertiles | | | | |
| Lowest Tertile | 341 (32.5) | 77 (35.3) | 1.00 | 1.00 |
| Middle Tertile | 351 (33.4) | 68 (31.2) | 0.86 (0.49, 1.51) | 0.86 (0.49, 1.51) |
| Highest Tertile | 358 (34.1) | 73 (33.5) | 1.00 (0.46, 2.18) | 1.02 (0.47, 2.25) |

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| | | | | |
|---|---|---|---|---|
| Deep WML volume in tertiles | | | | |
| Lowest Tertile | 352 (33.5) | 67 (30.7) | 1.00 | 1.00 |
| Middle Tertile | 340 (32.4) | 77 (35.3) | 1.24 (0.84, 1.84) | 1.22 (0.82, 1.81) |
| Highest Tertile | 358 (34.1) | 74 (33.9) | 1.21 (0.76, 1.94) | 1.18 (0.73, 1.90) |

*Adjusted for age, sex, smoking status, alcohol consumption, physical activity, body mass index, history of hypertension, history of diabetes, history of cardiovascular disease, history of peripheral artery disease, history of leg operation, and history of edema/swelling of legs and ankles.

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STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation | Page |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1, 5 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 5 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 7 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 8 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 8 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 8-11 |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants | 8 |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case | NA |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 8-11 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 8-11 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 11,12 |
| Study size | 10 | Explain how the study size was arrived at | 8, 11 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 8-11 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 11-12 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | NA |
| | | (*c*) Explain how missing data were addressed | 12 |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy | NA |
| | | (*e*) Describe any sensitivity analyses | NA |

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| | | | |
|---|---|---|---|
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 8,11, Table 1 |
| | | (b) Give reasons for non-participation at each stage | 8, 11 |
| | | (c) Consider use of a flow diagram | NA |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | Table 1 |
| | | (b) Indicate number of participants with missing data for each variable of interest | 12 |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) | NA |
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time | |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure | |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures | Table 2 |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 12-13, Table 2 |
| | | (*b*) Report category boundaries when continuous variables were categorized | 9-12 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | NA |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | NA |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 13 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 15 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 13-15 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 15 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 2-3 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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# Structural Brain Lesions and Restless Legs Syndrome: A cross-sectional population-based study

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# Structural Brain Lesions and Restless Legs Syndrome: A cross-sectional population-based study

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**Key words:** Epidemiology, Restless Legs Syndrome, MRI

**Running head:** Structural Brain Lesions and Restless Legs Syndrome

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**Abstract**

**Objective:** To evaluate the association between white matter lesion volume, silent infarcts, and restless legs syndrome in a population-based study of elderly individuals.

**Design:** Cross-sectional study

**Setting:** Population-based Three-City study

**Participants:** 1035 individuals from the Dijon, France center of the Three-City study who had available information on volume of white matter lesions (WML) from MRI scans and who answered questions about the prevalence of restless legs syndrome

**Primary Outcome Measure:** Prevalence of restless legs syndrome

**Results:** White matter lesion volume was measured using an automated tissue segmentation method. Logistic regression was used to evaluate adjusted associations between tertiles of WML volume and restless legs syndrome and between silent infarcts and restless legs syndrome. 218 individuals (21.1%) were determined to have restless legs syndrome. Compared to those in the first tertile of WML volume, individuals in the second tertile (OR=1.09; 95% CI: 0.75, 1.60) or third tertile (OR=1.17; 95% CI: 0.79, 1.74) did not have an increased prevalence of restless legs syndrome. We also did not observe associations between the volume of deep or periventricular WML and restless legs syndrome; nor did we observe an association between silent brain infarcts and RLS (OR=0.74; 95% CI: 0.40, 1.39). These findings were not modified by age or gender.

**Conclusion:** Higher volume of WML and the presence of silent infarcts were not associated with an increased prevalence of restless legs syndrome in this population-based cohort of elderly individuals.

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## Article Summary

### Article focus

- The aim of this study is to evaluate the association between white matter lesion volume, silent infarcts, and restless legs syndrome (RLS) in a population-based study of elderly individuals.

### Key messages

- We found no cross-sectional association between WML volume or brain infarcts and RLS. The results of this study do not support an association between RLS and vascular brain lesions.

### Strengths and limitations of this study

- Strengths include the population-based setting with available brain imaging, the size of the cohort, standardized assessment of RLS using criteria from the International Restless Legs Study Group and use of an automated measurement procedure to quantify and localize WML.

- Limitations to this study include its cross-sectional design, lack of information on kidney disease or iron deficiency for participants, and self-reported information on RLS. Additionally, RLS status was not assessed at baseline and participants who were still in the study at restless legs assessment may be healthier than participants who died or dropped out prior to RLS assessment.

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## Introduction

Restless legs syndrome (RLS) is a neurological disorder that originates in the motoneurons of the brain and is characterized by an urge to move the legs and unpleasant leg sensations. Population based studies using the minimal diagnostic criteria from the International Restless Legs Syndrome Study Group[1 2] have found the prevalence of RLS ranges from 6% to 12% with women being affected twice as often as men.[3] In addition, the prevalence of RLS increases with age.[3] While the causes of RLS are unknown, some studies have suggested that dysfunction of the dopaminergic system may contribute to disease development. However, neuroimaging studies have not shown any primary neurodegeneration of dopaminergic neurons in the substantia nigra.[4] A large genetics consortium study has found some genetic variants that may be associated with the development of RLS.[5]

Several studies have found associations between cardiovascular risk factors including smoking[6-10], diabetes[7 9 11], hypercholerolemia[9 10], exercise[9], body mass index[6 9 12 13] and hypertension[7 14 15] and RLS. These associations have led to the hypothesis that RLS may be a risk factor for cardiovascular disease. However, evidence for an association between RLS and incident cardiovascular disease is mixed.[6 9 11 15-20] One recent longitudinal study did not find evidence of an association between RLS and incident cardiovascular disease[21] while another found an association between RLS and coronary heart disease[22] and mortality.[23] Several possible biological mechanisms have been proposed to explain why RLS may be associated with high blood pressure, heart disease, and stroke. RLS is associated with sympathetic hyperactivity which may lead to daytime hypertension which itself is a risk factor for heart disease and stroke. Alternatively, even if the sympathetic hyperactivity does not lead to daytime hypertension, it may impact atherosclerotic plaque formation and rupture which could

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lead to heart disease and stroke. Other comorbidities associated with RLS may also be risk factors for heart disease and stroke.[24] In addition to its association with cardiovascular risk factors, RLS is also associated with migraine in both men and women.[25 26]

Given the association of RLS with several vascular risk factors as well as with migraine, we were interested to know if white matter lesions (WML), which are also strongly related to vascular risk factors, including migraine, and cardiovascular disease, were associated with RLS. Using a population-based cohort of elderly individuals residing in France, we examined the cross-sectional association between white matter lesion volume and RLS prevalence.

**Methods**

The Three-City (3C) is a longitudinal cohort study enrolling subjects living in three French cities (Bordeaux, Dijon, and Montpellier) designed to estimate the risk of dementia and cognitive impairment attributable to vascular risk factors.[27] The present analysis only uses data from subjects living in Dijon because data on RLS were only collected in that city. Each subject signed an informed consent statement and the Ethical Committee of the University Hospital of Kremlin-Bicêtre approved the methods and procedures of the 3C study.

To be eligible for the 3C study, the participant needs to live in Dijon, be registered on the electoral rolls in 1999, be 65 years of older, and not be institutionalized. A total of 4,931 individuals were recruited at the Dijon site between 1999 and 2001. For the MRI sub-study, all subjects recruited from the Dijon center who were <80 years of age and enrolled between June 1999 and September 2000 were eligible to participate. Of those eligible, 2,285 (82%) subjects agreed to participate in the MRI study, but only 1,924 scans could be performed at baseline due to financial constraints.

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The process of obtaining the MRI information has been described in detail elsewhere.[28 29] In brief, MRI scans were obtained using a 1.5T Magnetom (Siemens, Erlangen, Germany). A 3-dimensional (3D) high-resolution T1-weighted brain volume was obtained using a 3D inversion recovery fast spoiled-gradient echo sequence. T2- and proton density (PD)-weighted brain volumes were acquired using a 2-dimensional dual spin echo sequences with two echo times. Each subject dataset (T1, T2, PD) was reconstructed and visually checked for major artifacts before being stored.

A fully-automated image processing software was used to detect, measure, and localize WMLs. The process has been described in detail previously[28 29]. Based on the morphologic parameters (center of mass coordinates, Euclidian distance to the ventricular system, principal axes dimension), each WML was labeled as being either periventricular if the distance to the ventricular system was <10 mm or deep otherwise. Total volume of periventricular and deep WMLs were estimated by summing the volumes of all periventricular and deep lesions.

We log transformed the values of total WML, periventricular WML, deep WML, and total white matter volume as they were not normally distributed. We then divided the log transformed values into tertiles to allow for non-linear associations between WML volumes and RLS.

