

















## ORIGINAL RESEARCH

# High Prevalence of Cerebrovascular Calcifications and Clinical Correlates in Indigenous Bolivian Forager-Horticulturalists: A Population-Based Observational Study

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**BACKGROUND:** Intracranial arteriosclerosis (large- and small-vessel disease) is considered a risk factor for major neurological disorders, such as stroke, cognitive impairment, and dementia. While most studies investigating intracranial arteriosclerosis include individuals from industrialized populations, the prevalence and clinical meaning of intracranial vascular calcifications in populations with a subsistence lifestyle is unknown.

**METHODS:** In this population-based study evaluating data collected between 2017 and 2019 from Tsimane and Mose ten people, 2 indigenous populations of forager-horticulturalists living in the Bolivian Amazon, we used computed tomography to determine the prevalence of vascular calcifications in the intracranial internal carotid arteries, vertebral arteries, and lenticulostriate arteries within the basal ganglia, and their association with demographic characteristics, brain atrophy, cognitive performance, and clinical factors.

**RESULTS:** Our analysis included 1232 individuals who underwent a head computed tomography scan. Intracranial vascular calcifications were found in most individuals (>90%) and their prevalence was higher than that reported for age-equivalent industrialized populations. These calcifications were significantly associated with higher age, brain atrophy, worse cognitive performance, and parkinsonian symptoms.

**CONCLUSIONS:** Despite the physically active subsistence lifestyle and the low rates of typical cardiovascular risk factors and coronary artery disease, intracranial vascular calcifications are common in these Bolivian Amerindian people, suggesting that alternative factors may contribute to intracranial arteriosclerosis and a novel dementia phenotype.

**Key Words:** brain atrophy ■ cerebrovascular calcifications ■ cognitive performance ■ CT ■ Tsimane

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## RESEARCH PERSPECTIVE

### What Is New?

- Tsimane and Mosen, two indigenous populations of forager-horticulturalists living in the Bolivian Amazon with physically active subsistence lifestyle and low rates of typical cardiovascular risk factors and coronary artery disease, have a high prevalence of intracranial cerebrovascular calcifications.
- These calcifications were significantly associated with higher age, brain atrophy, worse cognitive performance, and parkinsonian symptoms.

### What Question Should Be Addressed Next?

- The factors contributing to the development of intracranial arteriosclerosis in this population remain unclear.

## Nonstandard Abbreviations and Acronyms

<b>BGVC</b>	basal ganglia vascular calcification
<b>BMD</b>	bone mineral density
<b>CVRF</b>	cardiovascular risk factors
<b>ICAC</b>	intracranial carotid artery vascular calcification
<b>THLHP</b>	Tsimane Health and Life History Project
<b>VAC</b>	vertebral artery vascular calcification

Intracranial arteriosclerosis (large- and small-vessel disease) is increasingly recognized as an important factor contributing to the pathogenesis of cognitive decline and dementia. Previous studies report an association of atherosclerosis affecting major intracranial arteries (such as the internal carotid arteries and vertebral arteries) with neuroimaging markers of vascular brain injury<sup>1</sup> and a higher risk of dementia and cognitive decline.<sup>2,3</sup> Additionally, ex vivo studies have shown the presence of cerebral arteriole and capillary pathology in patients with dementia.<sup>2,4-7</sup> These findings point toward a common and potentially preventable pathway through which cerebral vascular pathology may influence dementia risk and brain function.

Most studies on dementia and its contributing factors include individuals from industrialized populations, but given the significant prevalence and rise in dementia cases that is occurring in low- and middle-income countries,<sup>8</sup> it is compelling to perform more research in nonindustrialized populations.<sup>9</sup>

A recent preliminary analysis by our group of 155 individuals from indigenous South American populations observed a high rate of vascular calcifications in the intracranial carotid arteries based on visual assessment of brain computed tomography (CT) scans.<sup>10</sup> This result was surprising as the population has a largely nonindustrial lifestyle, with a high level of daily physical activity and a low-fat diet,<sup>11,12</sup> 5-fold lower prevalence of coronary artery calcium than industrial populations;<sup>13</sup> low prevalence of metabolic syndrome;<sup>14</sup> and few cardiovascular risk factors (CVRFs) such as smoking, obesity, and hypertension,<sup>13,15,16</sup> thereby suggesting that other factors might be implicated in the development of these calcifications. We previously observed that these calcifications assessed with visual rating qualitative scores were associated with a form of dementia characterized by parkinsonian symptoms and cognitive deficits in attention, visuospatial, and executive domains.<sup>10</sup> Additionally, recent studies highlighted the involvement of brain regions typically fed by branches of the internal carotid arteries and vertebral arteries (eg, frontal lobe and medial temporal lobe) in Parkinson disease.<sup>17-20</sup>

Previous research also reported associations between intracranial vascular calcifications in carotid arteries, cognitive impairment, and brain health.<sup>1,3,21</sup> It has been suggested that these calcifications might be indicators of subclinical small-vessel disease in the brain, which in turn can contribute to neurodegeneration through several mechanisms including chronic hypoperfusion, blood-brain barrier breakdown, and impaired brain clearance.<sup>22</sup>

In the current study, we performed a quantitative analysis of vascular calcifications in intracranial carotid arteries (ICACs), vertebral arteries (VACs), and basal ganglia (BGVCs) in a large cohort (n=1232) from the same 2 Amerindian populations in the Bolivian Amazon:

- Tsimane people, forager-horticulturalists, who live in small rural communities with minimal access to electricity, clean water, and medical services, and who engage in multiple types of subsistence activities such as slash-and-burn farming, hunting, fishing, and collection of wild fruits.
- Mosen people, who are culturally similar and linguistically related to the Tsimane people and share an agricultural subsistence lifestyle but have greater access to the market economy and services such as schools, clean water supply, sanitation, electricity, and health care.<sup>23</sup>

Here, we aim to characterize the prevalence and distribution of intracranial vascular calcifications and their relationships with demographic and clinical factors, neurological function, global and regional brain

atrophy, cardiovascular calcifications, and bone density. Differences between the populations in terms of prevalence and the above relationships are also evaluated. We hypothesized that greater calcification would be associated with greater brain atrophy, greater impairment in cognitive performance, more pronounced parkinsonian symptoms, lower bone density, and higher degree of calcifications in extracranial vascular beds.

