

The Epidermal Growth Factor Domain of the Mutation Does Not Appear to Influence Disease Progression in CADASIL When Brain Volume and Sex Are Taken into Account

J. Leberberg, J.-P. Guichard, A. Guillonnet, D. Hervé, N. Alili, A. Taleb, N. Dias-Gastellier, H. Chabriat, and E. Jouvent



ABSTRACT

BACKGROUND AND PURPOSE: By studying the evolution of brain volume across the life span in male and female patients, we aimed to understand how sex, brain volume, and the epidermal growth factor repeat domain of the mutation, the 3 major determinants of disability in CADASIL, interact in driving disease evolution.

MATERIALS AND METHODS: We used validated methods to model the evolution of normalized brain volume with age in male and female patients using nonparametric regression in a large, monocentric cohort with prospectively collected clinical and high-resolution MR imaging data. We used k-means clustering to test for the presence of different clinical course profiles.

RESULTS: We included 229 patients (mean age, 53 [SD, 12] years; 130 women). Brain volume was larger in women (mean size, 1024 [SD, 62] cm³ versus 979 [SD, 50] cm³; $P < .001$) and decreased regularly. In men, the relationship between brain volume and age unexpectedly suggested an increase in brain volume around midlife. Cluster analyses showed that this finding was related to the presence of a group of older male patients with milder symptoms and larger brain volumes, similar to findings of age-matched women. This group did not show specific epidermal growth factor repeat domain distribution.

CONCLUSIONS: Our results demonstrate a detrimental effect of male sex on brain volume throughout life in CADASIL. We identified a subgroup of male patients whose brain volume and clinical outcomes were similar to those of age-matched women. They did not have a specific distribution of the epidermal growth factor repeat domain, suggesting that yet-unidentified predictors may interact with sex and brain volume in driving disease evolution.

ABBREVIATIONS: EGFR = epidermal growth factor repeat; MDRS = Mattis Dementia Rating Scale; MMSE = Mini-Mental State Examination; SVD = small-vessel disease; TMTAT = Trail-Making Test A Time; TMTBT = Trail-Making Test B Time; WMH = white matter hyperintensities

CADASIL is the most frequent monogenic cerebral small-vessel disease (SVD). In this disorder, mutations lead to an odd number of cysteine residues within 1 of the 34 epidermal growth factor repeat (EGFR) domains of the *NOTCH3* gene.¹ Mutations in EGFR domains 7–34 were recently shown to be associated with less severe clinical outcomes.¹ Larger brain volumes were also associated with less severe clinical outcomes and predict

better clinical evolution.^{2,3} In addition, female patients show milder clinical phenotypes.^{2,4} However, this beneficial effect in female patients no longer appears significant in predictive models when brain volume is considered.² This finding suggests systematic innate (sex-related) and/or acquired differences in brain volume between male and female patients.³ While the identification of the EGFR domain as a predictor of disease evolution clearly improved our understanding of the disease, a considerable uncertainty remains regarding its evolution, and it is still unknown how sex, brain volume, the EGFR domain, and other MR imaging predictors, ie, lacunes, white matter hyperintensities (WMH), and cerebral microbleeds, interact in driving clinical outcomes. To better understand these interactions, we used a specifically designed approach to model life span brain volume in male and female patients from systematic high-resolution MR imaging scans obtained in a unique, large, prospective cohort of patients with CADASIL.

Received October 1, 2021; accepted after revision March 13, 2022.

From the Department of Neurology (E.J.), Neuroradiology (J.P.G., A.G.) and the Centre de Neurologie Vasculaire Translationnel (J.L., D.H., N.A., A.T., N.D.-G., H.C.), Assistance Publique–Hôpitaux de Paris, Hôpital Lariboisière, Paris, France; L'Institut National de la Santé et de la Recherche Médicale INSERM U1141, Université Paris Cité, Paris, France; and Federation Hospitalo-Universitaire NeuroVasc (J.L., N.D.-G., D.H., H.C., E.J.), Paris, France.

