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Case Report

Extensive Vascular Mineralization in the Brain of a Chimpanzee (*Pan troglodytes*)

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Spontaneous vascular mineralization (deposition of iron or calcium salts) has been observed in marble brain syndrome, mineralizing microangiopathy, hypothyroidism, Fahr syndrome, Sturge–Weber syndrome, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, and calciphylaxis in humans and as an aging or idiopathic lesion in the brains of horses, cats, nonhuman primates, mice, rats, cattle, white-tailed deer, and dogs. Here we present a 27-y-old, adult male chimpanzee (*Pan troglodytes*) with spontaneous, extensive vascular mineralization localized solely to the brain. The chimpanzee exhibited tremors and weakness of the limbs, which progressed to paralysis before euthanasia. Magnetic resonance brain imaging in 2002 and 2010 (immediately before euthanasia) revealed multiple hypointense foci, suggestive of iron- and calcium-rich deposits. At necropsy, the brain parenchyma had occasional petechial hemorrhage, and microscopically, the cerebral, cerebellar and brain stem, gray and white matter had moderate to severe mural aggregates of a granular, basophilic material (mineral) in the blood vessels. In addition, these regions often had moderate to severe medial to transmural deposition of mature collagen in the blood vessels. We ruled out common causes of brain mineralization in humans and animals, but an etiology for the mineralization could not be determined. To our knowledge, mineralization in brain has been reported only once to occur in a chimpanzee, but its chronicity in our case makes it particularly interesting.

Spontaneous vascular mineralization (deposition of iron or calcium salts) has been reported to occur in the brains of both humans and animals. This symptom has been observed in marble brain syndrome, mineralizing microangiopathy, hypothyroidism, Fahr syndrome, Sturge–Weber syndrome, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, and calciphylaxis in humans[1,2,3,4] and as an aging or idiopathic lesion in the brains of horses, cats, nonhuman primates, mice, rats, cattle, white-tailed deer and dogs.[5,6,7, 8-11] Vessels of the internal capsule, globus pallidus, cerebellar dentate nucleus, and infrequently, hippocampus are affected preferentially in horses, cattle, and, less commonly, dogs.[7] Meningeal vessels in old cats, old horses, and cattle as well as vessels in the choroid plexus of old cats are other sites of vascular mineralization, but obvious ischemic damage is rarely associated with mineralization as a primary lesion in any animal species.[7] Here we present a chimpanzee (*Pan troglodytes*) with extensive vascular mineralization that was localized solely to the brain.

Case Report

This 27-y-old, adult male chimpanzee was born at the Yerkes National Primate Research Center. He was socially housed in outdoor-indoor enclosures in accordance with the *Guide for the Care and Use of Laboratory Animals*[12] and Animal Welfare Act and Regulations[13,14] and fed a low-fat, commercial primate diet (Monkey Diet Jumbo 5037, Purina Mills, St Louis, MO), with daily fruit and vegetable supplementation. In September 2010, approximately 13 d before he was euthanized, the chimpanzee presented with moderately decreased tone and strength of the left leg, and the thigh muscles of both legs were noted to be mildly atrophied. Subsequently, tremors of both arms (more prominent in the left arm) occurred over the next 7 d. On day 8, limb tremors continued, and impaired mobility and decreased appetite were present. By day 12, both hindlegs were flaccid, joints were lax, and muscles of both hindlimbs (thigh and calf) appeared atrophied. In addition, the chimpanzee had obvious motor deficits (severe wobbling when attempting to ambulate) in both legs, with the left leg apparently affected more severely. MRI performed on the same day revealed multiple radiolucent hypointense foci in the brain; some of the lesions were located in the motor cortex. The animal was unable to recover from anesthesia after imaging, and euthanasia was elected due to his deteriorating condition and poor prognosis.