## METHODS

### Data-Sharing Statement

Individual-level data are stored in the Tsimane Health and Life History Project (THLHP) Data Repository and are available through restricted access for ethical reasons. The THLHP's highest priority is the safeguarding of human subjects and minimization of risk to study participants. The THLHP adheres to the "CARE Principles for Indigenous Data Governance" (Collective Benefit, Authority to Control, Responsibility, and Ethics), which assure that the Tsimane and Mosesten people (1) have sovereignty over how data are shared, (2) are the primary gatekeepers determining ethical use, (3) are actively engaged in the data generation, and (4) derive benefit from data generated and shared for use whenever possible. The THLHP is also committed to the "FAIR Guiding Principles for scientific data management and stewardship" (Findable, Accessible, Interoperable, Reusable). Requests for individual-level data should take the form of an application that details the exact uses of the data and the research questions to be addressed, procedures that will be used for data security and individual privacy, potential benefits to the study communities, and procedures for assessing and minimizing stigmatizing interpretations of the research results (see <https://tsimane.anth.ucsb.edu/data.html> for links to the data-sharing policy and data request forms).

Requests for individual-level data will require institutional review board approval (even if exempt) and will be reviewed by an Advisory Council composed of Tsimane community leaders, community members, Bolivian scientists, and the THLHP leadership. A similar structure exists for the Mosesten data. The study authors and the THLHP leadership are committed to open science and are available to assist interested investigators in preparing data access requests.

### Study Population

The THLHP has been investigating the Tsimane population's health and aging since 2002. Since 2011, the THLHP has covered ≈100 Tsimane villages, and in 2015, 10 Mosesten villages were added to the project. All individuals aged ≥60 years were invited to enroll, while those aged 40 to 59 were randomly

selected on the basis of community stratification.<sup>13,24</sup> Medical assessments and evaluations were performed at ≈2-year intervals by THLHP physicians and Tsimane anthropologists and included neurological examinations and cognitive testing, hearing and vision tests, body mass index, blood pressure assessments, and blood panels. All participants included in this study also underwent a noncontrast CT scan of the brain between July 2017 and December 2019.

All phases of the study were approved by the ethics committee of the San Simon University School of Medicine (Cochabamba, Bolivia), the institutional review boards of the University of New Mexico Health Sciences Center and the University of California, Santa Barbara (which currently serves as the designated THLHP institutional review board). The Tsimane and Mosesten governments, village leaders, and study participants approved all protocols. All participants provided informed consent in their native language. When incidental findings arose during brain CT scans, participants were advised and supported to receive medical treatment.

### CT Scan Acquisition, Processing, and Measurements

Noncontrast CT scans were performed at German Busch Hospital in the city of Trinidad, Bolivia, by a licensed radiological technologist using a 16-detector row multislice CT (GE Brightspeed, Milwaukee, WI) under the supervision of project clinicians, using a 0.625-mm slice thickness. ICACs, VACs, and BGVCs in the caudate and lentiform nuclei were quantified as previously described.<sup>1,25</sup> Intracranial internal carotid arteries and vertebral arteries were first manually delineated with ImageJ (National Institutes of Health, Bethesda, MD) by 3 raters trained by a physician-scientist with 10 years of experience in neuroradiology research. The 3 raters included 2 medical students and 1 neuroimaging master's student with 3 to 4 years of previous experience in neuroimaging research. The training sessions focused on understanding the anatomy and appearance of internal carotid arteries and vertebral arteries with different degrees of calcifications on CT and practice exercises on drawing the regions of interest with direct feedback from the instructor. After training, each of the 3 raters was assigned separate sets of data. Each individual case was then independently reviewed by the physician-scientist in a blinded fashion. Thus, each case was assessed by at least 2 people: a trained rater and an expert. Calcification volumes (mm<sup>3</sup>) were then computed by multiplying the number of voxels within the delineated area ≥130 Hounsfield units by the voxel size. This threshold corresponds to the traditional threshold of the Agatston score<sup>26</sup> and has been consistently used in previous studies on

ICACs and VACs.<sup>1,3,25,27</sup> The total volume of ICACs was calculated by summing the calcification volumes of the left and right intracranial carotid arteries. Similarly, VAC volume was calculated as the sum of the calcification volumes of the left and right vertebral arteries. Ten randomly selected CT scans were used to determine if calcification volume could be obtained reliably by the 3 different raters: the estimated intraclass correlation coefficient was excellent (0.97 [95% CI, 0.91–0.99];  $P < 0.001$ ).

Calcifications in the caudate and lentiform nuclei of the basal ganglia were segmented and quantified with the following fully automated approach. We transformed the voxel densities of the CT scans from Hounsfield units to Cormack units with a publicly available tool (<https://github.com/neurolabusc/Clinical>)<sup>28</sup> and registered the transformed CT images to a CT template as previously published<sup>29</sup> with a 12-parameter affine registration with the Linear Image Registration Tool of the Functional Magnetic Resonance Imaging of the Brain Software Library.<sup>30</sup> The affine registration matrix was subsequently inverted and applied to the template's basal ganglia masks to transform them to the native space of the original CT scan. Quality assessment of basal ganglia masks was performed in a blinded fashion. Finally, the volume ( $\text{mm}^3$ ) of vascular-perivascular BGVC mask was computed by multiplying the voxel size by the number of voxels  $\geq 70$  Hounsfield units. We used this threshold because the blood vessels and calcifications in basal ganglia are smaller than ICACs and VACs, and we observed that the traditional threshold of 130 Hounsfield units was too high to fully and accurately capture calcifications in the basal ganglia (Figure S1). As a limitation, with this approach we were unable to precisely discriminate between parenchymal and vascular calcifications in the basal ganglia mask, but we noted that in almost all cases the segmented calcifications have a tubular shape resembling the vascular type of calcifications and follow the typical course of the lenticulostriate arteries (Figure S1).

To estimate measures of cortical thickness (mm) and of gray matter and white matter volumes ( $\text{mm}^3$ ) from CT scans, we used a public artificial intelligence tool<sup>31,32</sup> and performed a brain parcellation based on the Desikan–Killiany atlas.<sup>33,34</sup>

Quality assessment of all vascular and brain segmentation masks was visually performed in a blinded fashion. The brain parcellation failed in 4 cases, which were excluded from subsequent analyses.