Infarcts were rated on T1, T2, and PD-weighted images and defined as focal lesions ≥3 mm in diameter with the same signal characteristic as cerebrospinal fluid on all sequences. They were discriminated from dilated Virchow-Robin spaces using multi-planar reformatting. Those with a typical vascular shape and following the orientation of perforating vessels were classified as dilated Virchow-Robin spaces and not as infarcts.[30]

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*Restless Legs Syndrome Assessment*

RLS was assessed at the fifth and sixth follow-up waves of the study. Participants were asked a series questions designed to address the four minimal diagnostic criteria of the International Restless Legs Study Group which has been established and validated in previous studies.[31 32] The participants were asked: "Have if ever felt unpleasant sensation in the legs (restlessness, tingling, tension, annoyance, annoyances, contractions, twitching, numbness, electricity, etc.) with the irresistible need or want to move?" Response options were yes or no. If the participant responded "yes", he or she was further asked: "Do these unpleasant sensations occur solely or mainly at rest (when you are sitting or lying down, without moving your legs) and do they improve with movement?" and "Are these unpleasant sensations more intense in the evening or at night than in the morning?" Response options for these questions were yes or no. If the participant responded yes to all three questions, he or she was defined as having RLS.

*Covariates*

Trained psychologists collected sociodemographic and medical data on participants during home visits. Participants were asked if there were treated for various co-morbidities at baseline and in the fifth and sixth wave of the study. We used all available information from baseline to RLS assessment to determine a subject's co-morbidity history. History of cardiovascular disease was defined as a history of myocardial infarction, stroke, angina, percutaneous transluminal coronary angioplasty, or coronary artery bypass surgery. History of diabetes was defined as glycemia ≥7 mmol/L or use of anti-diabetic treatment.[33] High blood pressure was defined as measured systolic blood pressure ≥140 mmHg or measured diastolic blood pressure ≥90 mmHg. High cholesterol was defined as lipid lowering treatment or

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cholesterol ≥6.2 mmol/L. Information was also collected on history of peripheral artery disease, history of leg operation, and history of edema/swelling of legs and ankles.

For the following covariates, we used values from the follow-up waves during which RLS was assessed: body mass index, smoking status, alcohol consumption and physical activity. If this value was missing, we used values from baseline. Height and weight were used to calculate body mass index. Smoking status was reported as never, past, or current, and alcohol consumption was measured in grams per day. Due to changes in the questionnaires on physical activity over time, we classified participants as active versus non-active.

*Statistical Analysis*

Of the 1924 individuals in the MRI sub-study, 1189 were still alive and participating in the 3C study during the waves in which RLS was assessed. We excluded 97 participants who did not answer any of the questions about RLS and the 57 participants who did not have data on total WML volume, leaving a total of 1035 participants eligible for our analyses of which 218 (21.1%) reported RLS (Figure 1).

We performed a cross-sectional analysis using logistic regression to calculate odds ratios and 95% confidence intervals of reporting RLS for each tertile of the WML volumes using the lowest tertile as the reference group. Analyses examining the association between tertiles of total WML volume and RLS adjusted for tertiles of total white matter as the likelihood of WML correlates with the size of the total white matter. Analyses examining the association between tertiles of periventricular or deep WML and RLS adjusted for tertiles of WML. We also used logistic regression to examine the association between any brain silent infarct and RLS. For the infarct analyses, we excluded participants with a brain tumor detected at MRI.

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We performed age- and sex-adjusted analyses and multivariable-adjusted analyses. Our multivariable analyses adjusted for age (continuous), sex, smoking status (never, past, or current smoker), alcohol consumption (0, 0 to ≤12, 12 to ≤24, and >24 grams/day), physical activity (active versus not active), body mass index (<25 kg/m$^2$, 25 to <30 kg/m$^2$, and ≥30 kg/m$^2$), history of hypertension (yes/no), history of diabetes (yes/no), history of cardiovascular disease (yes/no), history of peripheral artery disease (yes/no), history of leg operation (yes/no), and history of edema/swelling of legs and ankles (yes/no). Further adjustment for measures of sleep quality, difficulty sleeping and taking sleep medications did not affect our results (results not shown). All covariates were measured at baseline. Less than 39 people were missing information on any covariate, except for difficulty sleeping and were assigned to the reference value of that covariate. We created a separate category for those missing information on difficulty sleeping. We also performed separate age-adjusted analyses stratified by sex or mean age (72 years).

We considered a two-tailed p value of <0.05 as statistically significant and used SAS 9.3 as statistical software (Cary, NC).

**Results**

The characteristics of the participants by RLS status can be seen in Table 1. Those who reported RLS were more likely to be female, never smokers, non-drinkers, and were less physically active than those who did not report RLS.

We did not observe an association between tertiles of WML and RLS (Table 2). Compared to those in the lowest tertile of WML, the multivariable-adjusted odds ratio of reporting RLS was 1.09 (95% CI: 0.75, 1.60) for those in the second tertile and 1.17 (95% CI:

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0.79, 1.74) for those in the top tertile. We also did not observe an association between tertiles of deep or periventicular WML and RLS (Table 2).

We also performed age-adjusted analyses stratified by sex or mean age (72 years). Among men, compared to those in the lowest tertile of WML, the age-adjusted odds ratio of reporting RLS was 0.74 (95% CI: 0.35, 1.56) for those in the second tertile and 0.85 (95% CI: 0.41, 1.77) for those in the top tertile. Among women, compared to those in the lowest tertile of WML, the age-adjusted odds ratio of reporting RLS was 1.17 (95% CI: 0.76, 1.81) for those in the second tertile and 1.13 (95% CI: 0.72, 1.78) for those in the top tertile. For those under 72 years of age, the age-adjusted odds ratio of reporting RLS was 0.99 (95% CI: 0.61, 1.62) for those in the second tertile and 1.03 (95% CI: 0.61, 1.75) for those in the top tertile compared to those in the lowest tertile of WML. For those 72 years of age or older, the age-adjusted odds ratio of reporting RLS was 1.10 (95% CI: 0.61, 1.98) for those in the second tertile and 1.20 (95% CI: 0.68, 2.10) for those in the top tertile compared to those in the lowest tertile of WML.

We also explored whether there was an association between infarcts and RLS. Of the 1330 people with information on brain infarcts, 131 had a brain infarct and 225 reported RLS. The age- and sex-adjusted odds ratio between infarcts and RLS was 0.42 (95% CI: 0.15, 1.21). The multivariable-adjusted odds ratios between infarcts and RLS was 0.74 (95% CI: 0.40, 1.39).

**Discussion**

In this large, population-based study of elderly individuals, we found no cross-sectional association between WML volume or brain infarcts and RLS. The results of this study do not support an association between RLS and vascular brain lesions.

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Previous research on the association between WML volume and RLS is limited. A small study of 45 patients found that white matter hyperintensities were correlated with total limb movements per hour of sleep after adjusting for hypertension (r=0.66, p=0.01).[34] The authors suggest that leg movements may be associated with poor quality sleep which may contribute to episodes of nocturnal hypertension. Although nocturnal hypertension has been associated with the development of white matter hyperintensities even among those with daytime hypertension[35], this study did not present results on the association between RLS and white matter hyperintensities. Additionally, it is unclear if the authors adjusted for other potential confounders including age and sex.

Another study using data from the Memory and Morbidity in Augsburg Elderly Study (MEMO) examined the association between RLS and brain lesions detected using MRI. They found a non-significant increase risk of silent infarction (OR=2.11, 95% CI: 0.71-6.27) and subcortical brain lesions greater than or equal to 10mm (OR=1.35, 95% CI: 0.56-3.22) in those who reported RLS compared to those without RLS.[24] The small size of this study (26 RLS cases and 241 controls) and limited power to control for confounding by cardiovascular risk factors may explain some of the differences between the results of the MEMO study and our study.

While evidence for a direct association between WML and RLS is limited, some cross-sectional studies have suggested that RLS may be associated with hypertension[14], stroke[9 36], and migraine[25 26], which are associated with WML. However, a recent longitudinal study found no association between RLS and incident cardiovascular disease [21] and not all cross-sectional studies have found associations between hypertension and RLS.[9 11] Our study did not find an association between RLS and structural brain lesions which are strongly related to

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vascular risk factors and cardiovascular disease. This observation aligns with the previous study that did not find an association between RLS and incident cardiovascular disease.

This study has several strengths including the population-based setting with available brain imaging, the size of the cohort, and standardized assessment of RLS using criteria from the International Restless Legs Study Group.[1 2] We also used an automated measurement procedure to quantify and localize WML. Compared to visual scale, automated procedures are not subject to a ceiling effect, permit better discrimination of lesion volume, and are more sensitive in detecting small group differences.[37]

Limitations to this study include its cross-sectional design which prevents us from determining the temporal ordering of RLS and WML or examining how RLS may impact WML progression over time. RLS was first assessed in the fifth and sixth waves of the study (approximately 10 years after baseline). Participants who were still in the study then may be healthier than participants who died or dropped out prior to RLS assessment. We did not have information on kidney disease or iron deficiency for participants, which may be related to RLS. Information on RLS was self-reported and potential misclassification is possible. However, we used the best available questionnaire for population-level assessment of RLS and this questionnaire has been validated in previous cohorts.[31 32] Additionally, our questionnaire did not assess RLS severity or periodic limb movements association with RLS so we are unable to determine if the severity of RLS or presence of periodic limb movements may modify the association between RLS and WMH.