The history of this chimpanzee at our facility included a behavioral study in which his motor skills and performance were measured.[15] In terms of motor skills, the chimpanzee had 2 noteworthy limitations. First, he was very reluctant to use his left hand for grasping small food items, which is very unusual because all chimpanzees typically are able to use either hand, with nearly equal effort, for grasping.[16] Second, he exhibited poor tool-use behavior. As enrichment, chimpanzees at the center are provided with artificial tool-use devices that

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require them to use small sticks for probing into a hole to extract food, a task designed to simulate termite fishing by wild apes. Of the 188 chimpanzees examined by using this task, this animal had the worst performance.[15] In addition, even though he did fairly well cognitively on a series of projects that measured his ability to solve tasks based on physical cues such as spatial memory, understanding how tools work, and quantity discrimination, this animal did relatively poorly on tasks that assessed his understanding of social cues, notably the following of gaze and comprehension of communicative signals. However, even though this chimpanzee understood social cues very poorly, it is important to understand that 27% of the 97 animals tested also failed these tasks, so his performance, although poor, was not an outlier.[17]

In 2002, as part of a survey of normal chimpanzee brain morphology, this animal underwent MRI on a 1.5-T machine. T1-weighted images collected in the transverse plane by using a gradient echo protocol revealed extensive, irregular territories of very low signal intensity in both hemispheres, which were concentrated in the gray and white matter of the cerebral cortex, basal ganglia, and internal capsule and the gray and white matter of the cerebellum (Figure 1). In 2010, the chimpanzee was scanned again immediately prior to euthanasia, by using a broader array of protocols including T1-weighted, T2-weighted, susceptibility-weighted, and MR angiographic imaging protocols. Scanning was done on a 3-T machine (Trio, Siemens, Deerfield, IL) in the Yerkes Imaging Center. The T1-weighted images were collected in the transverse plane with a gradient echo protocol. Susceptibility-weighted scans, which are especially sensitive to angiopathy, hemorrhage, deposition of iron-containing blood products (ferritin and hemosiderin), and calcification,[18,19,20] showed damaged tissue and iron- and calcium-rich deposits as hypointense foci. The scanning results were consistent with lesions resulting from micro bleeds. Axonal damage itself might have contributed to the hypointense signal. The susceptibility-weighted images showed dark regions that often corresponded to those on the 2002 T1-weighted series, although the affected areas were typically much more extensive on susceptibility-weighted images. Comparison of the T1-weighted images collected in 2002 and 2010 revealed that many of the same lesions were evident in both scans, although the amount of affected tissue appeared greater in the 2002 scans than in the later scans.

The levels of calcium, phosphorus, magnesium, vitamin D, parathyroid hormone, and folic acid in the blood samples collected from this animal immediately before euthanasia were within reference ranges. At necropsy, the animal was in good body condition with adequate fat depots. The brain appeared grossly normal, with a normal pattern of major cerebral fissures. Gross lesions in other organs included multiple mild patchy foci of fibrosis in the myocardium and occasional white streaks in the renal cortices. Intact brain and all the other tissue sections were collected in 10% neutral buffered formalin. After 2 wk of fixation, brain hemispheres were blocked. Visual inspections of the blocks revealed occasional, petechial hemorrhage. Examination of the corresponding regions in susceptibility-weighted images obtained by MRI revealed hypointensities matching the location of the hemorrhage, but additional territories of hypointense signal occurred in places that lacked obvious hemorrhagic foci (Figure 2).

Multiple brain sections from cerebrum, cerebellum, and brain stem corresponding to the lesions on MRI were processed for histopathologic analysis. Formalin-fixed specimens were embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. According to the results of the microscopic analysis, the sections of brain were treated with von Kossa (for mineral), Alizarin red (calcium), Congo red (amyloid), Masson trichrome (collagen), and Perls iron (ferritin or iron) stains.