Measurements of coronary artery calcification and thoracic aortic calcium, including the ascending and descending aorta and the aortic arch, and thoracic bone mineral density (BMD) were performed on ECG-gated CT scans of the chest. Coronary artery calcification scoring was obtained using a semiautomatic

software on a Siemens workstation as described by Agatston and colleagues.<sup>26</sup> Thoracic aortic calcium was measured between the carina and the inferior surface of the heart with Siemens calcium scoring software following the approach described by Budoff et al.<sup>35</sup> A radiologist with  $>20$  years of experience manually measured BMD in each of 3 consecutive thoracic vertebrae (T7–T10 range): a circular region of interest was positioned at the center of each vertebra while excluding any area with large vessels, bone islands, fractures, and calcified herniated disks. The mean BMD for the 3 consecutive thoracic vertebrae was then calculated. Conversion of Hounsfield units to BMD ( $\text{mg}/\text{cm}^3$ ) was done via a calibration phantom of known density or a scanner-specific mean calibration factor for the T7 to T10 vertebrae from scans performed without the phantom.

Measurements of coronary artery calcification and thoracic aortic calcium were performed by the Core Laboratory at St. Luke's Mid America Heart Institute in Kansas City, whereas BMD measurements were performed by the Los Angeles Biomedical Research Institute. All measurements occurred blinded to participant data.

## Assessment of Cognition and Dementia

Trained Tsimane THLHP researchers administered to all participants a cognitive battery adapted from the Mexican Health and Aging Study<sup>36</sup> and the Indianapolis–Ibadan Dementia Project.<sup>26</sup> The battery included Visual Scan (searching for a target symbol among distractor symbols), Digit Span Forward, Immediate and Delayed Word Recall, Semantic Fluency (naming animals and fish), Tactile Digit Forward (a variation of the Corsi block tapping task), and Stick Design Test<sup>37</sup> (a measure of visuo-constructional ability). The median interval between CT and cognitive battery was 0.16 (interquartile range [IQR], 0.06–0.42) years.

An experienced Bolivian physician, who received additional training and consultation with a US-based team composed of a neurologist and a clinical psychologist, both experts in dementia, interviewed all participants aged  $\geq 60$  years and their family informants. Older adults' interviews used the Modified Mini-Mental State Examination (range, 0–100)<sup>38</sup> adapted to account for illiteracy, lack of ability to count, and unfamiliarity with dates.<sup>10</sup> The informant interview included the Kimberly Indigenous Cognitive Assessment<sup>39</sup> and the Blessed Dementia Scale.<sup>40</sup> Tsimane individuals were interviewed in the Tsimane language using a translator, and Mosen individuals were interviewed in Spanish. Neurological signs associated with parkinsonism or stroke were evaluated. The median interval between CT and interviews was 0.36 (IQR, 0–1.10) years.

Based on these assessments, for those aged  $\geq 60$  years, clinical diagnoses of mild cognitive impairment (MCI) or dementia were assigned following *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*, criteria for major and mild neurocognitive disorder<sup>41</sup> by a consensus process with independent diagnoses by 2 Bolivian physicians and the US-based team including a neurologist, clinical psychologist, 2 neuroradiologists, and 2 diagnostic radiologists. Disagreements between the 2 independent diagnoses ( $\approx 5\%$  of all cases) were resolved by discussion between the Bolivia-based team and US-based team.

## Statistical Analysis

All calcium volumes were highly right-skewed; data transformations did not sufficiently normalize them. Summary statistics on these measures therefore used medians and IQRs (or 25th and 75th percentiles). Multivariable quantile regression was used to assess associations of demographic variables (age, sex, and population), with median ICAC, VAC, and BGVC volumes used as dependent variables. Linear mixed models for repeated measures were used to assess the relationship between brain atrophy (total gray matter volume, total white matter volume, and mean cortical thickness) as dependent variables and cerebrovascular calcification volumes as independent variables, controlling for age, sex, population, and total intracranial volume. Subgroup analysis was also performed to explore effect modifications across levels of age, sex, and population. Statistically significant associations were further assessed in regional analyses evaluating the relationship between atrophy in brain parcellations (based on the Desikan–Killiany atlas<sup>34</sup>) and the cerebrovascular calcifications controlling for the same covariates used in the main models. In brain regional analyses, we performed correction for multiple comparisons across all the analyzed regions (86 regions) with the Holm–Bonferroni procedure.<sup>42</sup>

Quantile regression was used to compare median vascular calcification volumes across cognitive diagnoses (dementia, MCI, normal cognition) and the presence or absence of individual parkinsonian symptoms (tremor, bradykinesia, hypomimia, rigidity, parkinsonian gait), adjusted for age, sex, and population. A multinomial logistic regression model controlling for age, sex, and population was also used to evaluate the association between vascular calcifications and cognitive status (dementia, MCI, and normal cognition); odds ratios (MCI compared with normal cognition, dementia compared with normal cognition) were calculated per  $20\text{ mm}^3$  of each vessel calcification volume.

We used linear regression with cognitive test scores as dependent variables to evaluate associations with

**Table 1. Demographic Characteristics of Study Sample**

Age, y, mean $\pm$ SD	58.5 $\pm$ 10.5
40–44, n (%)	73 (5.9)
45–49, n (%)	232 (18.8)
50–54, n (%)	188 (15.3)
55–59, n (%)	206 (16.7)
60–64, n (%)	183 (14.8)
65–69, n (%)	162 (13.2)
70–74, n (%)	94 (7.6)
75–79, n (%)	53 (4.3)
$\geq 80$ , n (%)	41 (3.3)
Sex, n (%)	
Male	640 (51.9)
Female	592 (48.1)
Population, n (%)	
Tsimane	794 (64.4)
Moseten	438 (35.6)
Cognitive status, n (%) (n=529)*	
Normal cognition	477 (90.2)
MCI	48 (9.1)
Dementia	4 (0.8)

MCI indicates mild cognitive impairment.

\*Cognitive status was evaluated in 529 individuals aged  $\geq 60$  years.