This work was funded by the RHU TRT_cSVD project (ANR-16-RHUS-004) and the Association de Recherche en Neurologie Vasculaire, Hôpital Lariboisière, France.

Please address correspondence to Eric Jouvent, MD, PhD, Hôpital Lariboisière, 2 rue Ambroise Paré, 75010 Paris, France; e-mail: eric.jouvent@aphp.fr

Indicates article with online supplemental data.

<http://dx.doi.org/10.3174/ajnr.A7499>

MATERIALS AND METHODS

Patients

Since 2003, patients with genetically confirmed *NOTCH3* mutations followed in the French referral center for rare vascular disease of the eye and the brain (CERVCO, <https://www.cervco.fr>) and willing to participate are included in a prospective cohort. They are systematically evaluated both clinically and with standardized MR imaging every 18–24 months. The EGFR domain is recorded in each patient at the time of genetic testing. The level of education is systematically recorded at inclusion.³ Beginning at inclusion and at each visit, patients undergo brain MR imaging, including 3D T1 high-resolution, FLAIR, and T2* sequences (scans were acquired on a 1.5T MR imaging scanner until 2014 and on a 3T thereafter) as well as comprehensive neurologic and neuropsychological assessments performed by experienced neurologists and neuropsychologists, respectively. In the present study, in line with previous reports,⁵ we used the Mini-Mental State Examination (MMSE: range, 0–30; with higher scores being better) and the Mattis Dementia Rating Scale (MDRS: range, 0–144; with higher scores being better) as proxies of global cognitive functions; the time to complete part B of the Trail-Making Test (TMTBT, recorded in seconds, shorter times are better) as a marker of executive functioning; and the time to complete part A (TMTAT, in seconds as well) as a marker of processing speed. Finally, we used the mRS (from 0, asymptomatic, to 5, bedridden) as a measure of disability.

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by an independent ethics committee (updated agreement CEEI-IRB-17/388) and conducted in accordance with the Declaration of Helsinki and guidelines for Good Clinical Practice and General Data Protection Regulation (GDPR) in Europe.

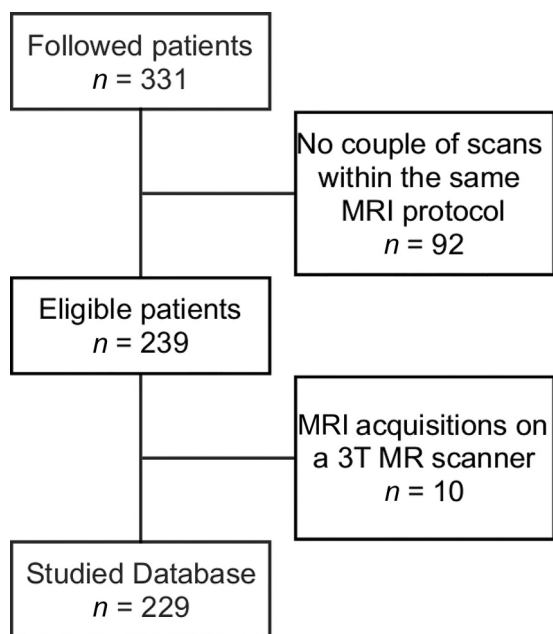


FIG 1. Flow diagram of patients included in the study.