Frequently, the blood vessels in the gray matter and occasionally in the white matter of the cerebral parenchyma had transmural aggregates of a granular, basophilic material (mineral) accompanied by extensive gliosis and loss of neuropil (Figure 3 A, B, and F). Moderate to severe expansion of the vascular wall due to deposition of mature collagen in the tunica media often was present in the cerebral gray matter and intermixed with axonal spheroids and aggregates of a brown pigment (hemosiderin; Figure 3 B and D). Similar lesions were present in the basal ganglia and cerebellum. Frequently, vessels in the choroid plexus had similar deposition of collagen, which often occluded the vascular lumen (Figure 3 C and E). Congo red failed to reveal any amyloid deposits in the brain. The gray matter of the thoracic spinal cord had focal extensive infarction intermixed with moderate number of gitter cells, swollen axons with distended myelin sheaths, and axonal spheroids (Figure 3 H). The lumbar spinal cord had small, rare foci of hemorrhage in the gray and white matter. Lesions in other organs included moderate multifocal interstitial nephritis with renal amyloidosis and mild myocardial fibrosis.

Genetic sequencing analysis was performed to rule out marble brain disease and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy in this animal, given that the carbonic anhydrase II and Notch 3 genes have been implicated in vascular mineralization in these conditions, respectively, in humans.[421,22] Sequencing primers were designed by using the whole-genome DNA sequence of the common chimpanzee (*Pan troglodytes*).[23] Exonic sequence data were obtained from the UCSC genome browser (http://genome.ucsc.edu/cgi-bin/hgGateway), and primers to span each exon were designed by using Primer3 (http://frodo.wi.mit.edu). Although differences between the sequenced subject and the chimpanzee reference genome (CSAC 2.1.4/panTRo4) were noted within the exons of the carbonic anhydrase II gene, none of the single-nucleotide polymorphisms led to a nonsynonymous amino acid change and thus was unlikely to be the causative factor for the observed phenotype. Given that the Notch 3 gene has 33 coding exons, we focused our search in areas already shown to be polymorphic in chimpanzees[23] and sequenced only exons 1, 2, 3, and 4. In addition, mutations in these exons have been associated with disease in humans.[22] No differences were observed between the coding regions of our subject and the reference chimpanzee genome in these regions. It is possible that variation within other regions of the Notch3 gene exists but has not been assessed to date.

Discussion

This case report presents severe, spontaneous, vascular mineralization in the brain of a chimpanzee. This lesion was first detected on MRI scans performed in 2002 and then again in 2010; these scans revealed extensive foci of mineralization in the brain without any associated clinical signs over the intervening 8 y. Interestingly, the amount of the affected tissue appeared larger in the 2002 T1-weighted series than in the 2010 T1-weighted