ICAC, VAC, and BGVC volumes, adjusted for age, sex, and population.

Rank-based partial correlations of intracranial vascular calcification volumes (ICAC, VAC, and BGVC) with calcification volumes in coronary and thoracic arteries, bone density, blood pressure, and physical activity (daily step count) were calculated, adjusted for age, sex, and population; given the large number of correlations assessed, false discovery rate–adjusted  $q$  values calculated with the Simes method<sup>43</sup> are reported.

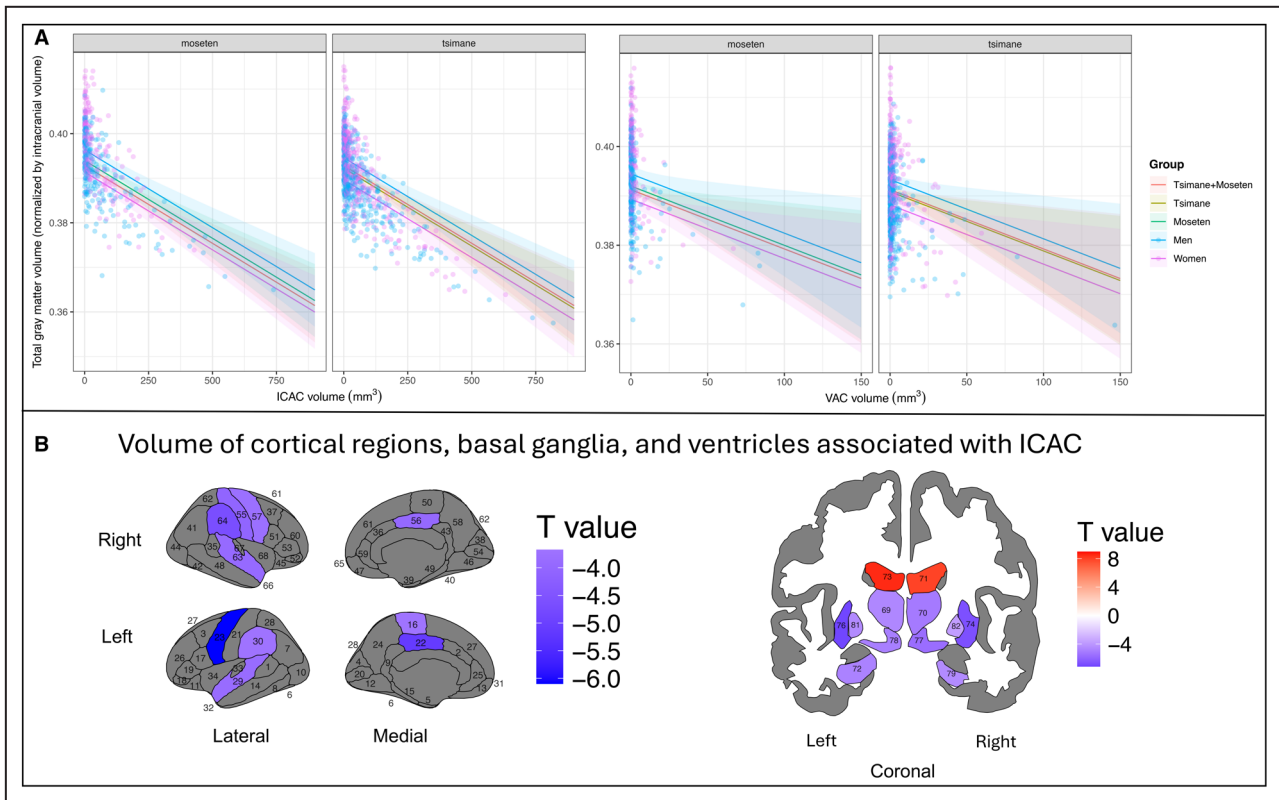
## RESULTS

### Study Population

A total of 1232 individuals, including 438 Moseten (208 women, 47.5%) and 794 Tsimane (384 women, 48.4%) individuals, were included in this study (Table 1). Tsimane individuals were slightly older than Moseten individuals (age,  $59.9\pm 10.3$  and  $55.9\pm 10.3$ ;  $P < 0.001$ ).

### Prevalence of Cerebrovascular Calcifications

ICAC was present in 1197 individuals (97.2%) (422 [96.3%] Moseten and 775 [97.6%] Tsimane individuals). A few of these calcifications were punctate and consistent with atherosclerosis, whereas the majority were annular and consistent with middle layer (medial



**Figure 1. Association of ICAC and VAC with brain atrophy.**

**A**, Predicted total gray matter volume from ICAC (left panels) and VAC volume (right panels), after controlling for age, sex, population, and total intracranial volume. Shaded areas indicate 95% CIs. **B**, ICAC volume was significantly associated with the volume of multiple brain regions. Only estimated T values for regions that remained statistically significant after correction for multiple comparisons (86 comparisons) are shown; nonsignificant regions are grayed out. Numbers within the assessed brain regions indicate the name of the corresponding region reported in Table S1. Statistically significant regions (region #): left paracentral (16), left and right posterior cingulate (22 and 56), left and right precentral (23 and 57), left and right superior temporal (29 and 63), left and right supramarginal (30 and 64), right postcentral (55), left and right thalamus (69 and 70), left and right lateral ventricles (71 and 73), left and right hippocampus (72 and 79), left and right putamen (76 and 74), left and right ventral diencephalon (77 and 78), and left and right globus pallidus (81 and 82). ICAC indicates intracranial carotid artery vascular calcification; and VAC, vertebral artery vascular calcification.

arterial calcification.<sup>44</sup> The median ICAC volume overall was 17.65 (IQR, 4.02–66.87) mm<sup>3</sup>. ICAC volume was significantly associated with age ( $\beta=2.53$  [SE, 0.18] mm<sup>3</sup> per year of age;  $P<0.001$ ) and population ( $\beta=-7.75$  [SE, 3.87] mm<sup>3</sup> for Tsimane individuals compared with Moseten individuals;  $P<0.001$ ) but not with sex [ $\beta=-1.79$  SE, 3.65] mm<sup>3</sup> for men compared with women;  $P=0.62$ ).