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While our data do not support a strong association between structural brain lesions and RLS, further targeted research is warranted to evaluate whether subgroups of patients with RLS exist who are at increased risk for structural brain lesions.

**Authorship Contributions**

Pamela M. Rist: drafting/revising the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data

Christophe Tzourio: obtaining funding, interpretation of data, revising the manuscript for content, and supervision

Alexis Elbaz: interpretation of data and revising the manuscript for content

Aicha Soumare: interpretation of data and revising the manuscript for content

Carole Dufouil: interpretation of data and revising the manuscript for content

Bernard Mazoyer: interpretation of data and revising the manuscript for content

Tobias Kurth: Conception and design, interpretation of data, revising the manuscript for content, and supervision

All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Tobias Kurth has received within the last 2 years investigator-initiated research funding from the French National Research Agency, and the US National Institutes of Health. Further, he has

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received honoraria from the American Academy of Neurology for educational lectures and from the *BMJ* and *Cephalalgia* for editorial services.

Alexis Elbaz has received within the last 2 years investigator-initiated research funding unrelated to this project from the French National Research Agency, EU Joint Programme – Neurodegenerative Disease Research (JPND), the French Agency for Drug Security (ANSM), and the French Institute for Research in Public Health (IRESP).

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Bernard Mazoyer has no disclosures.

**Data sharing:**

No additional data available.

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## References

1. Allen RP, Picchietti D, Hening WA, et al. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep medicine 2003;**4**(2):101-19

2. Walters AS. Toward a better definition of the restless legs syndrome. The International Restless Legs Syndrome Study Group. Movement disorders : official journal of the Movement Disorder Society 1995;**10**(5):634-42 doi: 10.1002/mds.870100517[published Online First: Epub Date]|.

3. Berger K, Kurth T. RLS epidemiology--frequencies, risk factors and methods in population studies. Movement disorders : official journal of the Movement Disorder Society 2007;**22 Suppl 18**:S420-3 doi: 10.1002/mds.21589[published Online First: Epub Date]|.

4. Paulus W, Dowling P, Rijsman R, et al.Pathophysiological concepts of restless legs syndrome. Movement disorders : official journal of the Movement Disorder Society 2007;**22**(10):1451-6 doi: 10.1002/mds.21533[published Online First: Epub Date]|.

5. Winkelmann J, Schormair B, Lichtner P, et al. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Nature genetics 2007;**39**(8):1000-6 doi: 10.1038/ng2099[published Online First: Epub Date]|.

6. Ohayon MM, Roth T. Prevalence of restless legs syndrome and periodic limb movement disorder in the general population. Journal of psychosomatic research 2002;**53**(1):547-54

7. Phillips B, Young T, Finn L, et al. Epidemiology of restless legs symptoms in adults. Archives of internal medicine 2000;**160**(14):2137-41

For peer review only

8. Sevim S, Dogu O, Camdeviren H, et al. Unexpectedly low prevalence and unusual characteristics of RLS in Mersin, Turkey. Neurology 2003;**61**(11):1562-9

9. Winter AC, Schurks M, Glynn RJ, et al. Vascular risk factors, cardiovascular disease, and restless legs syndrome in women. The American journal of medicine 2013;**126**(3):220-7, 27 e1-2 doi: 10.1016/j.amjmed.2012.06.040[published Online First: Epub Date]|.

10. Schlesinger I, Erikh I, Avizohar O, et al. Cardiovascular risk factors in restless legs syndrome. Movement disorders : official journal of the Movement Disorder Society 2009;**24**(11):1587-92 doi: 10.1002/mds.22486[published Online First: Epub Date]|.

11. Winter AC, Berger K, Glynn RJ, et al. Vascular risk factors, cardiovascular disease, and restless legs syndrome in men. The American journal of medicine 2013;**126**(3):228-35, 35 e1-2 doi: 10.1016/j.amjmed.2012.06.039[published Online First: Epub Date]|.

12. Kim J, Choi C, Shin K, et al. Prevalence of restless legs syndrome and associated factors in the Korean adult population: the Korean Health and Genome Study. Psychiatry and clinical neurosciences 2005;**59**(3):350-3 doi: 10.1111/j.1440-1819.2005.01381.x[published Online First: Epub Date]|.

13. Gao X, Schwarzschild MA, Wang H, et al.Obesity and restless legs syndrome in men and women. Neurology 2009;**72**(14):1255-61 doi: 10.1212/01.wnl.0000345673.35676.1c[published Online First: Epub Date]|.

14. Batool-Anwar S, Malhotra A, Forman J, et al. Restless legs syndrome and hypertension in middle-aged women. Hypertension 2011;**58**(5):791-6 doi: 10.1161/HYPERTENSIONAHA.111.174037[published Online First: Epub Date]|.

15. Ulfberg J, Nystrom B, Carter N, et al. Prevalence of restless legs syndrome among men aged 18 to 64 years: an association with somatic disease and neuropsychiatric symptoms.

For peer review only

Movement disorders : official journal of the Movement Disorder Society 2001;**16**(6):1159-63

16. Allen R, Chen C, Soaita A, et al. A randomized, double-blind, 6-week, dose-ranging study of pregabalin in patients with restless legs syndrome. Sleep medicine 2010;**11**(6):512-9 doi: 10.1016/j.sleep.2010.03.003[published Online First: Epub Date]|.
17. Juuti AK, Laara E, Rajala U, et al. Prevalence and associated factors of restless legs in a 57-year-old urban population in northern Finland. Acta neurologica Scandinavica 2010;**122**(1):63-9 doi: 10.1111/j.1600-0404.2009.01262.x[published Online First: Epub Date]|.
18. Wesstrom J, Nilsson S, Sundstrom-Poromaa I, et al.Restless legs syndrome among women: prevalence, co-morbidity and possible relationship to menopause. Climacteric : the journal of the International Menopause Society 2008;**11**(5):422-8 doi: 10.1080/13697130802359683[published Online First: Epub Date]|.
19. Winkelman JW, Finn L, Young T. Prevalence and correlates of restless legs syndrome symptoms in the Wisconsin Sleep Cohort. Sleep medicine 2006;**7**(7):545-52 doi: 10.1016/j.sleep.2006.01.004[published Online First: Epub Date]|.
20. Winkelman JW, Shahar E, Sharief I, et al.Association of restless legs syndrome and cardiovascular disease in the Sleep Heart Health Study. Neurology 2008;**70**(1):35-42 doi: 10.1212/01.wnl.0000287072.93277.c9[published Online First: Epub Date]|.
21. Winter AC, Schurks M, Glynn RJ, et al. Restless legs syndrome and risk of incident cardiovascular disease in women and men: prospective cohort study. BMJ open 2012;**2**(2):e000866 doi: 10.1136/bmjopen-2012-000866[published Online First: Epub Date]|.

For peer review only

22. Li Y, Walters AS, Chiuve SE, et al. Prospective study of restless legs syndrome and coronary heart disease among women. Circulation 2012;**126**(14):1689-94

23. Li Y, Wang W, Winkelman JW, et al. Prospective study of restless legs syndrome and mortality among men. Neurology 2013;**81**(1):52-9

24. Walters AS, Moussouttas M, Siddiqui F, et al. Prevalence of stroke in Restless Legs Syndrome: Initial Results Point to the Need for More Sophisticated Studies. The open neurology journal 2010;**4**:73-7 doi: 10.2174/1874205X01004010073[published Online First: Epub Date]|.

25. Schurks M, Winter AC, Berger K, et al. Migraine and restless legs syndrome in women. Cephalalgia;**32**(5):382-9

26. Winter AC, Schurks M, Berger K, et al. Migraine and restless legs syndrome in men. Cephalalgia;**33**(2):130-5

27. Group CS. Vascular factors and risk of dementia: design of the Three-City Study and baseline characteristics of the study population. Neuroepidemiology 2003;**22**(6):316-25

28. Maillard P, Delcroix N, Crivello F, et al. An automated procedure for the assessment of white matter hyperintensities by multispectral (T1, T2, PD) MRI and an evaluation of its between-centre reproducibility based on two large community databases. Neuroradiology 2008;**50**(1):31-42 doi: 10.1007/s00234-007-0312-3[published Online First: Epub Date]|.

29. Soumare A, Elbaz A, Zhu Y, et al. White matter lesions volume and motor performances in the elderly. Annals of neurology 2009;**65**(6):706-15 doi: 10.1002/ana.21674[published Online First: Epub Date]|.

For peer review only

30. Zhu YC, Dufouil C, Soumare A, et al. High degree of dilated Virchow-Robin spaces on MRI is associated with increased risk of dementia. Journal of Alzheimer's disease : JAD 2010;**22**(2):663-72 doi: 10.3233/JAD-2010-100378[published Online First: Epub Date]|.

31. Berger K, von Eckardstein A, Trenkwalder C, et al. Iron metabolism and the risk of restless legs syndrome in an elderly general population--the MEMO-Study. Journal of neurology 2002;**249**(9):1195-9 doi: 10.1007/s00415-002-0805-2[published Online First: Epub Date]|.

32. Hogl B, Kiechl S, Willeit J, et al. Restless legs syndrome: a community-based study of prevalence, severity, and risk factors. Neurology 2005;**64**(11):1920-4 doi: 10.1212/01.WNL.0000163996.64461.A3[published Online First: Epub Date]|.

33. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes care 1997;**20**(7):1183-97

34. Nocturnal limb movements are correlated with cerebral white matter hyperintensities. American Academy of Neurology; 2012; New Orleans, LA, USA.

35. Henskens LH, Kroon AA, van Oostenbrugge RJ, et al. Associations of ambulatory blood pressure levels with white matter hyperintensity volumes in hypertensive patients. Journal of hypertension 2009;**27**(7):1446-52 doi: 10.1097/HJH.0b013e32832b5204[published Online First: Epub Date]|.

36. Elwood P, Hack M, Pickering J, et al. Sleep disturbance, stroke, and heart disease events: evidence from the Caerphilly cohort. Journal of epidemiology and community health 2006;**60**(1):69-73 doi: 10.1136/jech.2005.039057[published Online First: Epub Date]|.

37. van Straaten EC, Fazekas F, Rostrup E, et al. Impact of white matter hyperintensities scoring method on correlations with clinical data: the LADIS study. Stroke; a journal of cerebral

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circulation 2006;**37**(3):836-40 doi: 10.1161/01.STR.0000202585.26325.74[published Online First: Epub Date]|.

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Table 1. Characteristics of participants by RLS status.

| Characteristic (%) | No RLS (n=817) | RLS (n=218) |
|---|---|---|
| Age (mean, SD) | 71.6 (3.9) | 71.6 (3.8) |
| Sex (% female) | 59.7 | 72.9 |
| Smoking status (%) | | |
| Never | 61.333.5 | 67.0 |
| Past | 5.1 | 26.66.4 |
| Current | | |
| Alcohol consumption (%) | 18.144.619.215.3 | 22.021.419.311.5 |
| Non-drinker | | |
| 0 to ≤12 g/day | | |
| 12 to ≤24 g/day | | |
| >24 g/day | | |
| Physically active (%) | 55.9 | 63.8 |
| Body mass index | 51.438.89.8 | 56.032.611.5 |
| <25 kg/m$^2$ | | |
| 25 to < 30 kg/m$^2$ | | |
| ≥30 kg/m$^2$ | | |
| History of high blood pressure (%) | 73.3 | 71.6 |
| History of high cholesterol (%) | 58.0 | 51.4 |
| History of diabetes (%) | 13.0 | 9.2 |

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| | | |
|---|---|---|
| History of cardiovascular disease (%) | 11.3 | 7.8 |
| History of stroke (%) | 4.5 | 1.4 |
| History of peripheral artery disease (%) | 2.1 | 2.8 |
| History of leg operation (%) | 0.6 | 0.5 |
| History of edema/swelling of legs and ankles (%) | 19.1 | 25.2 |
| Difficulty sleeping | 19.652.613.112.5 | 14.246.819.316.5 |
| Never | | |
| Rarely | | |
| Regularly | | |
| Often | | |
| Quality of sleep | 46.930.49.4 | 31.238.513.8 |
| Good | | |
| Average | | |
| Mediocre or bad | | |
| Take medications for sleep | 31.2 | 36.7 |
| Volume of white matter hyperintensities | 3.9 (2.7 – 5.8) | 4.0 (2.7 – 6.0) |
| ($cm^3$, median and interquartile range) | | |

*Numbers may not add to 100% due to missing data.

List of abbreviations: RLS=restless legs syndrome, SD=standard deviation

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Table 2. Cross-sectional age- and sex-adjusted and multivariable-adjusted* associations between RLS and brain white matter lesion volumes.

| | No restless legs syndrome (n=817) | Restless legs syndrome (n=218) | | |
|---|---|---|---|---|
| Total WML volume in tertiles | N (%) | N (%) | Age and sex-adjusted odds ratio (95% CI) | Multivariable-adjusted* odds ratio (95% CI) |
| Lowest Tertile | 269 (32.9) | 73 (33.5) | 1.00 | 1.00 |
| Middle Tertile | 271 (33.2) | 71 (32.6) | 1.04 (0.72, 1.52) | 1.09 (0.75, 1.60) |
| Highest Tertile | 277 (33.9) | 74 (33.9) | 1.09 (0.74, 1.59) | 1.17 (0.79, 1.74) |
| Periventricular WML volume in tertiles | | | | |
| Lowest Tertile | 267 (32.7) | 74 (33.9) | 1.00 | 1.00 |

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| | | | | |
|---|---|---|---|---|
| Middle Tertile | | | 0.86 | 0.85 |
| | 273 (33.4) | 69 (31.7) | (0.49, 1.51) | (0.48, 1.50) |
| Highest Tertile | | | | |
| | 277 (33.9) | 75 (34.4) | 0.96 | 0.91 |
| | | | (0.44, 2.10) | (0.41, 2.01) |
| Deep WML volume in tertiles | | | | |
| Lowest Tertile | | | 1.00 | 1.00 |
| | 275 (33.7) | 66 (30.3) | | |
| Middle Tertile | | | | |
| | 266 (32.6) | 77 (35.3) | 1.27 | 1.24 |
| | | | (0.85, 1.90) | (0.82, 1.87) |
| Highest Tertile | | | | |
| | 276 (33.8) | 75 (34.4) | 1.27 | 1.33 |
| | | | (0.78, 2.05) | (0.81, 2.17) |

*Adjusted for age, sex, smoking status, alcohol consumption, physical activity, body mass index, history of hypertension, history of diabetes, history of cardiovascular disease, history of peripheral artery disease, history of leg operation, and history of edema/swelling of legs and ankles.

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# Structural Brain Lesions and Restless Legs Syndrome: A cross-sectional population-based study

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Bernard Mazoyer has no disclosures.

**Key words:** Epidemiology, Restless Legs Syndrome, MRI

**Running head:** Structural Brain Lesions and Restless Legs Syndrome

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**Abstract**

**Objective:** To evaluate the association between white matter lesion volume, silent infarcts, and restless legs syndrome in a population-based study of elderly individuals.

**Design:** Cross-sectional study

**Setting:** Population-based Three-City study

**Participants:** ~~1268~~ 1035 individuals from the Dijon, France center of the Three-City study who had available information on volume of white matter lesions (WML) from MRI scans and who answered questions about the prevalence of restless legs syndrome

**Primary Outcome Measure:** Prevalence of restless legs syndrome

**Results:** White matter lesion volume was measured using an automated tissue segmentation method. Logistic regression was used to evaluate adjusted associations between tertiles of WML volume and restless legs syndrome and between silent infarcts and restless legs syndrome. 218 individuals (~~17.2~~21.1%) were determined to have restless legs syndrome. Compared to those in the first tertile of WML volume, individuals in the second tertile (OR=~~0.95~~1.09; 95% CI: 0.75~~66~~, 1.60~~37~~) or third tertile (OR=1.17~~00~~; 95% CI: 0.79~~69~~, 1.74~~46~~) did not have an increased prevalence of restless legs syndrome. We also did not observe associations between the volume of deep or periventricular WML and restless legs syndrome; nor did we observe an association between silent brain infarcts and RLS (OR=0.~~61~~74; 95% CI: 0.~~33~~40, 1.39~~11~~). These findings were not modified by age or gender.

**Conclusion:** Higher volume of WML and the presence of silent infarcts were not associated with an increased prevalence of restless legs syndrome in this population-based cohort of elderly individuals.

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## Article Summary

### Article focus

- The aim of this study is to evaluate the association between white matter lesion volume, silent infarcts, and restless legs syndrome (RLS) in a population-based study of elderly individuals.

### Key messages

- We found no cross-sectional association between WML volume or brain infarcts and RLS. The results of this study do not support an association between RLS and vascular brain lesions.

### Strengths and limitations of this study

- Strengths include the population-based setting with available brain imaging, the size of the cohort, standardized assessment of RLS using criteria from the International Restless Legs Study Group and use of an automated measurement procedure to quantify and localize WML.

- Limitations to this study include its cross-sectional design, lack of information on kidney disease or iron deficiency for participants, and self-reported information on RLS. Additionally, RLS status was not assessed at baseline and participants who were still in the study at restless legs assessment may be healthier than participants who died or dropped out prior to RLS assessment.