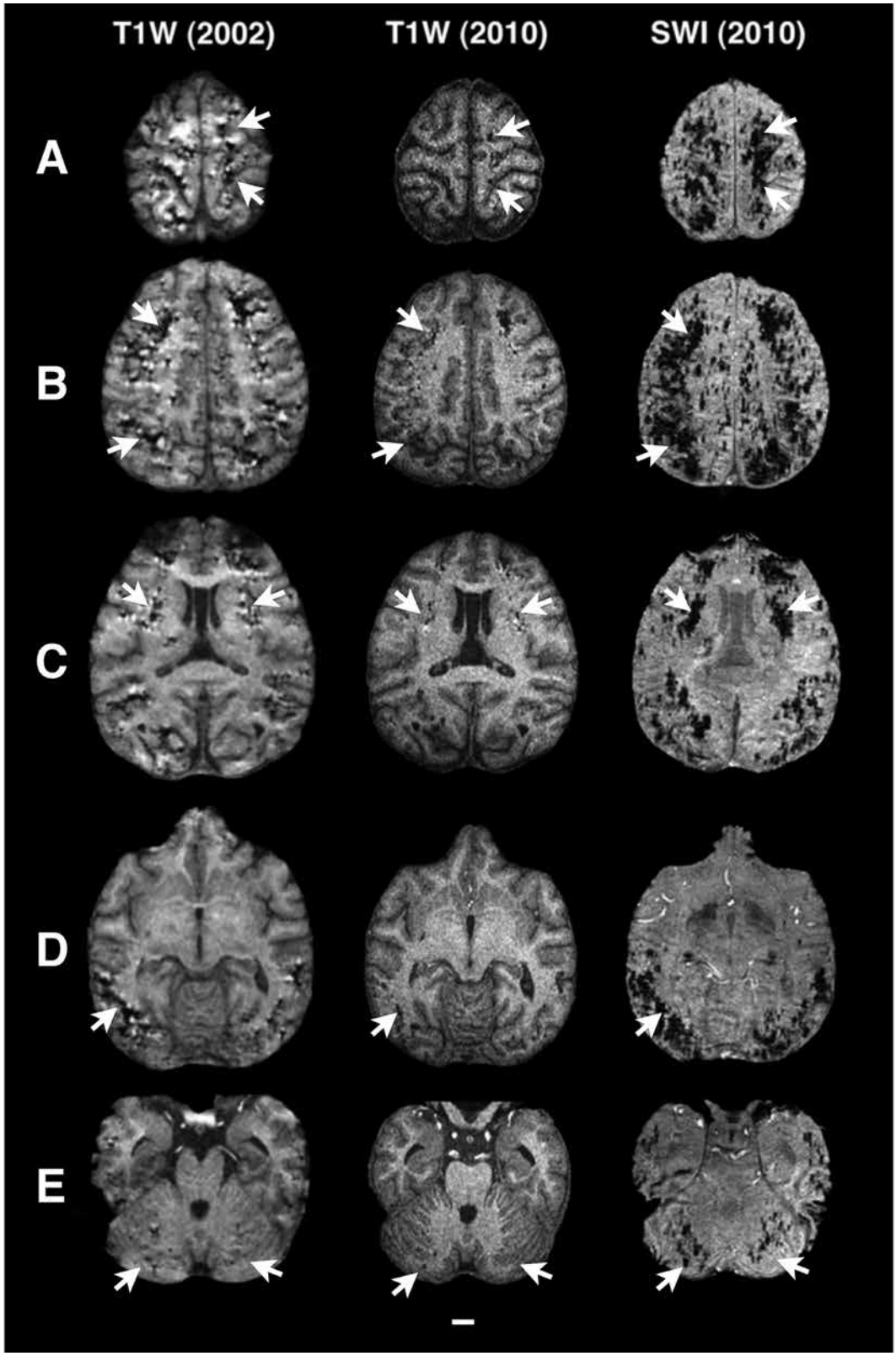

Figure 1. Series of axial MRI scans, including T1-weighted images taken in 2002 and T1- and susceptibility-weighted images taken in 2010, showing large territories of abnormal signal that appear dark in both T1- and susceptibility-weighted imaging. At each level, arrowheads denote the location of abnormalities in matched locations from the 2002 and 2010 T1-weighted and 2010 susceptibility-weighted scans. The abnormalities denoted with arrowheads are located in (A, B, D) the gray and white matter of the cerebral cortex, (C) the basal ganglia, and (E) the cerebellum. In the T1-weighted scans, the abnormal, dark territories appear to be larger in 2002 than in 2010. The susceptibility-weighted scans from 2010, however, show larger abnormalities than those in either of the T1-weighted series. Scale, 1 cm.