VAC was observed in 883 individuals (71.7%) (297 [67.8%] Moseten and 586 [73.8%] Tsimane individuals). The median VAC volume overall was 0.60 (IQR, 0.00–1.94) mm<sup>3</sup> and was significantly associated with age ( $\beta=0.017$  [SE, 0.005] per year of age;  $P<0.001$ ) and population ( $\beta=0.25$  [SE, 0.10] for Tsimane compared with Moseten individuals;  $P=0.015$ ) but not with sex ( $\beta=-0.15$  [SE, 0.10] for men compared with women;  $P=0.10$ ).

BGVC was observed in most individuals, 1212 (98.4%) (436 [99.5%] Moseten and 776 [97.7%] Tsimane

individuals). Based on their tubular morphology in coronal sections, we localized these calcifications to the lenticulostriate arteries rather than the parenchyma (Figure S1). The median BGVC volume was 6.38 (IQR, 2.38–15.68) mm<sup>3</sup> and was positively associated with age ( $\beta=0.11$  SE, 0.04] per year of age;  $P=0.003$ ) and population ( $\beta=2.81$  [SE, 0.78] for Tsimane compared with Moseten individuals;  $P<0.001$ ) but not sex ( $\beta=0.88$  [SE, 0.73] for men compared with women;  $P=0.23$ ).

### Association of Cerebrovascular Calcifications With Brain Atrophy

ICAC and VAC volumes were negatively associated with total gray matter volume ( $\beta=-46.4$  [SE, 6.6] mm<sup>3</sup>;  $P<0.001$ , and  $\beta=-159.6$  [SE, 60.2] mm<sup>3</sup>;  $P=0.008$ , respectively) after controlling for age, sex, population, and the total intracranial volume (Figure 1A). Subgroup analysis revealed that the relationship between VAC

**Table 2. Associations of Cerebrovascular Calcifications With Cognitive Impairment**

	Normal cognition, median (95% CI) (n=477)	MCI, median (95% CI) (n=48)	Dementia, median (95% CI) (n=4)	P value*
ICAC volume, mm <sup>3</sup>	64.4 (54.6–74.2)	90.6 (59.1–122.1)	127.7 (18.6–236.8)	0.18
VAC volume, mm <sup>3</sup>	0.79 (0.53–1.06)	2.28 (1.43–3.14)	23.37 (20.40–26.33)	<0.0001
BGVC volume, mm <sup>3</sup>	9.36 (7.80–10.93)	11.77 (6.75–16.79)	20.02 (2.62–37.43)	0.35

BGVC indicates basal ganglia vascular calcification; ICAC, intracranial carotid artery vascular calcification; MCI, mild cognitive impairment; and VAC, vertebral artery vascular calcification.

\*P values by quantile regression for continuous measures of ICAC, VAC, and BGVC volumes. All models are adjusted for age, sex, and population.

and total gray matter volume was observed in men ( $\beta=-150.4$  [SE, 67.6] mm<sup>3</sup>;  $P=0.026$ ), individuals aged >65 years ( $\beta=-172.9$  [SE, 62.2] mm<sup>3</sup>;  $P=0.006$ ), and Tsimane individuals ( $\beta=-172.9$  [SE, 62.2] mm<sup>3</sup>;  $P=0.006$ ), whereas no significant association was found between VAC and total gray matter volume in women ( $\beta=-175.7$  [SE, 121.7] mm<sup>3</sup>;  $P=0.149$ ), individuals aged <65 years ( $\beta=120.2$  [SE, 142.9] mm<sup>3</sup>;  $P=0.401$ ), or Mosen individuals ( $\beta=-151.0$  [SE, 110.3] mm<sup>3</sup>;  $P=0.172$ ). ICAC volume was also negatively associated with the mean cortical thickness ( $\beta=-0.37$  [SE, 0.06] mm<sup>3</sup>;  $P<0.001$ ); similarly, a negative trend was observed for VAC ( $\beta=-0.98$  SE, 0.54] mm<sup>3</sup>;  $P=0.07$ ). In regional analysis, ICAC volume was significantly associated with atrophy of multiple gray matter regions, including the cortex in the frontoparietal area, the basal ganglia, and the hippocampus bilaterally, and with enlargement of the lateral ventricles (Figure 1B and Table S1). Similar results were obtained for cortical thickness (Figure S2 and Table S1). In the adjusted linear models assessing the association between regional atrophy and VAC volume, none of the regions survived multiple comparison correction. No significant associations with white matter volume were found.

**Table 3. Multinomial Logistic Regression Evaluating the Relationship Between Cerebrovascular Calcifications and Cognitive Status**

	Adjusted OR (95% CI)	P value
ICAC (per 20mm <sup>3</sup> )		
MCI vs normal cognition	1.04 (1.00–1.09)	0.075
Dementia vs normal cognition	0.99 (0.87–1.13)	0.88
VAC (per 20mm <sup>3</sup> )		
MCI vs normal cognition	1.43 (1.00–2.04)	0.049
Dementia vs normal cognition	2.05 (1.5–4.01)	0.035
BGVC (per 20mm <sup>3</sup> )		
MCI vs normal cognition	1.02 (0.96–1.09)	0.49
Dementia vs normal cognition	1.05 (0.89–1.24)	0.57

ORs and 95% CIs adjusted for age, sex, and population. BGVC indicates basal ganglia vascular calcification; ICAC, intracranial carotid artery vascular calcification; MCI, mild cognitive impairment; OR, odds ratio; and VAC, vascular calcifications in vertebral arteries.

BGVC was not significantly associated with any brain atrophy measure in the overall model, although subgroup analysis revealed statistically significant associations in Mosen individuals, showing a negative relationship with total gray matter volume ( $\beta=-139.0$  [SE, 43.98] mm<sup>3</sup>;  $P=0.002$ ) and with white matter volume ( $\beta=-82.6$  [SE, 41.32] mm<sup>3</sup>;  $P=0.046$ ), which was not observed in Tsimane individuals (gray matter volume:  $\beta=-5.03$  [SE, 9.34] mm<sup>3</sup>;  $P=0.591$ ; white matter volume:  $\beta=11.5$  [SE, 9.52] mm<sup>3</sup>;  $P=0.228$ ).