MR Imaging Processing

We used BIANCA from the FSL suite (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BIANCA/Userguide>) to extract masks of WMH in all patients, as previously reported.^{6,7} The volume of WMH was obtained by multiplying the number of voxels in WMH masks by the corresponding voxel size. In agreement with recent guidelines,⁸ we registered these masks on 3D T1 images and replaced the intensity of the corresponding voxels in the native image with that of normal-appearing white matter. To obtain brain volume and brain volume changes, we used SIENAX (<http://support.qment.com/knowledge/sienax-2.6/-/fsl-6.0>) and SIENA (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/SIENA/UserGuide>) from FSL, respectively.^{9,10} Their reliability in the context of SVD is considered good.¹¹ Given the long-term follow-up (17 years at the time of this writing), the MR imaging protocol underwent several alterations with concerns regarding the reliability of quantitative measures. Given the known influence of the sequence characteristics on segmentation results, we used SIENA only between time points obtained with the same 3D T1 sequence.¹² Because the number of longitudinal acquisitions largely differed among patients (from 1 to 10), we randomly selected for each patient 1 couple of MR imaging acquisitions to avoid overrepresentation of data from patients with the longest follow-ups, who are likely to be the least severe. We multiplied the baseline volume, obtained with SIENAX, by the associated percentage of brain volume change with SIENA, to obtain brain volume at follow-up. To control for interindividual variability, we systematically corrected brain volumes for head size, in line with current recommendations.⁸ Finally, lacunes and cerebral microbleeds, defined according to the STRIVE criteria (<https://harness-neuroimaging.org/strive-standards>),¹³ were manually identified by an experienced reader (E.J.), with previously validated methods.⁵

Statistical Analysis

All analyses were performed with Python 3.7¹⁴ and R 3.5 (<http://www.r-project.org/>).¹⁵ We used mean or median for continuous variables and SDs or interquartile range as measures of dispersion, depending on variable type and distribution. Group differences were evaluated with the Mann-Whitney test for continuous variables or the χ^2 test of independence for categorical variables. We built regression models to estimate the relationships between brain volume and age. Given the known effect of sex on disease severity, we built separate models for men and women. Considering possible nonlinearities with age, the evolution of brain volume with age across the whole life span was modeled using a nonparametric locally weighted regression approach (statmodels 0.12.1 in Python; <https://www.statsmodels.org/v0.12.1/>).^{16,17} When considered necessary, cluster analysis with the k-means algorithm (scikit-learn library 0.22.1; <https://zenodo.org/record/3596890#.Ykcc9SjMJPY>)¹⁸ was performed to determine whether different subgroups could be identified on the basis of age at baseline, baseline brain volume, and annual percentage of brain volume change. We computed Silhouette scores (https://scikit-learn.org/stable/modules/generated/sklearn.metrics.silhouette_score.html), defined as the normalized difference between the minimal intercluster dissimilarity and the mean intracluster dissimilarity, to select the optimal number of clusters.¹⁹ The optimal number of clusters provides the best trade-off between the

Table 1: Baseline characteristics of the study sample^a

| | Study Sample (n = 229) | Nonincluded Patients (n = 102) | P Value |
|--|----------------------------|--------------------------------|---------|
| Women ^b | 130 (57%) | 50 (49%) | .24 |
| Age (mean) (range) (yr) | 52.8 (SD 12.0) (24.1–79.4) | 53.8 (SD, 12.1) (25.6–78.8) | .19 |
| Genetic profile ^b | | | .19 |
| Mutation in EGFR domains 1–6 | 164 (72%) | 65 (64%) | |
| Mutation in EGFR domains 7–34 | 65 (28%) | 37 (36%) | |
| Level of education (yr) ^{b,c,d} | | | .08 |
| 1–3 | 40 (17%) | 16 (16%) | |
| 4–6 | 122 (53%) | 46 (45%) | |
| 7 | 66 (29%) | 32 (31%) | |
| mRS ^{b,e} (median) (IQR) | 1 (0–2) | 1 (0–2) | .22 |
| MDRS ^f (mean) | 133.9 (SD, 17.1) | 133.0 (SD, 17.5) | .33 |
| MMSE ^g (mean) | 27.1 (SD, 4.3) | 26.4 (SD, 4.9) | .43 |
| TMTAT ^h (mean) | 47.3 (SD, 33.9) | 55.6 (SD, 50.2) | .48 |
| TMTBT ⁱ (mean) | 123.9 (SD, 99.7) | 113.5 (SD, 90.4) | .45 |
| Normalized brain volume (cm ³) | 1004.4 (SD, 61.1) | 1024.8 (SD, 80.9) | <.001 |

Note: —IQR indicates interquartile range.