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## Introduction

Restless legs syndrome (RLS) is a neurological disorder that originates in the motoneurons of the brain and is characterized by an urge to move the legs and unpleasant leg sensations. Population based studies using the minimal diagnostic criteria from the International Restless Legs Syndrome Study Group[1 2] have found the prevalence of RLS ranges from 6% to 12% with women being affected twice as often as men.[3] In addition, the prevalence of RLS increases with age.[3] While the causes of RLS are unknown, some studies have suggested that dysfunction of the dopaminergic system may contribute to disease development. However, neuroimaging studies have not shown any primary neurodegeneration of dopaminergic neurons in the substantia nigra.[4] A large genetics consortium study has found some genetic variants that may be associated with the development of RLS.[5]

Several studies have found associations between cardiovascular risk factors including smoking[6-10], diabetes[7 9 11], hypercholerolemia[9 10], exercise[9], body mass index[6 9 12 13] and hypertension[7 14 15] and RLS. These associations have led to the hypothesis that RLS may be a risk factor for cardiovascular disease. However, evidence for an association between RLS and incident cardiovascular disease is mixed.[6 9 11 15-20] One recent longitudinal study did not find evidence of an association between RLS and incident cardiovascular disease[21] while another found an association between RLS and coronary heart disease[22] and mortality.[23] Several possible biological mechanisms have been proposed to explain why RLS may be associated with high blood pressure, heart disease, and stroke. RLS is associated with sympathetic hyperactivity which may lead to daytime hypertension which itself is a risk factor for heart disease and stroke. Alternatively, even if the sympathetic hyperactivity does not lead to daytime hypertension, it may impact atherosclerotic plaque formation and rupture which could

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lead to heart disease and stroke. Other comorbidities associated with RLS may also be risk factors for heart disease and stroke.[24] In addition to its association with cardiovascular risk factors, RLS is also associated with migraine in both men and women.[25 26]

Given the association of RLS with several vascular risk factors as well as with migraine, we were interested to know if white matter lesions (WML), which are also strongly related to vascular risk factors, including migraine, and cardiovascular disease, were associated with RLS. Using a population-based cohort of elderly individuals residing in France, we examined the cross-sectional association between white matter lesion volume and RLS prevalence.

**Methods**

The Three-City (3C) is a longitudinal cohort study enrolling subjects living in three French cities (Bordeaux, Dijon, and Montpellier) designed to estimate the risk of dementia and cognitive impairment attributable to vascular risk factors.[27] The present analysis only uses data from subjects living in Dijon because data on RLS were only collected in that city. Each subject signed an informed consent statement and the Ethical Committee of the University Hospital of Kremlin-Bicêtre approved the methods and procedures of the 3C study.

To be eligible for the 3C study, the participant needs to live in Dijon, be registered on the electoral rolls in 1999, be 65 years of older, and not be institutionalized. A total of 4,931 individuals were recruited at the Dijon site between 1999 and 2001. For the MRI sub-study, all subjects recruited from the Dijon center who were <80 years of age and enrolled between June 1999 and September 2000 were eligible to participate. Of those eligible, 2,285 (82%) subjects agreed to participate in the MRI study, but only 1,924 scans could be performed at baseline due to financial constraints.

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The process of obtaining the MRI information has been described in detail elsewhere.[28 29] In brief, MRI scans were obtained using a 1.5T Magnetom (Siemens, Erlangen, Germany). A 3-dimensional (3D) high-resolution T1-weighted brain volume was obtained using a 3D inversion recovery fast spoiled-gradient echo sequence. T2- and proton density (PD)-weighted brain volumes were acquired using a 2-dimensional dual spin echo sequences with two echo times. Each subject dataset (T1, T2, PD) was reconstructed and visually checked for major artifacts before being stored.

A fully-automated image processing software was used to detect, measure, and localize WMLs. The process has been described in detail previously[28 29]. Based on the morphologic parameters (center of mass coordinates, Euclidian distance to the ventricular system, principal axes dimension), each WML was labeled as being either periventricular if the distance to the ventricular system was <10 mm or deep otherwise. Total volume of periventricular and deep WMLs were estimated by summing the volumes of all periventricular and deep lesions.

We log transformed the values of total WML, periventricular WML, deep WML, and total white matter volume as they were not normally distributed. We then divided the log transformed values into tertiles to allow for non-linear associations between WML volumes and RLS.

Infarcts were rated on T1, T2, and PD-weighted images and defined as focal lesions ≥3 mm in diameter with the same signal characteristic as cerebrospinal fluid on all sequences. They were discriminated from dilated Virchow-Robin spaces using multi-planar reformatting. Those with a typical vascular shape and following the orientation of perforating vessels were classified as dilated Virchow-Robin spaces and not as infarcts.[30]

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*Restless Legs Syndrome Assessment*

RLS was assessed at the fifth and sixth follow-up waves of the study. Participants were asked a series questions designed to address the four minimal diagnostic criteria of the International Restless Legs Study Group which has been established and validated in previous studies.[31 32] The participants were asked: "Have if ever felt unpleasant sensation in the legs (restlessness, tingling, tension, annoyance, annoyances, contractions, twitching, numbness, electricity, etc.) with the irresistible need or want to move?" Response options were yes or no. If the participant responded "yes", he or she was further asked: "Do these unpleasant sensations occur solely or mainly at rest (when you are sitting or lying down, without moving your legs) and do they improve with movement?" and "Are these unpleasant sensations more intense in the evening or at night than in the morning?" Response options for these questions were yes or no. If the participant responded yes to all three questions, he or she was defined as having RLS.

*Covariates*

Trained psychologists collected sociodemographic and medical data on participants during home visits. Participants were asked if there were treated for various co-morbidities at baseline and in the fifth and sixth wave of the study. We used all available information from baseline to RLS assessment to determine a subject's co-morbidity history. History of cardiovascular disease was defined as a history of myocardial infarction, stroke, angina, percutaneous transluminal coronary angioplasty, or coronary artery bypass surgery. History of diabetes was defined as glycemia ≥7 mmol/L or use of anti-diabetic treatment.[33] High blood pressure was defined as measured systolic blood pressure ≥140 mmHg or measured diastolic blood pressure ≥90 mmHg. High cholesterol was defined as lipid lowering treatment or

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cholesterol ≥6.2 mmol/L. Information was also collected on history of peripheral artery disease, history of leg operation, and history of edema/swelling of legs and ankles.

For the following covariates, we used values from the follow-up waves during which RLS was assessed: body mass index, smoking status, alcohol consumption and physical activity. If this value was missing, we used values from baseline. Height and weight were used to calculate body mass index. Smoking status was reported as never, past, or current, and alcohol consumption was measured in grams per day. Due to changes in the questionnaires on physical activity over time, we classified participants as active versus non-active.

*Statistical Analysis*

Of the 1924 individuals in the MRI sub-study, 1189 were still alive and participating in the 3C study during the waves in which RLS was assessed. ~~w~~We excluded ~~581~~ 97 participants who did not answer any of the questions about RLS and the 5775 participants who did not have data on total WML volume, leaving a total of ~~1268~~ 1035 participants eligible for our analyses of which 218 (~~17.2~~21.1%) reported RLS (Figure 1).

We performed a cross-sectional analysis using logistic regression to calculate odds ratios and 95% confidence intervals of reporting RLS for each tertile of the WML volumes using the lowest tertile as the reference group. Analyses examining the association between tertiles of total WML volume and RLS adjusted for tertiles of total white matter as the likelihood of WML correlates with the size of the total white matter. Analyses examining the association between tertiles of periventricular or deep WML and RLS adjusted for tertiles of WML. We also used logistic regression to examine the association between any brain silent infarct and RLS. For the infarct analyses, we excluded participants with a brain tumor detected at MRI.

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We performed age- and sex-adjusted analyses and multivariable-adjusted analyses. Our multivariable analyses adjusted for age (continuous), sex, smoking status (never, past, or current smoker), alcohol consumption (0, 0 to ≤12, 12 to ≤24, and >24 grams/day), physical activity (active versus not active), body mass index (<25 kg/m$^2$, 25 to <30 kg/m$^2$, and ≥30 kg/m$^2$), history of hypertension (yes/no), history of diabetes (yes/no), history of cardiovascular disease (yes/no), history of peripheral artery disease (yes/no), history of leg operation (yes/no), and history of edema/swelling of legs and ankles (yes/no). Further adjustment for measures of sleep quality, difficulty sleeping and taking sleep medications did not affect our results (results not shown). All covariates were measured at baseline. Less than ~~14~~ 39 people were missing information on any covariate, except for difficulty sleeping and were assigned to the ~~most frequent~~reference value of that covariate. We created a separate category for those missing information on difficulty sleeping. We also performed separate age-adjusted analyses stratified by sex or mean age (72 years).

We considered a two-tailed p value of <0.05 as statistically significant and used SAS 9.~~1.~~3 as statistical software (Cary, NC).

**Results**

The characteristics of the participants by RLS status can be seen in Table 1. Those who reported RLS were more likely to be female, never smokers, non-drinkers, and were less physically active than those who did not report RLS.

We did not observe an association between tertiles of WML and RLS (Table 2). Compared to those in the lowest tertile of WML, the multivariable-adjusted odds ratio of reporting RLS was ~~0.95~~1.09 (95% CI: 0.75~~66~~, 1.60~~37~~) for those in the second tertile and 1.17~~00~~

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(95% CI: 0.7969, 1.7446) for those in the top tertile. We also did not observe an association between tertiles of deep or periventicular WML and RLS (Table 2).

We also performed age-adjusted analyses stratified by sex or mean age (72 years). Among men, compared to those in the lowest tertile of WML, the age-adjusted odds ratio of reporting RLS was 0.743 (95% CI: 0.356, 1.562) for those in the second tertile and 0.8578 (95% CI: 0.4138, 1.7756) for those in the top tertile. Among women, compared to those in the lowest tertile of WML, the age-adjusted odds ratio of reporting RLS was 1.1702 (95% CI: 0.7667, 1.8155) for those in the second tertile and 1.1307 (95% CI: 0.7269, 1.7866) for those in the top tertile. For those under 72 years of age, the age-adjusted odds ratio of reporting RLS was 0.880.99 (95% CI: 0.6155, 1.6242) for those in the second tertile and 1.030.99 (95% CI: 0.6159, 1.7566) for those in the top tertile compared to those in the lowest tertile of WML. For those 72 years of age or older, the age-adjusted odds ratio of reporting RLS was 1.1002 (95% CI: 0.6158, 1.9880) for those in the second tertile and 1.2005 (95% CI: 0.681, 2.101.78) for those in the top tertile compared to those in the lowest tertile of WML.