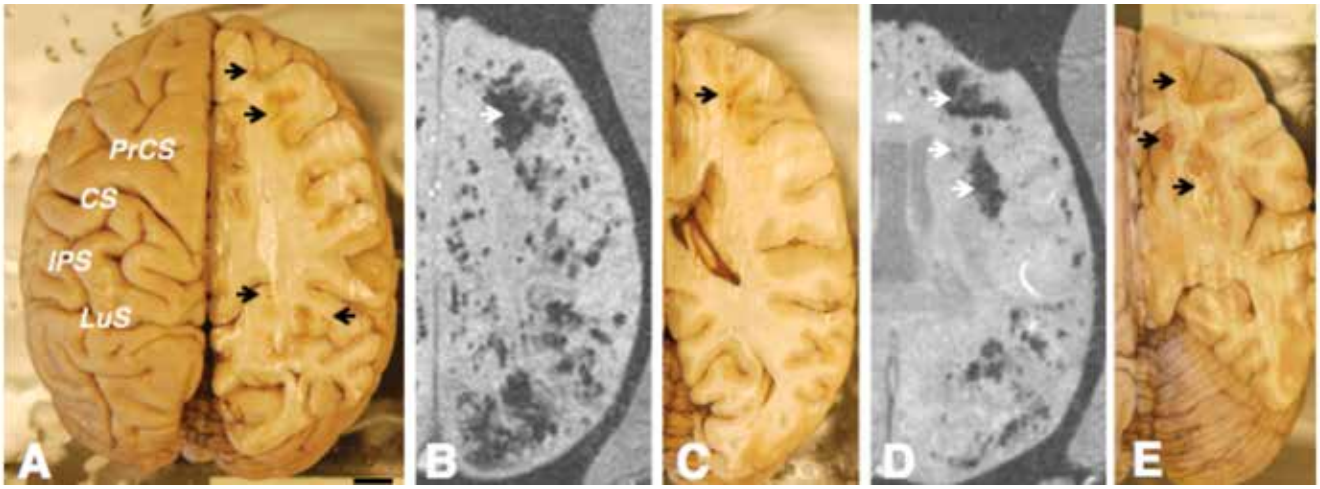

Figure 2. (A, C, E) Gross findings matched with (B, D) susceptibility-weighted images of the chimpanzee's brain. (A) The external morphology of the brain was unremarkable, showing the normal configuration of cortical gyri and sulci. On dissection, only small territories of blood could be seen, as denoted by the black arrowheads in panels A, C, and E. Susceptibility-weighted imaging, however, revealed far more extensive territories of abnormal tissue (white arrowheads in B and D), as well as additional territories of abnormal tissue that were not apparent from visual inspection. CS, central sulcus; IPS, intraparietal sulcus; LuS, lunate sulcus; and PrCS, precentral sulcus. Scale, 1 cm.