^aCharacteristics of included and nonincluded patients were compared according to variable type and distribution (Mann-Whitney test by default or χ^2 test of independence for categorical variables).

^bComparisons performed using the χ^2 test of independence.

^cOn 228 studied patients and 94 excluded patients.

^dReference levels of education: 0 = illiterate, 3 = incomplete secondary school (<9 years), 6 = secondary school (13 years), 7 = university (\geq 16 years).

^eOn 216 and 100 patients, respectively.

^fOn 200 and 94 patients, respectively.

^gOn 209 and 90 patients, respectively.

^hOn 194 and 85 patients, respectively.

ⁱOn 182 and 76 patients, respectively.

average Silhouette score to maximize, and the number of negative scores representing mislabeling. To determine whether patients in different clusters actually show different disease course profiles, we built regression models to predict clinical outcomes (MMSE, MDRS, TMTAT, TMTBT, and mRS) based on the subgroup to which the patient pertained, age, level of education, the EGFR domain of the mutation (categorized as EGFR domains 1–6 versus EGFR domains 7–34, according to previous data¹), brain volume, volume of WMH, and number of lacunes and microbleeds, all measured at baseline. Missing values were imputed, considering the standard span of values in the data base (package Amelia in R; <https://www.rdocumentation.org/packages/Amelia/versions/1.8.0>).²⁰ The threshold for significance was set at .05.

RESULTS

As of this writing, the whole database comprises 331 patients with available follow-up data (2230 patient-years). The age at inclusion ranges from 24.1 to 79.4 years (mean age, 53.2 [SD, 12.2] years). Among the 331 patients, 239 had at least 2 acquisitions with the same protocol (229 at 1.5T, 10 at 3T). Given that <5% of scans were acquired at 3T, we herein provide results corresponding to the data obtained at 1.5T only (the flow chart is presented in Fig 1). However, including the few data obtained at 3T did not alter our results (data not shown). Characteristics of the 229 patients in the study sample are detailed Table 1. On average, the time elapsed between the 2 MR imaging evaluations for these 229 patients was 20.8 (SD, 6.5) months. While they were in general, similar to nonincluded patients, included patients showed significantly lower brain volumes ($P < .001$).

The mean brain volume was significantly larger in female patients throughout the life span (1024 [SD, 62] cm³ versus 979 [SD, 50] cm³ in male patients; $P < .001$), while mean age was not significantly different between sexes (52.8 [SD, 13.0] years in

women versus 52.8 [SD, 10.6] years in men, $P = .41$). The spread of brain volume was relatively constant across age for women but steadily increased for men (Fig 2A). By comparison with the regression line in female patients, which suggested a continuous decrease in brain volume across the life span, the regression line in male patients presented an unexpected 3-step curve (Fig 2A and Online Supplemental Data). Given that we considered unlikely that brain volume increases after middle age in male patients, we performed a cluster analysis to determine whether this aspect may be related to the presence of different subgroups of patients. As suggested by the homogeneous regression curve in female patients, cluster analysis among women did not identify any relevant subgroup.

According to the Silhouette algorithm, 4 subgroups of male patients could be identified (Fig 3 and Table 2 and Online Supplemental Data). One group corresponded to most male patients younger than 50 years of age (the “young” group, 24 patients). Two others, thereafter denoted “middle aged” (30 patients) and “older” patients (23 patients), roughly followed the global trend for brain volume reduction with age observed in women, with a downward shift (Fig 2B). A fourth group of 22 older male patients showed strikingly different characteristics, with brain volumes close to that of age-matched females (“older men with larger brains” group in Fig 2B). When excluding this subgroup, brain volume in male patients showed a shape similar to that of female patients with a downward shift (Fig 2B).