We also explored whether there was an association between infarcts and RLS. Of the 1330 people with information on brain infarcts, 131 had a brain infarct and 225 reported RLS. The age- and sex-adjusted odds ratio between infarcts and RLS was 0.4259 (95% CI: 0.1532, 1.2107). The multivariable-adjusted odds ratios between infarcts and RLS was 0.7461 (95% CI: 0.4033, 1.3911).

**Discussion**

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In this large, population-based study of elderly individuals, we found no cross-sectional association between WML volume or brain infarcts and RLS. The results of this study do not support an association between RLS and vascular brain lesions.

Previous research on the association between WML volume and RLS is limited. A small study of 45 patients found that white matter hyperintensities were correlated with total limb movements per hour of sleep after adjusting for hypertension (r=0.66, p=0.01).[34] The authors suggest that leg movements may be associated with poor quality sleep which may contribute to episodes of nocturnal hypertension. Although nocturnal hypertension has been associated with the development of white matter hyperintensities even among those with daytime hypertension[35], this study did not present results on the association between RLS and white matter hyperintensities. Additionally, it is unclear if the authors adjusted for other potential confounders including age and sex.

Another study using data from the Memory and Morbidity in Augsburg Elderly Study (MEMO) examined the association between RLS and brain lesions detected using MRI. They found a non-significant increase risk of silent infarction (OR=2.11, 95% CI: 0.71-6.27) and subcortical brain lesions greater than or equal to 10mm (OR=1.35, 95% CI: 0.56-3.22) in those who reported RLS compared to those without RLS.[24] The small size of this study (26 RLS cases and 241 controls) and limited power to control for confounding by cardiovascular risk factors may explain some of the differences between the results of the MEMO study and our study.

While evidence for a direct association between WML and RLS is limited, some cross-sectional studies have suggested that RLS may be associated with hypertension[14], stroke[9 36],

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and migraine[25 26], which are associated with WML. However, a recent longitudinal study found no association between RLS and incident cardiovascular disease [21] and not all cross-sectional studies have found associations between hypertension and RLS.[9 11] Our study did not find an association between RLS and structural brain lesions which are strongly related to vascular risk factors and cardiovascular disease. This observation aligns with the previous study that did not find an association between RLS and incident cardiovascular disease.

This study has several strengths including the population-based setting with available brain imaging, the size of the cohort, and standardized assessment of RLS using criteria from the International Restless Legs Study Group.[1 2] We also used an automated measurement procedure to quantify and localize WML. Compared to visual scale, automated procedures are not subject to a ceiling effect, permit better discrimination of lesion volume, and are more sensitive in detecting small group differences.[37]

Limitations to this study include its cross-sectional design which prevents us from determining the temporal ordering of RLS and WML or examining how RLS may impact WML progression over time. RLS was first assessed in the fifth and sixth waves of the study (approximately 10 years after baseline). Participants who were still in the study then may be healthier than participants who died or dropped out prior to RLS assessment. We did not have information on kidney disease or iron deficiency for participants, which may be related to RLS. Information on RLS was self-reported and potential misclassification is possible. However, we used the best available questionnaire for population-level assessment of RLS and this questionnaire has been validated in previous cohorts.[31 32] Additionally, our questionnaire did not assess RLS severity or periodic limb movements association with RLS so we are unable to

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determine if the severity of RLS or presence of periodic limb movements may modify the association between RLS and WMH.

While our data do not support a strong association between structural brain lesions and RLS, further targeted research is warranted to evaluate whether subgroups of patients with RLS exist who are at increased risk for structural brain lesions.

**Authorship Contributions**

Pamela M. Rist: drafting/revising the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data

Christophe Tzourio: obtaining funding, interpretation of data, revising the manuscript for content, and supervision

Alexis Elbaz: interpretation of data and revising the manuscript for content

Aicha Soumare: interpretation of data and revising the manuscript for content

Carole Dufouil: interpretation of data and revising the manuscript for content

Bernard Mazoyer: interpretation of data and revising the manuscript for content

Tobias Kurth: Conception and design, interpretation of data, revising the manuscript for content, and supervision

All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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## References

1. Allen RP, Picchietti D, Hening WA, et al. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep medicine 2003;**4**(2):101-19

2. Walters AS. Toward a better definition of the restless legs syndrome. The International Restless Legs Syndrome Study Group. Movement disorders : official journal of the Movement Disorder Society 1995;**10**(5):634-42 doi: 10.1002/mds.870100517[published Online First: Epub Date]|.

3. Berger K, Kurth T. RLS epidemiology--frequencies, risk factors and methods in population studies. Movement disorders : official journal of the Movement Disorder Society 2007;**22 Suppl 18**:S420-3 doi: 10.1002/mds.21589[published Online First: Epub Date]|.

4. Paulus W, Dowling P, Rijsman R, Stiasny-Kolster K, Trenkwalder C, de Weerd A. Pathophysiological concepts of restless legs syndrome. Movement disorders : official journal of the Movement Disorder Society 2007;**22**(10):1451-6 doi: 10.1002/mds.21533[published Online First: Epub Date]|.

5. Winkelmann J, Schormair B, Lichtner P, et al. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Nature genetics 2007;**39**(8):1000-6 doi: 10.1038/ng2099[published Online First: Epub Date]|.

6. Ohayon MM, Roth T. Prevalence of restless legs syndrome and periodic limb movement disorder in the general population. Journal of psychosomatic research 2002;**53**(1):547-54

7. Phillips B, Young T, Finn L, Asher K, Hening WA, Purvis C. Epidemiology of restless legs symptoms in adults. Archives of internal medicine 2000;**160**(14):2137-41

For peer review only

8. Sevim S, Dogu O, Camdeviren H, et al. Unexpectedly low prevalence and unusual characteristics of RLS in Mersin, Turkey. Neurology 2003;**61**(11):1562-9

9. Winter AC, Schurks M, Glynn RJ, et al. Vascular risk factors, cardiovascular disease, and restless legs syndrome in women. The American journal of medicine 2013;**126**(3):220-7, 27 e1-2 doi: 10.1016/j.amjmed.2012.06.040[published Online First: Epub Date]|.

10. Schlesinger I, Erikh I, Avizohar O, Sprecher E, Yarnitsky D. Cardiovascular risk factors in restless legs syndrome. Movement disorders : official journal of the Movement Disorder Society 2009;**24**(11):1587-92 doi: 10.1002/mds.22486[published Online First: Epub Date]|.

11. Winter AC, Berger K, Glynn RJ, et al. Vascular risk factors, cardiovascular disease, and restless legs syndrome in men. The American journal of medicine 2013;**126**(3):228-35, 35 e1-2 doi: 10.1016/j.amjmed.2012.06.039[published Online First: Epub Date]|.

12. Kim J, Choi C, Shin K, et al. Prevalence of restless legs syndrome and associated factors in the Korean adult population: the Korean Health and Genome Study. Psychiatry and clinical neurosciences 2005;**59**(3):350-3 doi: 10.1111/j.1440-1819.2005.01381.x[published Online First: Epub Date]|.

13. Gao X, Schwarzschild MA, Wang H, Ascherio A. Obesity and restless legs syndrome in men and women. Neurology 2009;**72**(14):1255-61 doi: 10.1212/01.wnl.0000345673.35676.1c[published Online First: Epub Date]|.

14. Batool-Anwar S, Malhotra A, Forman J, Winkelman J, Li Y, Gao X. Restless legs syndrome and hypertension in middle-aged women. Hypertension 2011;**58**(5):791-6 doi: 10.1161/HYPERTENSIONAHA.111.174037[published Online First: Epub Date]|.

For peer review only

15. Ulfberg J, Nystrom B, Carter N, Edling C. Prevalence of restless legs syndrome among men aged 18 to 64 years: an association with somatic disease and neuropsychiatric symptoms. Movement disorders : official journal of the Movement Disorder Society 2001;**16**(6):1159-63

16. Allen R, Chen C, Soaita A, et al. A randomized, double-blind, 6-week, dose-ranging study of pregabalin in patients with restless legs syndrome. Sleep medicine 2010;**11**(6):512-9 doi: 10.1016/j.sleep.2010.03.003[published Online First: Epub Date]|.

17. Juuti AK, Laara E, Rajala U, et al. Prevalence and associated factors of restless legs in a 57-year-old urban population in northern Finland. Acta neurologica Scandinavica 2010;**122**(1):63-9 doi: 10.1111/j.1600-0404.2009.01262.x[published Online First: Epub Date]|.

18. Wesstrom J, Nilsson S, Sundstrom-Poromaa I, Ulfberg J. Restless legs syndrome among women: prevalence, co-morbidity and possible relationship to menopause. Climacteric : the journal of the International Menopause Society 2008;**11**(5):422-8 doi: 10.1080/13697130802359683[published Online First: Epub Date]|.

