Brief Report

Association of Low Lysosomal Enzymes Activity With Brain Arterial Dilatation

A Pilot Study

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Background and Purpose—Absent or diminished α-galactosidase A (GLA) and acid α-glucosidase (GAA) enzyme activity are core features of Fabry and Pompe disease, respectively. Patients with Fabry or Pompe disease may have dilated intracranial arteries but whether lower GLA or GAA enzyme activity relates to brain arterial dilatation in other populations is unknown.

Methods—Participants included Parkinson disease patients and nonblood-related controls, whose GLA and GAA enzymatic activities were measured in dried blood spots. Independent readers measured the axial arterial diameter of the ascending portion of the cavernous internal carotid arteries and the most proximal segment of the basilar artery in T2 black voids. Linear regression models were built to investigate the relationship between brain arterial diameters and lysosomal enzymatic activities.

Results—The cohort included 107 participants (mean age, 66.5±10.3; 67% men). In an adjusted linear regression model, lower GLA activity was associated with larger brain arterial diameters (B=0.50±0.23, *P*=0.03). The strength of association was the greatest for the basilar artery diameter (B=0.80±0.33, *P*=0.02). Similarly, lower GAA activity was associated with an increased basilar arterial diameter (B=0.73±0.35, *P*=0.04).

Conclusions—Lower GLA and GAA enzymatic activities were associated with larger brain arterial diameters, particularly the basilar artery diameter. Lower lysosomal enzymatic function in patients without Fabry or Pompe disease may play a role in brain arterial dilatation. **(*Stroke*. 2018;49:00-00. DOI: 10.1161/STROKEAHA.118.021964.)**

Key Words: dilatation ■ hypertension ■ inflammation ■ lysosomes ■ sphingolipids

Brain arterial dilatation is associated with higher risk of death, vascular events, and cognitive decline.[1,2] Acquired (eg, hypertension and smoking) and congenital risks (eg, Marfan or Ehlers-Danhlos syndromes) have been associated with brain arterial dilatation.[3]

Two lysosomal storage diseases, Fabry and Pompe disease, are also associated with brain arterial dilatation.[4,5] In Fabry disease, a deficiency of α-galactosidase A (GLA) leads to a buildup of sphingolipids that triggers pathogenic responses including inflammation, ischemia, fibrosis, and hypertrophy.[6,7] In Pompe disease, a deficiency of acid α-glucosidase (GAA) leads to progressive accumulation of lysosomal glycogen storage in heart, skeletal, and smooth muscle.[8] Because both enzymes are linked to diseases associated with brain arterial dilatation, we tested the hypothesis that GLA or GAA enzymatic activities are reduced in people with larger brain arterial diameters, in individuals whose enzymatic activities are above clinical criteria for Fabry or Pompe disease.

Methods

The data that support the finding of this study are available from the corresponding author on request. Participants included consecutive Parkinson disease patients and nonblood-related controls from Columbia University Medical Center who participated in a genetic-biomarker study.[9] The study was conducted under the approval of the institutional review board. All participants signed written informed consent. History of vascular risk factors was self-reported. Enzymatic activities of 5 lysosomal enzymes—glucocerebrosidase, acid sphingomyelinase, GLA, GAA, and galactocerebrosidase—were measured using a multiplex assay by Genzyme/Sanofi[10] in dried blood spots as previously described (Figure [B]).[11]

We measured the diameters of the basilar artery (BA) and the right and left internal carotid artery using the local picture archiving and communication system measuring tool. The longest axial diameter of

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each arterial black void was measured first. A second measurement was taken perpendicular to the first to account for naturally occurring variation in arterial curvature. Both measurements were averaged to obtain the axial diameter of each of the 3 arteries (Figure [A]). The interreader intraclass correlation coefficient was 0.74 for the right internal carotid artery, 0.67 for the left internal carotid artery, and 0.93 for the BA.

Statistical Analysis

Enzymatic activity was expressed first in µmol/L per hour and then adjusted for a batch effect.[12] The distribution of each enzyme was inverted and log-transformed. We ran collinearity diagnostics with the 5 lysosomal enzymes used for this study and found tolerance of <0.83 in all cases, variance inflation of <1.58, and a condition index of <14. Based on these, we analyzed the lysosomal enzymes individually and then together in 1 regression model. We created models with progressive adjustment for possible confounders and tested for nonlinear association using generalized additive models. The statistical analysis was performed with SAS software, version 9.4 (SAS Institute Inc, Cary, NC).