At the global level, analyses confirmed the previously reported relationships among age, lacunes, and brain volume and clinical outcomes as well as the inconsistent effect of the EGFR domain and sex on clinical outcomes when brain volume is considered (Online Supplemental Data). Among subgroups, male patients from the older with larger brain group showed significantly better clinical scores than the older male group (Table 2). Most noteworthy,

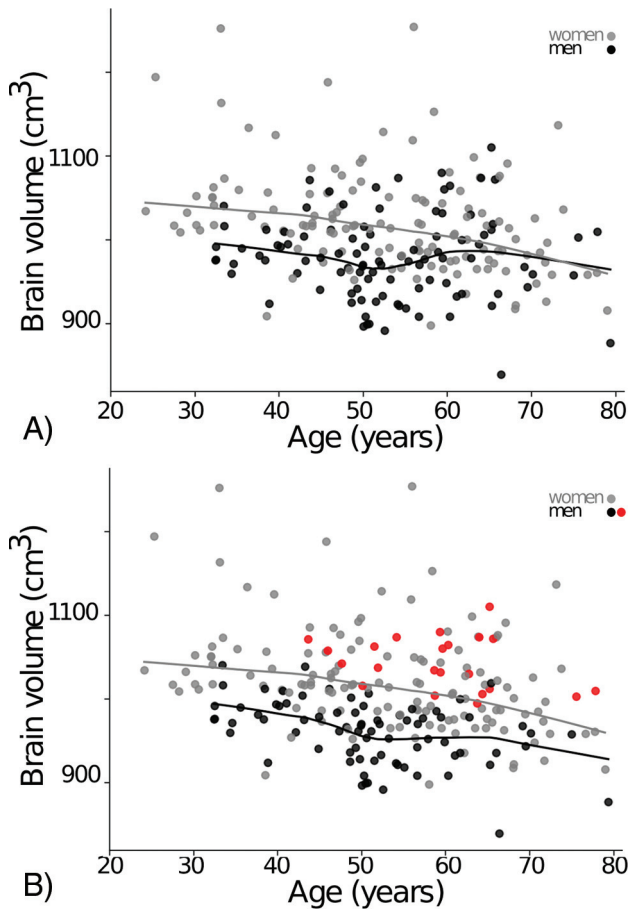


FIG 2. Evolution of brain volume with age. Scatterplot of brain volume measured at the first time point for each patient against age, with (A) and without (B) the “older with larger brains” group (red points in B). Weighted regressions were reconstructed separately for men and women, given the known detrimental effect of the disease in male patients (black and gray solid lines). When we built regression lines without considering the older with larger brains group (in red), they looked similar in male and female patients across the whole life span, though shifted downward in men, for yet-unknown reasons.

the difference between these groups was significant while considering the most well-known factors associated with clinical outcomes in CADASIL, namely age, level of education, and EGFR domain of the mutation. Further considering the burden of WMH, the number of lacunes or the number of microbleeds did not alter this finding. As expected however, the group effect was no longer significant when including brain volume as a covariate (Online Supplemental Data).

DISCUSSION

In the present study, we observed that in contrast to the general population,²¹ female patients have larger brain volumes than male patients across the life span in CADASIL, suggesting an innate or early-life difference in brain volume between male and female patients, long before the appearance on brain MR imaging of markers of SVD, which will be associated with the development of brain atrophy (Fig 2). We did not identify any obvious reason for such a detrimental effect of male sex on brain volume, in particular regarding the EGFR domain, which was not associated with sex. However, we identified a subgroup of

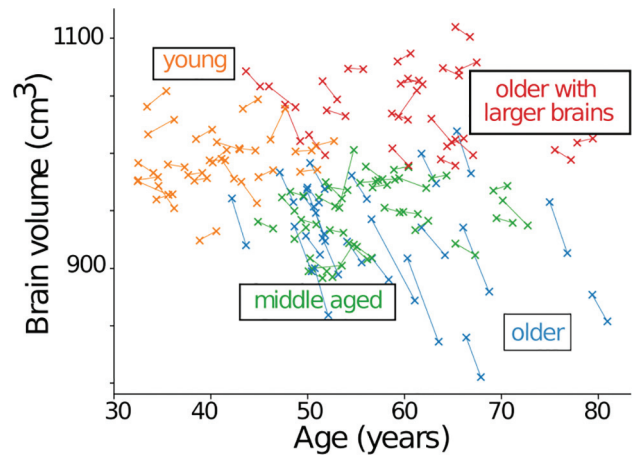


FIG 3. K-means clustering analysis in men. The clustering analysis used with the Silhouette criteria led to the definition of 4 distinct groups of patients. The group of young patients comprised nearly all individuals younger than 50 years of age. The middle-aged and older patient groups seemed to follow the age trend of female patients. In total contrast, the group of older with larger brains patients showed strikingly different characteristics, with larger brain volume and lower brain atrophy.