### Association of Cerebrovascular Calcifications With Cognitive Impairment and Extrapyramidal Signs

Cerebrovascular calcifications were most notable among the small number of people diagnosed with dementia. VAC volumes significantly differed among the diagnostic groups ( $P<0.0001$ ; Table 2). After adjusting for age, sex, and population, individuals with VAC had significantly higher odds of being diagnosed with MCI (odds ratio, 1.43 [95% CI, 1.00–2.04] per 20mm<sup>3</sup> volume;  $P=0.049$ ) or dementia (odds ratio, 2.05 [95% CI, 1.50–4.01] per 20mm<sup>3</sup> volume;  $P=0.035$ ; Table 3). Adjusted associations of intracranial carotid artery and basal ganglia volumes were not associated with clinical cognitive diagnoses.

Greater ICAC volume was significantly associated with poorer immediate word recall ( $\beta=-0.0008$  [SE, 0.0003];  $P=0.023$ ), VAC volume was associated with poorer delayed word recall ( $\beta=-0.0174$  [SE, 0.0072];  $P=0.016$ ), and BGVC volume was significantly associated with poorer performance on the digit span forward ( $\beta=-0.0016$  [SE, 0.0005];  $P=0.002$ ; Table S2).

With regard to parkinsonian symptoms (Table 4), ICAC volumes were 2-fold higher in patients with tremor or rigidity, whereas BGVC volumes were 2-fold higher in patients with hypomimia.

### Association of Cerebrovascular Calcifications With Cardiovascular Calcifications

ICAC (but not VAC or BGVC) volume was significantly and positively correlated with calcifications in the coronary arteries and the thoracic aorta (adjusted  $\rho=0.19$

**Table 4. Estimated Vascular Calcification Volumes (in mm<sup>3</sup>) by Presence/Absence of Extrapyramidal Signs**

Extrapyramidal signs	Extrapyramidal signs absent calcification	Extrapyramidal signs present calcification	Adjusted median difference (95% CI)	P value
ICAC volume, mm <sup>3</sup>				
Tremor	N=492	N=51	N=543	
Median (IQR)	47.7 (12.3 to 113.6)	109.1 (33.2 to 186.6)	47.8 (18.3 to 77.4)	0.002
Bradykinesia	N=285	N=251	N=536	
Median (IQR)	50.5 (13.5 to 114.7)	50.5 (13.1 to 131.4)	0.9 (−18.4 to 20.2)	0.92
Hypomimia	N=489	N=47	N=536	
Median (IQR)	48.6 (12.8 to 114.6)	93.1 (26.7 to 173.4)	28.1 (−4.5 to 60.8)	0.09
Rigidity	N=361	N=180	N=541	
Median (IQR)	44.6 (12.4 to 107.4)	68.2 (15.8 to 157.1)	20.7 (0.81 to 40.67)	0.04
Parkinsonian gait	N=28	N=21	N=49	
Median (IQR)	29.7 (13.5 to 66.5)	78.4 (50.8 to 131.4)	24.1 (−24.5 to 72.8)	0.32
VAC volume, mm <sup>3</sup>				
Tremor	N=492	N=51	N=543	
Median (IQR)	0.6 (0.1 to 2.5)	0.7 (0 to 2.7)	−0.1 (−0.7 to 0.5)	0.72
Bradykinesia	N=285	N=251	N=536	
Median (IQR)	0.6 (0 to 2.1)	0.7 (0.1 to 3.5)	0.03 (−0.4 to 0.4)	0.87
Hypomimia	N=489	N=47	N=536	
Median (IQR)	0.7 (0.1 to 2.5)	0.6 (0 to 3.4)	−0.3 (−1.0 to 0.3)	0.36
Rigidity	N=361	N=180	N=541	
Median (IQR)	0.6 (0 to 2.2)	0.8 (0.07 to 3.6)	0.1 (−0.3 to 0.5)	0.56
Parkinsonian gait	N=28	N=21	N=49	
Median (IQR)	0.1 (0 to 1.6)	0.4 (0 to 1.3)	0.15 (−0.8 to 1.1)	0.76
BGVC volume, mm <sup>3</sup>				
Tremor	N=492	N=51	N=543	
Median (IQR)	8.0 (2.5 to 21.6)	5.9 (2.5 to 19.7)	−4.3 (−9.3 to 0.7)	0.09
Bradykinesia	N=285	N=251	N=536	
Median (IQR)	7.3 (2.8 to 20.3)	8.9 (2.4 to 24.2)	−2.0 (−5.2 to 1.2)	0.22
Hypomimia	N=489	N=47	N=536	
Median (IQR)	7.4 (2.5 to 20.1)	19.8 (5.2 to 31.1)	7.9 (2.8 to 13.0)	0.002
Rigidity	N=361	N=180	N=541	
Median (IQR)	7.3 (2.5 to 20.5)	10.1 (2.6 to 23.5)	0.8 (−2.3 to 4.0)	0.60
Parkinsonian gait	N=28	N=21	N=49	
Median (IQR)	8.9 (3.3 to 34.34)	5.0 (2.5 to 9.8)	−5.6 (−19.1 to 7.8)	0.40

Difference in calcification volumes by parkinsonian symptom absent vs present, estimated by quantile regression adjusted for age, sex, and population. BGVC indicates basal ganglia vascular calcification; ICAC, intracranial carotid artery vascular calcification; IQR, interquartile range; and VAC, vertebral artery vascular calcification.