Results

The study included 107 participants, 98 Parkinson disease patients, and 9 controls. The median age was 67. Seventy-two were men, and 102 were non-Hispanic white (Table 1). Thirty-five participants (32.7%) had hypertension, 11 (10.3%) had diabetes mellitus, 45 (42.1%) had dyslipidemia, and 34 (31.8%) had a prior history of smoking. The associations between demographics, cardiovascular risk factors, and brain arterial diameters are reported in Table I in the online-only Data Supplement.

The scatterplots of both GLA enzymatic activity and GAA enzymatic activity by brain arterial diameters suggested a linear association, with the steepest slopes noted for the BA. For both enzymes, association with the BA diameter remained significant after adjusting for multiple covariates (Table 2).

The strength of association between BA diameter and lower GAA and GLA activities was similar in men and women but appeared greater among participants without Parkinson disease and when the time between the brain magnetic resonance imaging and the blood measurement was >1 year apart (average time difference overall was 9 months).

Discussion

In this study, we found a significant association between lower GLA or GAA activities and BA dilatation, independent of demographic, and vascular risk factors (Table 2; model 2). The stronger association of lower GLA and

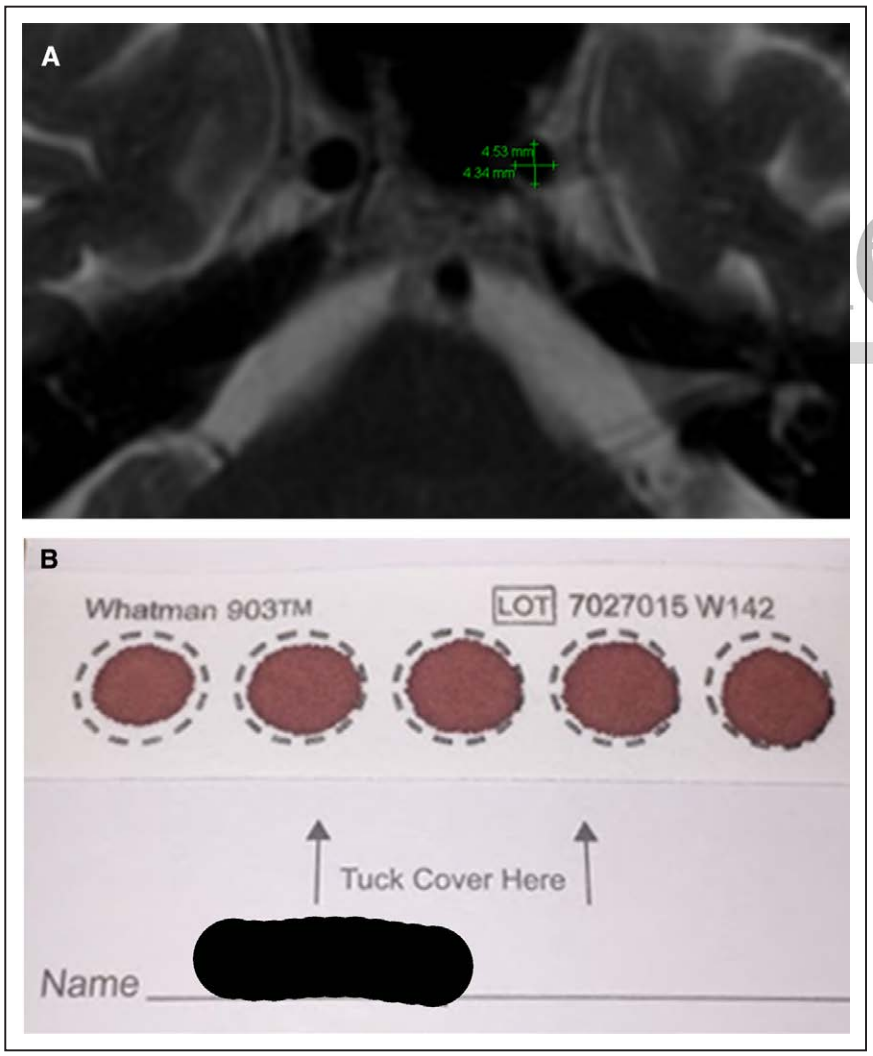

Figure. Example of the acquisition of brain arterial diameters (**A**; main outcome) and lysosomal enzymatic activity (**B**; main predictor). **A**, Green lines denote the left internal carotid artery. We averaged the 2 measurements to account for naturally occurring curvatures in brain arteries. **B**, We show an example of a dried blood spot card.