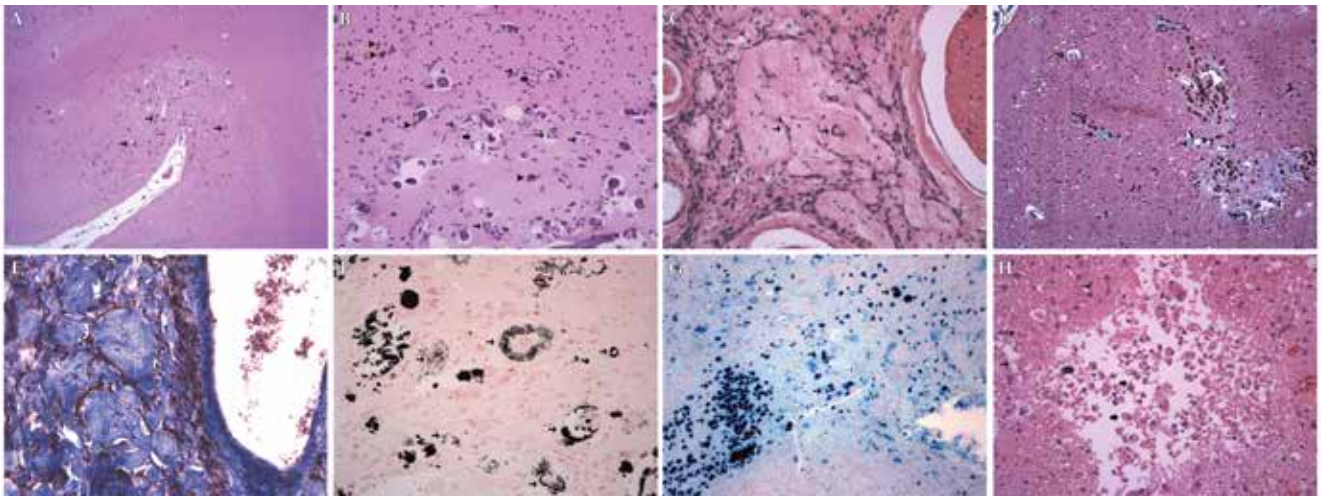

Figure 3. Histopathology. (A) Mostly gray and occasionally white matter in the cerebrum has extensive, basophilic, granular aggregates of mineral (arrows). (B) A higher magnification of the cerebral gray matter shows predominantly intravascular (thin arrows) and occasionally extravascular mineralization accompanied by severe medial to transmural deposition of mature collagen (block arrows) intermixed with hemosiderin deposits (arrowheads) and parenchymal loss. (C) Blood vessels in the choroid plexus with severe medial deposition of mature collagen, which often occludes the vascular lumen (arrows). (D) Blood vessels in cerebral gray matter showing vascular deposition of mature collagen. (E) Blood vessels in the choroid plexus with intravascular deposition of mature collagen. (F) Same section of cerebrum as in panel B with mineralization (arrows). (G) Cerebral gray and white matter showing hemosiderin deposits (arrows). (H) The gray matter of thoracic spinal cord shows focal extensive infarction intermixed with gitter cells (broad arrows), axonal spheroids and axons with distended myelin sheaths (thin arrows). Hematoxylin and eosin (A–C, H), Masson trichrome (D and E), von Kossa (F), and Perls iron (G) stains; magnification: 20× (A), 100× (D and G), and 200× (B, C, E, F, and H).

series, possibly due to cessation of the bleeding over the years or to resorption of some of the mineral deposits. The pattern of signal in the presented chimpanzee is highly unusual and has not been noted in any other chimpanzee that has been scanned at Yerkes Imaging Center. Therefore this animal's imaging characteristics were a subject of interest but did not stand out as being clinically significant.

Based on the animal's history, clinical signs, serology, pathology, and genetic analysis, we ruled out renal failure, hypoparathyroidism, Fahr syndrome, Sturge–Weber syndrome, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, and marble brain disease, which are associated with vascular calcification of the brain in humans. Hypercholesterolemia and hypertension are other factors associated with vascular calcification in humans.[24] However, even in the absence of any clinical sign, the mean total serum cholesterol level in captive chimpanzees at Yerkes is much higher than that seen in humans,[25] and our chimpanzee had normal cholesterol levels—in fact, levels that were low for his age group—so a high cholesterol level could not have accounted for the neural lesions. In addition, this animal did not exhibit hypertension, given that the blood pressure measurements

taken during routine annual examination were in the median to upper range of normotension.

The absence of vascular lesions in any organ except for the brain is perplexing and suggests that the etiology for the calcification was localized to the brain. In addition to the mural mineralization, the cerebral and choroid plexus blood vessels often showed moderate to severe deposition of mature collagen, a potential response to injury. This collagen abnormality could have caused vascular fragility, subsequently leading to nonfatal micro bleeds in the brain parenchyma, as evidenced by small, scattered foci of hemorrhage and aggregates of hemosiderin.