19. Winkelman JW, Finn L, Young T. Prevalence and correlates of restless legs syndrome symptoms in the Wisconsin Sleep Cohort. Sleep medicine 2006;**7**(7):545-52 doi: 10.1016/j.sleep.2006.01.004[published Online First: Epub Date]|.

20. Winkelman JW, Shahar E, Sharief I, Gottlieb DJ. Association of restless legs syndrome and cardiovascular disease in the Sleep Heart Health Study. Neurology 2008;**70**(1):35-42 doi: 10.1212/01.wnl.0000287072.93277.c9[published Online First: Epub Date]|.

21. Winter AC, Schurks M, Glynn RJ, et al. Restless legs syndrome and risk of incident cardiovascular disease in women and men: prospective cohort study. BMJ open

For peer review only

2012;**2**(2):e000866 doi: 10.1136/bmjopen-2012-000866[published Online First: Epub Date]|.

22. Li Y, Walters AS, Chiuve SE, Rimm EB, Winkelman JW, Gao X. Prospective study of restless legs syndrome and coronary heart disease among women. Circulation 2012;**126**(14):1689-94

23. Li Y, Wang W, Winkelman JW, Malhotra A, Ma J, Gao X. Prospective study of restless legs syndrome and mortality among men. Neurology 2013;**81**(1):52-9

24. Walters AS, Moussouttas M, Siddiqui F, et al. Prevalence of stroke in Restless Legs Syndrome: Initial Results Point to the Need for More Sophisticated Studies. The open neurology journal 2010;**4**:73-7 doi: 10.2174/1874205X01004010073[published Online First: Epub Date]|.

25. Schurks M, Winter AC, Berger K, Buring JE, Kurth T. Migraine and restless legs syndrome in women. Cephalalgia;**32**(5):382-9

26. Winter AC, Schurks M, Berger K, Buring JE, Gaziano JM, Kurth T. Migraine and restless legs syndrome in men. Cephalalgia;**33**(2):130-5

27. Group CS. Vascular factors and risk of dementia: design of the Three-City Study and baseline characteristics of the study population. Neuroepidemiology 2003;**22**(6):316-25

28. Maillard P, Delcroix N, Crivello F, et al. An automated procedure for the assessment of white matter hyperintensities by multispectral (T1, T2, PD) MRI and an evaluation of its between-centre reproducibility based on two large community databases. Neuroradiology 2008;**50**(1):31-42 doi: 10.1007/s00234-007-0312-3[published Online First: Epub Date]|.

For peer review only

29. Soumare A, Elbaz A, Zhu Y, et al. White matter lesions volume and motor performances in the elderly. Annals of neurology 2009;**65**(6):706-15 doi: 10.1002/ana.21674[published Online First: Epub Date]|.

30. Zhu YC, Dufouil C, Soumare A, Mazoyer B, Chabriat H, Tzourio C. High degree of dilated Virchow-Robin spaces on MRI is associated with increased risk of dementia. Journal of Alzheimer's disease : JAD 2010;**22**(2):663-72 doi: 10.3233/JAD-2010-100378[published Online First: Epub Date]|.

31. Berger K, von Eckardstein A, Trenkwalder C, Rothdach A, Junker R, Weiland SK. Iron metabolism and the risk of restless legs syndrome in an elderly general population--the MEMO-Study. Journal of neurology 2002;**249**(9):1195-9 doi: 10.1007/s00415-002-0805-2[published Online First: Epub Date]|.

32. Hogl B, Kiechl S, Willeit J, et al. Restless legs syndrome: a community-based study of prevalence, severity, and risk factors. Neurology 2005;**64**(11):1920-4 doi: 10.1212/01.WNL.0000163996.64461.A3[published Online First: Epub Date]|.

33. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes care 1997;**20**(7):1183-97

34. Nocturnal limb movements are correlated with cerebral white matter hyperintensities. American Academy of Neurology; 2012; New Orleans, LA, USA.

35. Henskens LH, Kroon AA, van Oostenbrugge RJ, et al. Associations of ambulatory blood pressure levels with white matter hyperintensity volumes in hypertensive patients. Journal of hypertension 2009;**27**(7):1446-52 doi: 10.1097/HJH.0b013e32832b5204[published Online First: Epub Date]|.

For peer review only

36. Elwood P, Hack M, Pickering J, Hughes J, Gallacher J. Sleep disturbance, stroke, and heart disease events: evidence from the Caerphilly cohort. Journal of epidemiology and community health 2006;**60**(1):69-73 doi: 10.1136/jech.2005.039057[published Online First: Epub Date]|.

37. van Straaten EC, Fazekas F, Rostrup E, et al. Impact of white matter hyperintensities scoring method on correlations with clinical data: the LADIS study. Stroke; a journal of cerebral circulation 2006;**37**(3):836-40 doi: 10.1161/01.STR.0000202585.26325.74[published Online First: Epub Date]|.

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Table 1. Characteristics of participants by RLS status.

| Characteristic (%) | No RLS (n=~~1050~~817) | RLS (n=218) |
|---|---|---|
| Age (mean, SD) | ~~72.1 (4.1)~~71.6 (3.9) | 71.6 (3.8) |
| Sex (% female) | 59.~~73~~ | 72.9 |
| Smoking status (%) | | |
| Never | ~~60.0~~61.3 | 67.0 |
| Past | ~~35.2~~33.5 | ~~29.4~~26.6 |
| Current | ~~4.8~~5.1 | ~~3.7~~6.4 |
| Alcohol consumption (%) | | |
| Non-drinker | ~~33.6~~18.1 | ~~39.9~~22.0 |
| 0 to ≤12 g/day | ~~41.1~~44.6 | ~~45.0~~21.4 |
| 12 to ≤24 g/day | ~~15.8~~19.2 | ~~11.0~~19.3 |
| >24 g/day | ~~9.2~~15.3 | ~~4.1~~11.5 |
| Physically active (%) | ~~21.3~~55.9 | ~~16.5~~63.8 |
| Body mass index | | |
| <25 kg/m$^2$ | ~~45.3~~51.4 | ~~44.5~~56.0 |
| 25 to < 30 kg/m$^2$ | ~~40.2~~38.8 | ~~40.8~~32.6 |
| ≥30 kg/m$^2$ | ~~14.5~~9.8 | ~~14.7~~11.5 |
| History of high blood pressure (%) | ~~86.6~~73.3 | ~~86.7~~71.6 |
| History of high cholesterol (%) | ~~63.6~~58.0 | ~~59.6~~51.4 |
| History of diabetes (%) | ~~11.8~~13.0 | 9.2 |

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| | | |
|---|---|---|
| History of cardiovascular disease (%) | ~~18.1~~11.3 | ~~17.9~~7.8 |
| History of stroke (%) | 4.5 | 1.4 |
| History of peripheral artery disease (%) | ~~4.6~~2.1 | ~~4.1~~2.8 |
| History of leg operation (%) | 0.~~6~~9 | 0.5 |
| History of edema/swelling of legs and ankles (%) | ~~29.0~~19.1 | ~~36.7~~25.2 |
| Difficulty sleeping | | |
| Never | ~~47.5~~19.6 | ~~44.0~~14.2 |
| Rarely | ~~30.2~~52.6 | ~~20.2~~46.8 |
| Regularly | ~~10.8~~13.1 | ~~17.0~~19.3 |
| Often | ~~10.8~~12.5 | ~~18.8~~16.5 |
| Quality of sleep | | |
| Good | ~~52.6~~46.9 | ~~45.0~~31.2 |
| Average | ~~31.8~~30.4 | ~~33.5~~38.5 |
| Mediocre or bad | ~~12.3~~9.4 | ~~21.6~~13.8 |
| Take medications for sleep | ~~35.2~~31.2 | ~~45.0~~36.7 |
| Volume of white matter hyperintensities | 3.9 (2.7 – 5.8) | 4.0 (2.7 – 6.0) |
| ($cm^3$, median and interquartile range) | | |

*Numbers may not add to 100% due to missing data.