elderly male patients having larger brain volumes and milder clinical outcomes, similar to that of age-matched female patients, suggesting the existence of yet-undefined disease-modifying factors.

While the follow-up of our cohort was exceptionally long (17 years), we could not fully use these longitudinal data to model brain volume evolution, given the large impact of sequence and scanner alterations on quantitative metrics. This mostly cross-sectional nature of our analyses may yield unexpected findings, such as the crude regression line suggesting an increase of brain volume in male patients between 50 and 60 years of age. Because systematic brain volume enlargement in middle-aged male patients is unlikely, the shape of the regression curve could be explained, in part, by the gradual departure of the patients with the most severe disease as they age, which could lead to an overrepresentation of elderly patients with less severe disease, resulting in a tendency for group-wide brain volume to increase in cross-sectional analyses. However, this could not explain the presence of older male patients with brain volumes similar to those in male patients 20 years younger. Figure 2B shows that this subgroup appears more similar to age-matched female patients. For whatever reason, these patients may not be affected by the detrimental effect of male sex on brain volume. We expected to find a larger representation of EGFR domains 7–34, associated with better clinical outcomes in this subgroup, but this was not the case, suggesting that other factors may temper the detrimental effect of male sex in CADASIL. Further studies will be needed to understand the mechanisms underlying this phenomenon.

In line with results suggesting that the presence of extensive WMH in the anterior temporal poles is associated with a better prognosis,²² in the present study, older male patients with larger brain volumes also tended to have larger volumes of WMH in these areas, but the difference did not reach significance (data not shown). What is noteworthy, we recently reported the case of a patient whose large extent of WMH, particularly in the anterior temporal poles, was strongly reduced while taking valpromide for severe depression

Table 2: Baseline characteristics of “older with larger brains” men and older men in CADASIL^a

| | Older with Larger Brains Men (n = 22) | Older Men (n = 23) | P Value |
|---|---------------------------------------|-----------------------------|---------|
| Age (mean) (range) (yr) | 59.3 (SD, 8.5) (43.6–77.8) | 56.4 (SD, 10.7) (42.1–79.4) | .12 |
| Genetic profile ^b | | | .92 |
| Mutation in EGFR domains 1–6 | 15 (68%) | 17 (74%) | |
| Mutation in EGFR domains 7–34 | 7 (32%) | 6 (26%) | |
| Level of education ^b (yr) | | | .30 |
| 1–3 | 2 (9%) | 6 (26%) | |
| 4–6 | 13 (59%) | 10 (43%) | |
| 7 | 7 (32%) | 7 (30%) | |
| mRS ^{b,c} (median) (IQR) | 1 (0–2) | 3 (1–5) | .009 |
| MDRS ^d (mean) | 136.2 (SD, 8.7) | 120.8 (SD, 28.5) | .04 |
| MMSE ^e (mean) | 28.2 (SD, 2.2) | 22.9 (SD, 6.7) | .45 |
| TMTAT ^f (mean) | 52.6 (SD, 30.3) | 42.9 (SD, 20.3) | .004 |
| TMTBT ^g (mean) | 125.4 (SD, 87.0) | 142.0 (SD, 97.7) | .003 |
| Normalized brain volume (mean) (cm ³) | 1043.5 (SD, 30.8) | 944.2 (SD, 40.1) | <.001 |
| Annual PBVC (%) | −0.4 (0.7) | −2.0 (0.7) | <.001 |
| Normalized WMH volume (%) | 0.1 (0.1) | 0.1 (0.0) | .03 |
| No. of lacunes | 6.8 (7.7) | 10.6 (8.0) | .01 |
| No. of microbleeds | 11.0 (23.4) | 8.6 (18.2) | .36 |

Note:—PBVC indicates percentage of brain volume change

^aCharacteristics of older men with larger brains and older men were compared with appropriate methods, depending on variable type and distribution (Mann-Whitney test by default or χ^2 test of independence for categorical variables).