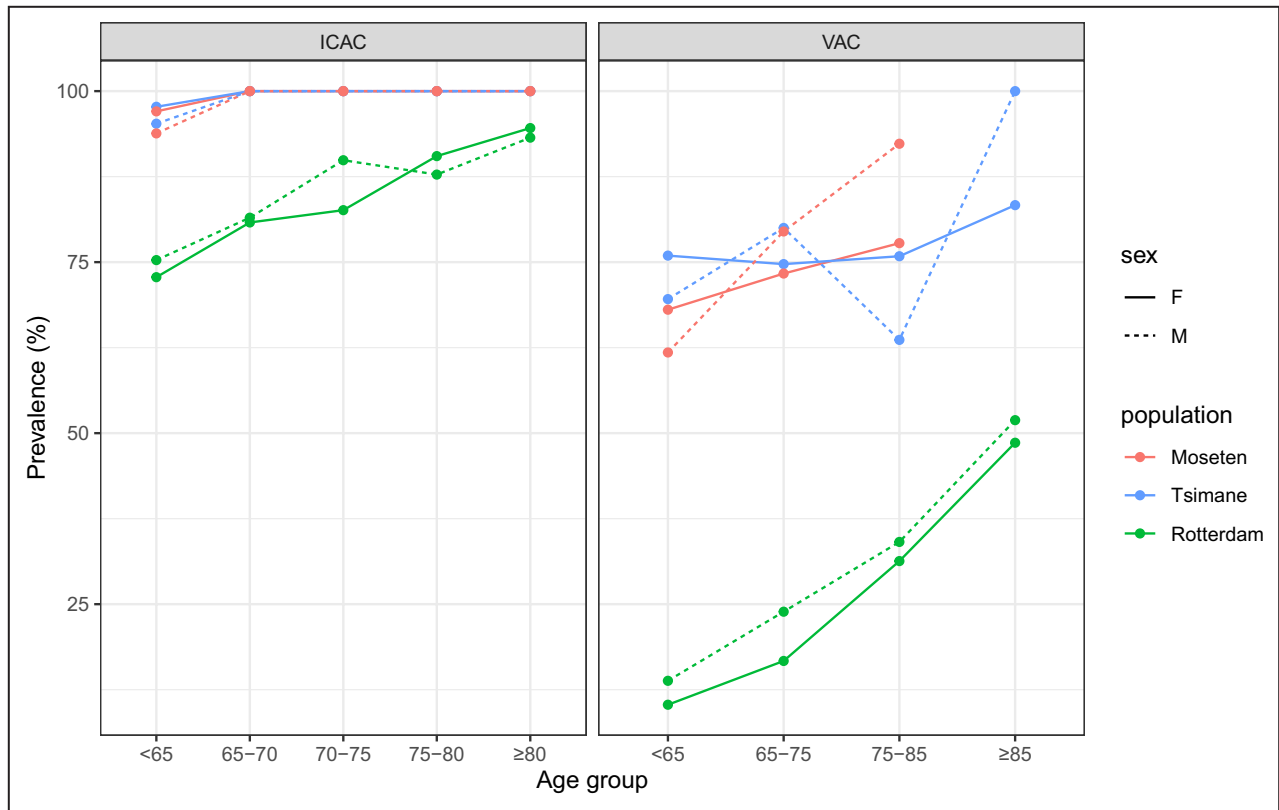
and 0.28, respectively; false discovery rate—adjusted  $q=0.0015$  for both; [Table S3](#)). Additionally, ICAC volume was significantly and negatively correlated with bone density in the thoracic spine (adjusted  $\rho=-0.19$ ,  $q=0.0015$ ) and daily step count (adjusted  $\rho=-0.11$ ,  $q=0.008$ ).

BGVC volume was positively correlated with body mass index (adjusted  $\rho=0.11$ ,  $q=0.001$ ) and inversely correlated with daily step count (adjusted  $\rho=-0.13$ ,  $q=0.001$ ).

No significant correlations were found with VAC volume.

### Comparison Between Cerebrovascular Calcification Prevalence in Amerindian Individuals and a European Population

Prevalence of ICAC (97.2%) and VAC (71.7%) in the 2 Bolivian Amerindian populations studied here is higher compared with that previously described in an elderly European population—based study (N=2495; mean age, 69 years), reporting 82.2%<sup>45</sup> and 21.0%<sup>46</sup> for ICAC and VAC, respectively. This difference was evident in both men and women across all age groups ([Figure 2](#)). Prevalence of BGVC in Tsimane and Mosen individuals is also higher



**Figure 2. Prevalence of ICAC and VAC in our Bolivian Amerindian cohort and the Rotterdam study cohort.**

Prevalence of ICAC (left panel) and VAC (right panel) according to age groups (x axis), sex (dashed line for women, solid line for men), and population (red lines for Moseten population, blue for Tsimane population, and green for Rotterdam study population). ICAC and VAC data for the Rotterdam study have been collected from Van Der Rijk<sup>45</sup> and van der Toorn,<sup>46</sup> respectively. ICAC indicates intracranial carotid artery vascular calcification; and VAC, vertebral artery vascular calcification.

compared with that observed in another European prospective multicenter cohort study of patients at risk of cerebrovascular disease (N=1133; mean age, 67.4 years), where BGVC prevalence in patients with or without an established diagnosis of transient ischemic attack or ischemic stroke at discharge were 29.7% and 28.8%, respectively,<sup>47</sup> compared with 98% in the Tsimane and Moseten individuals. Demographic information of the cohorts included in these 3 European studies are reported in Table S4.

## DISCUSSION

In this population-based study analyzing intracranial vascular calcifications (as a proxy of intracranial arteriosclerosis on nonenhanced CT<sup>1</sup>) in 2 Bolivian Amerindian populations, we found that most subjects presented with vascular calcifications in the intracranial carotid arteries (97.2%) and basal ganglia (98.4%), and about 70% in the vertebral arteries. This prevalence is higher compared with that previously described in European older adult studies (82.2% for intracranial carotid arteries,<sup>45</sup> 21.0% for vertebrobasilar arteries,<sup>46</sup>

and 29.7% for basal ganglia<sup>47</sup>). This result is notable because participants represent a nonindustrial lifestyle with 5-fold lower prevalence of coronary artery calcium than industrial populations<sup>13</sup> and low prevalence of metabolic syndrome<sup>14</sup> and CVRFs.<sup>13,15,16</sup>

Importantly, we observed that higher ICAC and VAC were associated with gray matter atrophy. In regional analyses, ICAC was associated with atrophy in regions typically supplied by the middle cerebral artery, the largest terminal branch of the internal carotid artery, suggesting a direct downstream effect. We did not observe similar regional relationships between VAC and atrophy in regions supplied by the posterior cerebral artery, possibly due to the relatively smaller number of VACs detectable in this population (mean VAC volume was <5mm<sup>3</sup>), which at that level might be insufficient for contributing to pathological changes in specific brain regions.

Higher cerebrovascular calcification volumes were observed in individuals with MCI or dementia. Higher ICAC and BGVC volumes were also associated with extrapyramidal signs, particularly tremor, rigidity, and hypomimia, which typically are associated with basal ganglia network dysfunction.