List of abbreviations: RLS=restless legs syndrome, SD=standard deviation

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Table 2. Cross-sectional age- and sex-adjusted and multivariable-adjusted* associations between RLS and brain white matter lesion volumes.

| | No restless legs syndrome (n=~~1050~~817) | Restless legs syndrome (n=218) | | |
|---|---|---|---|---|
| Total WML volume in tertiles | N (%) | N (%) | Age and sex-adjusted odds ratio (95% CI) | Multivariable-adjusted* odds ratio (95% CI) |
| Lowest Tertile | ~~343 (32.7)~~ 269 (32.9) | ~~76 (34.9)~~ 73 (33.5) | 1.00 | 1.00 |
| Middle Tertile | ~~348 (33.1)~~ 271 (33.2) | ~~70 (32.1)~~ 71 (32.6) | ~~0.95 (0.66, 1.36)~~ 1.04 (0.72, 1.52) | ~~0.95 (0.66, 1.37)~~ 1.09 (0.75, 1.60) |
| Highest Tertile | ~~359 (34.2)~~ 277 (33.9) | ~~72 (33.0)~~ 74 (33.9) | ~~1.00 (0.69, 1.45)~~ 1.09 (0.74, 1.59) | ~~1.00 (0.69, 1.46)~~ 1.17 (0.79, 1.74) |
| Periventricular WML volume in tertiles | | | | |

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| | | | | |
|---|---|---|---|---|
| Lowest Tertile | ~~341 (32.5)~~ | ~~77 (35.3)~~ | 1.00 | 1.00 |
| | 267 (32.7) | 74 (33.9) | | |
| Middle Tertile | ~~351 (33.4)~~ | ~~68 (31.2)~~ | 0.86 | 0.~~86~~85 |
| | 273 (33.4) | 69 (31.7) | (0.49, 1.51) | (0.~~49~~48, 1.~~51~~50) |
| Highest Tertile | ~~358 (34.1)~~ | ~~73 (33.5)~~ | ~~1.00~~ | ~~1.02~~ |
| | 277 (33.9) | 75 (34.4) | ~~(0.46, 2.18)~~ | ~~(0.47, 2.25)~~ |
| | | | 0.96 | 0.91 |
| | | | (0.44, 2.10) | (0.41, 2.01) |
| Deep WML volume in tertiles | | | | |
| Lowest Tertile | ~~352 (33.5)~~ | ~~67 (30.7)~~ | 1.00 | 1.00 |
| | 275 (33.7) | 66 (30.3) | | |
| Middle Tertile | ~~340 (32.4)~~ | ~~77 (35.3)~~ | ~~1.24~~ | ~~1.22~~ |
| | 266 (32.6) | 77 (35.3) | ~~(0.84, 1.84)~~ | ~~(0.82, 1.81)~~ |
| | | | 1.27 | 1.24 |
| | | | (0.85, 1.90) | (0.82, 1.87) |
| Highest Tertile | ~~358 (34.1)~~ | ~~74 (33.9)~~ | ~~1.21~~ | ~~1.18~~ |
| | 276 (33.8) | 75 (34.4) | ~~(0.76, 1.94)~~ | ~~(0.73, 1.90)~~ |
| | | | 1.27 | 1.33 |
| | | | (0.78, 2.05) | (0.81, 2.17) |

*Adjusted for age, sex, smoking status, alcohol consumption, physical activity, body mass index, history of hypertension, history of diabetes, history of cardiovascular disease, history of

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peripheral artery disease, history of leg operation, and history of edema/swelling of legs and ankles.

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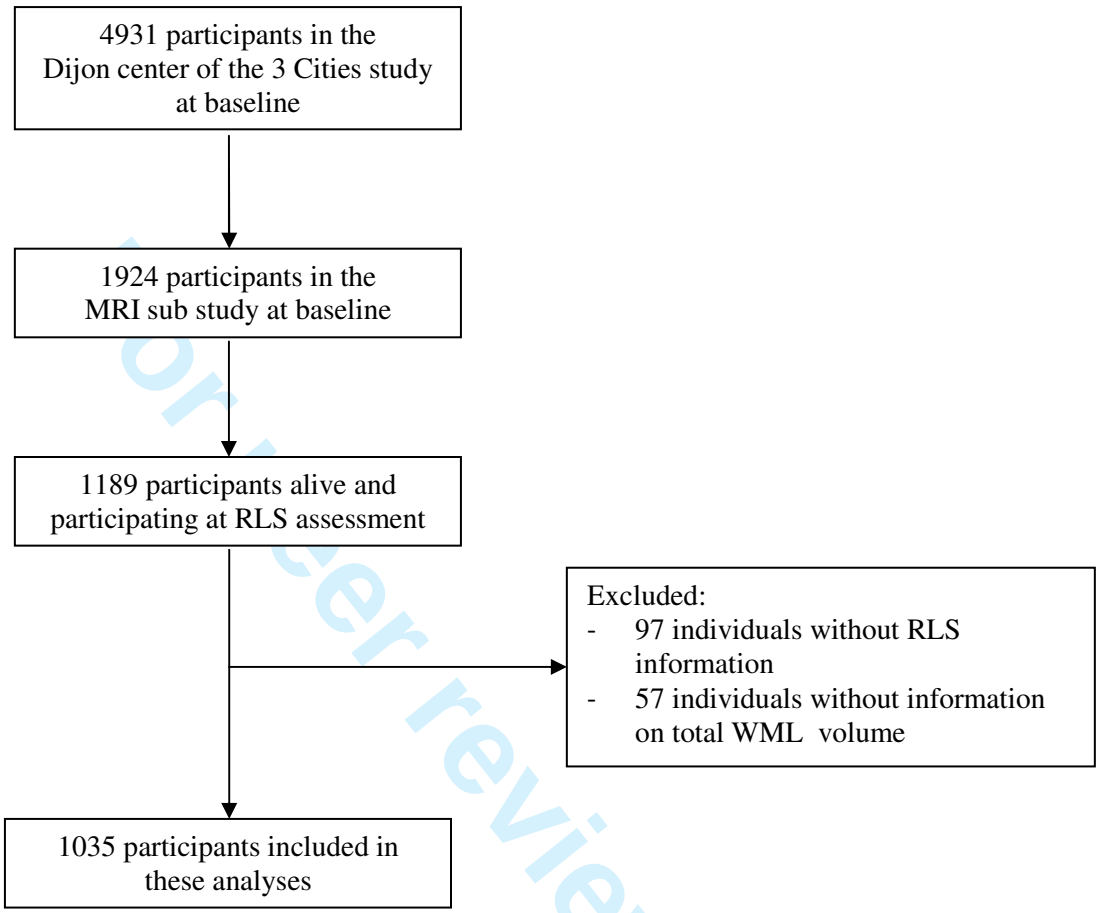
Figure 1. Flowchart of subjects included in this analysis.

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STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation | Page |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1, 5 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 5 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 7 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 8 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 8 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 8-11 |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants | 8 |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case | NA |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 8-11 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 8-11 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 11,12 |
| Study size | 10 | Explain how the study size was arrived at | 8, 11 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 8-11 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 11-12 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | NA |
| | | (*c*) Explain how missing data were addressed | 12 |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy | NA |
| | | (*e*) Describe any sensitivity analyses | NA |

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| | | | |
|---|---|---|---|
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 8,11, Table 1 |
| | | (b) Give reasons for non-participation at each stage | 8, 11 |
| | | (c) Consider use of a flow diagram | NA |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | Table 1 |
| | | (b) Indicate number of participants with missing data for each variable of interest | 12 |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) | NA |
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time | |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure | |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures | Table 2 |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 12-13, Table 2 |
| | | (*b*) Report category boundaries when continuous variables were categorized | 9-12 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | NA |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | NA |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 13 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 15 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 13-15 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 15 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 2-3 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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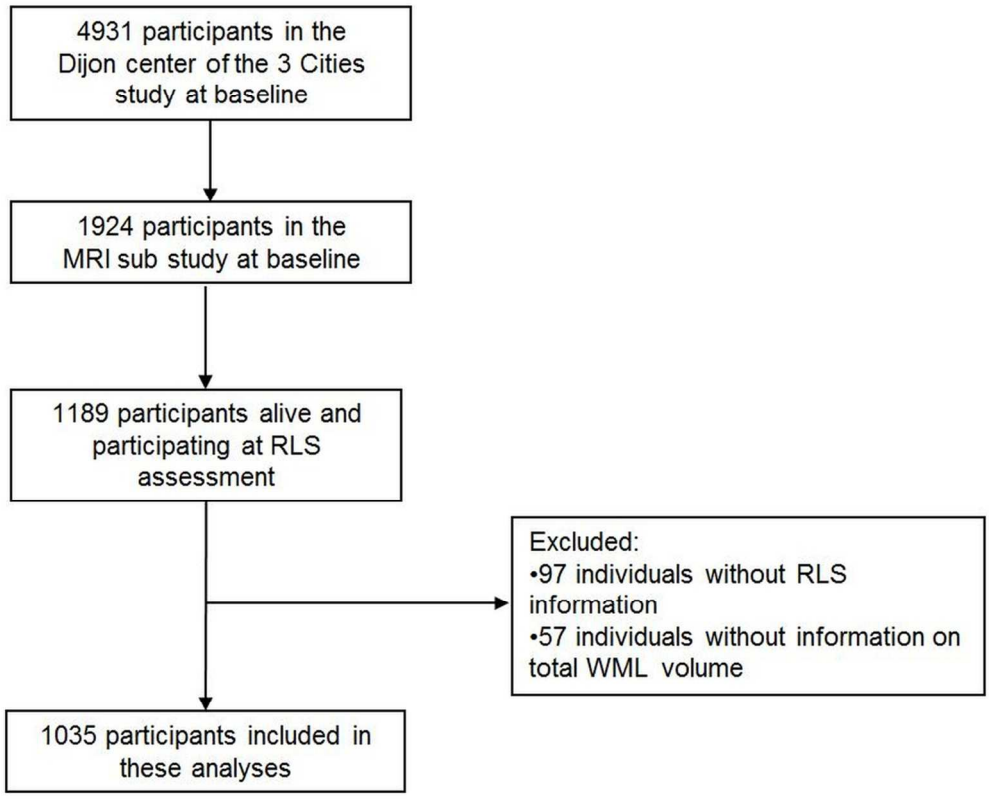


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