^bComparisons performed using the χ^2 test of independence.

^cOn 22 older with men larger brains and 21 older men.

^dOn 20 and 12 patients, respectively.

^eOn 22 and 16 patients, respectively.

^fOn 19 and 9 patients, respectively.

^gOn 17 and 9 patients, respectively.

before re-inflating after the treatment was switched to lithium.²³ These results support the hypothesis that mechanisms underlying WMH in CADASIL may not be related only to chronic hypoperfusion but may involve large variations of water influx into the different brain compartments. Whether these changes might affect male and female patients differently and whether they might alter the natural history of the disease remain unknown. Future studies will help determine whether certain medications or conditions may lead male patients to switch from one group to another. The results might potentially lead to the identification of new therapeutic approaches.

Our study has several strengths. We evaluated a large cohort of patients in whom the diagnosis was, in all cases, confirmed genetically, while following the current standard for processing brain images in the context of severe SVD. We designed a specific approach to process brain images acquired during a very long period of time while avoiding biases due to overfitting the patients with the longest follow-ups. Finally, we based our subgroup analyses on unsupervised approaches, whose results were, thereafter, confirmed by testing associations with clinical outcomes.

We must also acknowledge some limitations. We could not use all the data gathered during the past 17 years due to methodologic constraints related to mandatory changes in MR imaging scanners or sequences. The differences in brain volume between included and nonincluded patients shown in Table 1 illustrate the importance of MR imaging scanner on brain volume metrics. Indeed, while the 229 included and the 102 nonincluded patients did not differ regarding clinical variables, the brain volume of nonincluded patients was significantly larger. However, after excluding the data obtained on the 3T scanner (55 of 102 patients), this difference was no longer significant, suggesting that sequences acquired on the 3T scanner

overestimate brain volume compared with those obtained on the 1.5T scanner. Finally, we could not formally exclude any bias due to data selection because we chose not to fully exploit longitudinal data in some patients. Indeed, some patients in our cohort have been followed for >15 years. This individual approach may well provide additional information, and we are currently working on specific postprocessing approaches allowing the exploitation of such valuable data, as illustrated by a recent case report of our group.²³

CONCLUSIONS

The present study results show that brain volume, a major determinant of clinical outcomes, is larger in female patients throughout life in CADASIL, in total contrast to the general population. For yet-undetermined reasons, some elderly male patients may exhibit larger brain volumes and milder clinical outcomes similar to those of age-matched female patients, suggesting the possibility of identifying new therapeutic targets in this disorder.

ACKNOWLEDGMENTS

The authors thank all patients for their collaboration on this study.

Disclosure forms provided by the authors are available with the full text and PDF of this article at www.ajnr.org.

REFERENCES

1. Rutten JW, Van Eijnsden BJ, Duering M, et al. **The effect of NOTCH3 pathogenic variant position on CADASIL disease severity: NOTCH3 EGFR 1-6 pathogenic variant are associated with a more severe phenotype and lower survival compared**