Considering this chimpanzee's limited grasping skill with his left hand as well as his very poor motor skills, the MRI and histopathology data are quite consistent. We expected that the lesions would be more severe in his right hemisphere, but that was not obvious from the scans, and the resolution of the scans did not allow us to quantify the magnitude of the lesions in each hemisphere. It is unclear why his grasping problems were largely unilateral, because the scans and microscopic analysis showed significant bilateral lesions in the primary and premotor cortex. These lesions were located both in the gray matter and in the white matter underlying the cortical regions involved in individual digit and motor control. Damage to these regions decreases neural innervations of the individual digits of the hand, leading to poorer motor skill.[26] Lesions also were apparent in the basal ganglia and cerebellum, and both of these regions are involved in motor learning and execution. Therefore, we consider that the chimpanzee's clinical problems, in terms of impaired motor skill and extreme lateralization, were consistent with the location of the lesions.

With only a single subject, it is difficult to confirm our clinical opinion, but our chimpanzee's case was unusual, and his performance on the tool use tasks was unusually poor (in fact, he had the worst performance in the colony). Furthermore, the fact that he would not use his left hand for certain prehensile grasping tasks was and is very unusual. To our knowledge, he is one of only 2 chimpanzees that have ever been unwilling or unable to use one of their hands for simple grasping tasks in the absence of any overt problems with the limb.[15] The other chimpanzee to show this phenotype had a lesion in the caudate nucleus of the hemisphere opposite to the location of the lesion.[15]

To our knowledge, vascular calcification of the brain in a chimpanzee has been reported only once previously. That case involved a 4-y-old chimpanzee with progressive neurologic dysfunction, cerebral atrophy, and leucoencephalopathy.[11] Our current case is remarkable in light of the chronicity of the vascular mineralization (at least 8 y), accompanied by deposition of mature collagen, without any apparent debilitating clinical signs. Therefore, we conclude that, as seen in humans,[27] the extensive vascular calcification could have impaired the animal's motor skills and caused his terminal grand mal seizures, but the infarct in the spinal cord was responsible for his progressive paralysis.