Significant associations with cognitive test scores were consistent with the brain regions potentially affected by vascular calcifications. For example, the caudate nuclei and putamen are important for the digit span task,<sup>48</sup> which we found inversely associated with BGVC. ICAC volume was inversely associated with immediate word recall, a task typically processed by the prefrontal cortex, which in turn is fed by the main branches of the internal carotid arteries, that is, the anterior and middle cerebral arteries. Conversely, calcifications in the vertebral arteries may impair the posterior cerebral circulation downstream, which is critical for the posterior portion of the medial temporal lobe that is involved in the delayed word recall task and other episodic memory functions.<sup>49</sup>

Putative mechanisms through which intracranial arteriosclerosis may lead to brain damage are known and include a direct stenosis of the blood vessel lumen by calcified plaques and impairment of the vascular reactivity and hemodynamics, such as increased arterial stiffness, which reduces the ability of the arteries to dilate in response to increased demand for blood flow, resulting in hypoperfusion.<sup>50</sup>

However, the factors contributing to the development of intracranial arteriosclerosis in this population remain unclear. One hypothesis pertains to the high level of infections and inflammation that have been documented in this population.<sup>51</sup> Chronic parasitic infections are very common in subsistence populations, and it is possible that these vascular calcifications may represent a subclinical manifestation of infections and of their subsequent chronic inflammatory status.<sup>52</sup>

We found no correlation between cerebrovascular calcifications and blood pressure (Table S3), suggesting the limited influence of this CVRF on the measured burden of intracranial arteriosclerosis. Consistently, we observed no difference in cerebrovascular calcifications between women and men, in contrast with previous studies in European older adults reporting lower ICAC and VAC in women than men,<sup>45,46</sup> which was interpreted as a lower arteriosclerotic vulnerability to conventional CVRFs related to the protective effect of endogenous female sex steroids.<sup>53–55</sup> The lack of sex-related differences in our cohort further supports the hypothesis that the development of intracranial vascular calcifications in these Amerindian populations might not depend on traditional CVRFs.

We also noted an inverse correlation between ICAC and bone density in the thoracic spine, in agreement with previous studies in industrialized populations reporting a link between vascular calcifications and bone health.<sup>56–58</sup> Despite evidence suggesting that biological factors associated with bone metabolism may promote vascular calcifications, to date it remains unclear whether the co-occurrence of vascular calcification and osteoporosis could be biologically linked.<sup>57</sup>

In our cohort, ICAC correlated with calcifications in the coronaries and thoracic aorta, in agreement with previous studies in industrialized populations showing significant correlations for calcifications in different vascular beds.<sup>46,59,60</sup> However, the correlation coefficients were relatively low and were not significant for VAC and BGVC; hence, location-specific differences in the development of arteriosclerosis may exist.

Finally, we noted significant differences in ICAC, VAC, and BGVC between Tsimane and Mosesten individuals, with Tsimane individuals estimated to have around 25% lower ICAC and 50% higher VAC and BGVC than Mosesten individuals. These vessel-specific differences further suggest that distinctive pathogenetic mechanisms may exist for these intracranial vascular calcifications. This finding might be related to dissimilarities in diet and lifestyle between these 2 ethnic groups. In fact, despite sharing a language and close genetic ancestry, Tsimane and Mosesten individuals differ in their level of acculturation and access to the market economy, clean water, and health care because of their different relationship and integration with the Bolivian society.<sup>11,23</sup> For example, while both Tsimane and Mosesten diets are characterized by similar low levels of fat and salt, the Mosesten people rely more on domesticated foods and purchased meat than wild fish and game, and consume >3 and 5 times higher daily intake of sugar and cooking oil, respectively, compared with the Tsimane people.<sup>11</sup> The nutrition transition observed in the Mosesten people mirrors a global phenomenon and has the potential to dramatically change the epidemiology of several noncommunicable chronic diseases, including arteriosclerosis, diabetes, and dementia.<sup>11</sup> Conversely, the Mosesten people's higher access to clean water and medical services may result in lower burden of infectious diseases compared with the Tsimane people.

Limitations of this study include the ability to assess exclusively vascular calcifications on nonenhanced CT scans, not vascular pathology per se, although previous autopsy studies showed that calcifications satisfactorily reflect the underlying arteriosclerosis burden.<sup>61,62</sup> Moreover, our quantitative measurements were not able to discriminate between medial versus intimal calcifications, although on visual inspection most of the calcification patterns appeared similar to what has previously been described as medial-type calcifications.<sup>44</sup> Characterization of other features of the calcified blood vessels (eg, stenosis or ulceration) requires more advanced imaging modalities such as contrast-enhanced CT and/or magnetic resonance imaging. The quantitative analyses using cortical volume and thickness obtained with the artificial intelligence algorithm (Figure 1B and Table S1) should be considered exploratory, given the current technical difficulties in obtaining highly accurate cortical measurements from

CT and the use of this algorithm, which is fairly new and not yet well established; nonetheless, it should be noted that if the assessed volumes were subject to random measurement error, then any associations with ICAC/VAC/BGVC volume will be downwardly biased toward the null (ie, associations would be biased toward 0). The small number of dementia cases, reflecting both low prevalence of dementia<sup>10</sup> and difficulty to recruit those dementia cases for imaging, may in turn underestimate the investigated associations. The cross-sectional nature of this study prevented establishing a cause-and-effect relationship.

Strengths of our study include the large population size, the population-based setting, and the consistency in the methodology used for the quantitative assessment of the calcifications.

In conclusion, we observed a high prevalence of ICAC, VAC, and BGVC in 2 Amerindian populations of the Bolivian Amazon, which are higher than what is reported in population-based European studies. ICAC is associated with brain atrophy, neurological symptoms, calcifications in coronary arteries and thoracic aorta, and lower bone density. Our results suggest that intracranial vascular calcifications might represent a neglected or overlooked indication of pathology that may have important chronic clinical implications for brain health and the development of cognitive impairment and extrapyramidal disorders, despite being incidental, silent, and asymptomatic per se.

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## Disclosures

None.

## Supplemental Material

Tables S1–S4

Figures S1–S2

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