- with EGFR 7-34 pathogenic variant. *Genet Med* 2019;21:676–82 [CrossRef Medline](#)
2. Chabriat H, Hervé D, Duering M, et al. Predictors of clinical worsening in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: prospective cohort study. *Stroke* 2016;47:4–11 [CrossRef Medline](#)
 3. Jouvent E, Duchesnay E, Hadj-Selem F, et al. Prediction of 3-year clinical course in CADASIL. *Neurology* 2016;87:1787–95 [CrossRef Medline](#)
 4. Gunda B, Hervé D, Godin O, et al. Effects of gender on the phenotype of CADASIL. *Stroke* 2012;43:137–41 [CrossRef Medline](#)
 5. Viswanathan A, Guichard JP, Gschwendtner A, et al. Blood pressure and haemoglobin A1c are associated with microhaemorrhage in CADASIL: a two-centre cohort study. *Brain* 2006;129:2375–83 [CrossRef Medline](#)
 6. Griffanti L, Zamboni G, Khan A, et al. BIANCA (Brain Intensity AbNormality Classification Algorithm): a new tool for automated segmentation of white matter hyperintensities. *Neuroimage* 2016;141:191–205 [CrossRef Medline](#)
 7. Ling Y, Jouvent E, Cousyn L, et al. Validation and optimization of BIANCA for the segmentation of extensive white matter hyperintensities. *Neuroinformatics* 2018;16:269–81 [CrossRef Medline](#)
 8. De Guio F, Duering M, Fazekas F, et al. Brain atrophy in cerebral small vessel diseases: Extent, consequences, technical limitations and perspectives: the HARNESS initiative. *J Cereb Blood Flow Metab* 2020;40:231–45 [CrossRef Medline](#)
 9. Smith SM, Zhang Y, Jenkinson M, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *Neuroimage* 2002;17:479–89 [CrossRef Medline](#)
 10. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 2004;23(Suppl 1):S208–19 [CrossRef Medline](#)
 11. De Guio F, Jouvent E, Biessels GJ, et al. Reproducibility and variability of quantitative magnetic resonance imaging markers in cerebral small vessel disease. *J Cereb Blood Flow Metab* 2016;36:1319–37 [CrossRef Medline](#)
 12. Opfer R, Ostwaldt AC, Walker-Egger C, et al. Within-patient fluctuation of brain volume estimates from short-term repeated MRI measurements using SIENA/FSL. *J Neurol* 2018;265:1158–65 [CrossRef Medline](#)
 13. Wardlaw JM, Smith EE, Biessels GJ, et al. Standards for Reporting Vascular changes on neuroimaging (STRIVE v1). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol* 2013;12:822–38 [CrossRef Medline](#)
 14. van Rossum G, de Boer J. Interactively testing remote servers using the Python programming language. *CWI Quarterly* 1991;4:283–304
 15. R Core Team. *R: A Language Environment for Statistical Computing*. R Foundation for Statistical Computing; 2020. <https://www.R-project.org>.
 16. Cleveland WS. Robust locally weighted regression and smoothing scatterplots. *J Am Stat Assoc* 1979;74:829–36 [CrossRef](#)
 17. Seabold S, Perktold J. Statsmodels: econometric and statistical modeling with Python. In: *Proceedings of the Ninth Python in Science Conference*, Austin, Texas; November 15, 2010
 18. Pedregosa F, Varoquaux G, Gramfort A, et al. Scikit-learn: machine learning in Python. *J Mach Learn Res* 2011;12:2825–30
 19. Rousseeuw PJ. Silhouettes: a graphical aid to the interpretation and validation of cluster analysis. *J Comput Appl Math* 1987;20:53–65 [CrossRef](#)
 20. Honaker J, King G, Blackwell M. Amelia II: a program for missing data. *J Stat Software* 2011;45:1–47 [CrossRef](#)
 21. Ruigrok AN, Salimi-Khorshidi G, Lai MC, et al. A meta-analysis of sex differences in human brain structure. *Neurosci Biobehav Rev* 2014;39:34–50 [CrossRef Medline](#)
 22. Duchesnay E, Hadj Selem F, De Guio F, et al. Different types of white matter hyperintensities in CADASIL. *Front Neurol* 2018;9:526 [CrossRef Medline](#)
 23. Jouvent E, Alili N, Herve D, et al. Vanishing white matter hyperintensities in CADASIL: a case report with insight into disease mechanisms. *J Alzheimers Dis* 2020;78:907–10 [CrossRef Medline](#)