Table 1. Characteristics of the Studied Population (n=107)

Age, y, mean±SD, median, range	66.5±10.3, 67, 39–91
Men, n (%)	72 (67.29%)
Non-Hispanic white, n (%)	102 (95.33%)
Height, inches, mean±SD, median, range	67.7±3.8, 68.0, 59.0–76.0
Hypertension, n (%)	35 (32.71%)
Diabetes mellitus, n (%)	11 (10.28%)
Dyslipidemia, n (%)	45 (42.06%)
Smoking, n (%)	34 (31.78%)
Parkinson disease, n (%)	98 (91.59%)

GAA activities with BA dilatation, compared with internal carotid artery dilatation, could be because of differences in architecture between the anterior and posterior intracranial arteries, with posterior arteries containing less elastin, thinner vessel walls, and increased concentric intimal thickening.[13]

These results should be interpreted in the context of its strengths and limitations. We used a convenience sample of consecutive participants in a Parkinson biomarker study and data on cardiovascular risk factors was self-reported. Although subgroup analysis suggested an even greater association for controls (without Parkinson), a larger sample of controls should be tested. In addition, magnetic resonance imaging measurements were made using clinical scans from T2 black voids, which may not be as precise as time-of-flight magnetic resonance angiography or computed tomographic angiography to measure the actual luminal diameter. This lack of precision increases measurement error and type II error. Consequently, we may have underestimated the strength of the reported associations.

Table 2. Association Between Brain Arterial Diameters and Lysosomal Enzymatic Activities

	Average Carotid and Basilar Arterial Normalized Diameters	Average Carotid Arterial Normalized Diameters	Average Basilar Artery Normalized Diameter
	β-coefficient±SE, *P* value		
α-galactosidase A (inverted log-transformed)			
Model 0	0.49±0.23, *P*=0.04	0.34±0.29, *P*=0.25	0.81±0.31, *P*=0.01
Model 1	0.55±0.22, *P*=0.02	0.42±0.29, *P*=0.14	0.85±0.32, *P*=0.009
Model 2	0.50±0.23, *P*=0.03	0.36±0.29, *P*=0.21	0.77±0.33, *P*=0.02
Model 3	0.50±0.23, *P*=0.03	0.36±0.29, *P*=0.21	0.80±0.33, *P*=0.02
Acid α-glucosidase (inverted log-transformed)			
Model 0	0.30±0.25, *P*=0.24	0.14±0.31, *P*=0.65	0.61±0.33, *P*=0.07
Model 1	0.34±0.25, *P*=0.17	0.19±0.31, *P*=0.55	0.65±0.34, *P*=0.06
Model 2	0.36±0.25, *P*=0.15	0.20±0.30, *P*=0.52	0.68±0.34, *P*=0.05
Model 3	0.37±0.25, *P*=0.14	0.20±0.31, *P*=0.52	0.73±0.35, *P*=0.04

Model 0 was adjusted for the magnet strength of the magnetic resonance imaging (MRI), MRI vendor, and the duration between the brain MRI and the enzymatic activity reading. Model 1 was adjusted for model 0 plus age, sex, ethnicity, and height. Model 2 was adjusted for model 1 plus hypertension, diabetes mellitus, dyslipidemia, and smoking. Model 3 was adjusted for model 2 plus Parkinson disease.

Summary

We found a significant association between increased BA diameter and lower GLA and GAA enzymatic activities, which suggest a role for lysosomes in brain arterial dilatation in nonsyndromic populations. If these results are confirmed in larger samples, therapies aimed at modifying lysosomal enzymatic activity may conceivably be tested among patients with dolichoectasia.

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SUPPLEMENTAL MATERIAL

Supplementary Table I: Multivariate analysis of predictors of brain arterial diameters* in this sample			
Variable	**Beta Estimate**	**Standard Error**	**P value|**
Age (In years)	0.003	0.007	0.650
Male sex	0.134	0.196	0.495
Non-Hispanic white	0.165	0.334	0.621
Height	0.026	0.025	0.302
Hypertension	0.212	0.159	0.187
Diabetes	-0.051	0.239	0.831
Hypercholesterolemia	-0.339	0.144	0.021
Current smoking	0.181	0.156	0.247
Siemens versus not	-0.052	0.175	0.769
tesla1.5 versus 3.0	0.009	0.155	0.955

*Average carotid and basilar arterial normalized diameters