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References

1. **Boltshauser E, Wilson J, Hoare RD.** 1976. Sturge–Weber syndrome with bilateral intracranial calcification. J Neurol Neurosurg Psychiatry **39:**429–435.
2. **Jouvent E, Viswanathan A, Mangin JF, O'Sullivan M, Guichard JP, Gschwendtner A, Cumurciuc R, Buffon F, Peters N, Pachai C, Bousser MG, Dichgans M, Chabriat H.** 2007. Brain atrophy is related to lacunar lesions and tissue microstructural changes in CADASIL. Stroke **38:**1786–1790.
3. **Kobayashi S, Yamadori I, Miki H, Ohmori M.** 1987. Idiopathic nonarteriosclerotic cerebral calcification (Fahr's disease): an electron microscopic study. Acta Neuropathol **73:**62–66.
4. **Shanley DJ.** 1995. Mineralizing microangiopathy: CT and MRI. Neuroradiology **37:**331–333.
5. **Fankhauser R, Luginbuhl H, McGrath JT.** 1965. Cerebrovascular disease in various animal species. Ann N Y Acad Sci **127:**817–860.
6. **Hamir AN, Palmer MV, Kunkle RA.** 2008. Wasting and neurologic signs in a white-tailed deer (*Odocoileus virginianus*) not associated with abnormal prion protein. J Wildl Dis **44:**1045–1050.
7. **Storts RH, Montgomery DL.** 2001. The nervous system, 381-459.In: M. Donald McGavin, William W. Carlton, James F. Zachary. Thomson's Special Veterinary Pathology. St. Louis (Missouri): Mosby
8. **Yanai T, Masegi T, Ishikawa K, Sakai H, Iwasaki T, Moritomo Y, Goto N.** 1996. Spontaneous vascular mineralization in the brain of horses. J Vet Med Sci **58:**35–40.
9. **Yanai T, Masegi T, Ueda K, Manabe J, Teranishi M, Takaoka M, Matsunuma N, Fukuda K, Goto N.** 1993. Spontaneous globoid mineralization in the cerebellum of rats. J Comp Pathol **109:**447–451.
10. **Institute for Laboratory Animal Research.** 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academies Press.
11. **Zoller M, Grevot A, Matz-Rensing K, Hofmann P, Jurek V, Schulz-Schaeffer W, Kaup FJ.** 2007. Leucoencephalopathy with cerebral calcinosis in a young chimpanzee (*Pan troglodytes*)—a case report. J Med Primatol **36:**385–390.
12. **Institute for Laboratory Animal Research.** 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academies Press.
13. **Animal Welfare Act as Amended.** 2008.7 USC §2131–2159.
14. **Animal Welfare Regulations.** 2008. 9 CFR § 3.75–3.92.
15. **Hopkins WD, Russell JL, Schaeffer JA, Gardner M, Schapiro SJ.** 2009. Handedness for tool use in captive chimpanzees (*Pan troglodytes*): sex differences, performance, heritability, and comparison to the wild. Behaviour **146:**1463–1483.
16. **Hopkins WD, Russell JL.** 2004. Further evidence of a right-hand advantage in motor skill by chimpanzees (*Pan troglodytes*). Neuropsychologia **42:**990–996.
17. **Russell JL, Lyn H, Schaeffer JA, Hopkins WD.** 2011. The role of socio-communicative rearing environments in the development of social and physical cognition in apes. Dev Sci **14:**1459–1470.
18. **Sehgal V, Delproposto Z, Haddar D, Haacke EM, Sloan AE, Zamorano LJ, Barger G, Hu J, Xu Y, Prabhakaran KP, Elangovan IR, Neelavalli J, Reichenbach JR.** 2006. Susceptibility-weighted imaging to visualize blood products and improve tumor contrast in the study of brain masses. J Magn Reson Imaging **24:**41–51.
19. **Thomas B, Somasundaram S, Thamburaj K, Kesavadas C, Gupta AK, Bodhey NK, Kapilamoorthy TR.** 2008. Clinical applications of susceptibility-weighted MR imaging of the brain—a pictorial review. Neuroradiology **50:**105–116.
20. **Tsui YK, Tsai FY, Hasso AN, Greensite F, Nguyen BV.** 2009. Susceptibility-weighted imaging for differential diagnosis of cerebral vascular pathology: a pictorial review. J Neurol Sci **287:**7–16.
21. **Almstedt K, Lundqvist M, Carlsson J, Karlsson M, Persson B, Jonsson BH, Carlsson U, Hammarstrom P.** 2004. Unfolding a folding disease: folding, misfolding, and aggregation of the marble brain syndrome-associated mutant H107Y of human carbonic anhydrase II. J Mol Biol **342:**619–633.

22. **Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, Alamowitch S, Domenga V, Cecillion M, Marechal E, Maciazek J, Vayssiere C, Cruaud C, Cabanis EA, Ruchoux MM, Weissenbach J, Bach JF, Bousser MG, Tournier-Lasserve E.** 1996. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature **383:**707–710.
23. **Chimpanzee Sequencing and Analysis Consortium.** 2005. Initial sequence of the chimpanzee genome and comparison with the human genome. Nature **437:**69–87.
24. **Tang FT, Chen SR, Wu XQ, Wang TQ, Chen JW, Li J, Bao LP, Huang HQ, Liu PQ.** 2006. Hypercholesterolemia accelerates vascular calcification induced by excessive vitamin D via oxidative stress. Calcif Tissue Int **79:**326–339.
25. **Herndon JG, Tigges J.** 2001. Hematologic and blood biochemical variables of captive chimpanzees: cross-sectional and longitudinal analyses. Comp Med **51:**60–69.
26. **Nudo RJ, Jenkins WM, Merzenich MM, Prejean T, Grenda R**. 1992. Neurophysiological correlates of hand preference in primary motor cortex of adult squirrel monkeys. J Neurosci **12**:2918-2947.
27. **Loeb JA, Sohrab SA, Huq M, Fuerst DR.** 2006. Brain calcifications induce neurological dysfunction that can be reversed by a bone drug. J Neurol Sci **243:**77–81.