PROGRESSIVE BRAIN CALCIFICATIONS AND SIGNS IN A FAMILY WITH THE L9R MUTATION IN THE PDGFB GENE

Primary familial brain calcifications (PFBC) are a heterogeneous group of rare autosomal dominant disorders. Mutations in the PDGFB gene are the second most common cause of PFBC. A model for PDGFB-associated PFBC, hypomorphic PDGFB<ret/ret> mouse, displays impaired blood-brain barrier (BBB), progressive brain calcifications and increased flux of the oxysterol 24S-hydroxycholesterol from the brain into the circulation.1,2 Only 8 families and 2 sporadic cases with PDGFB mutations have been identified so far, one of them a Swedish-Finnish family previously described as F13.3–6 Very little is known about the natural history of PDGFB-associated PFBC. Here, we provide a comprehensive long-term follow-up of the F13 family.

Methods. The study was approved by the local ethics committee. The F13 family harbors the c.26T>G (L9R) mutation in the PDGFB gene1 (pedigree in figure e-1 at Neurology.org/ng). Participants consented to physical examination, cognitive assessment, radiologic studies, and biochemical analyses. Biomarkers for neuronal and BBB damage in plasma and CSF, including oxysterols, were analyzed at a single point.

Imaging. Dual-energy computed scans and brain MRIs were performed according to details provided in appendix e-1. The degree of calcification was measured with the Total Calcification Score (TCS).3 We also used software for image coregistration (Integrated Registration, GE AW server). In brief, the region of interest placed on the baseline CT is propagated in a semiautomated fashion to the follow-up examinations. Changes in Hounsfield units are measured as relative and absolute values.

Results. The progression of clinical and radiologic features is summarized in appendix e-1. Mean time of clinical follow-up was 5.5 years, and the time between 2 successive brain CT scans was 4.8 years.

Clinical features. In brief, all the affected had a diagnosis of migraine with aura and displayed subtle movement disorders and mild eye movement abnormalities. Patient III:1, the proband, was diagnosed with mild language impairment (anomia, paraphasias, and impaired repetition ability) and cognitive deficits (reduced working memory) and has developed chorea and posturing. Her father, II:3, has developed mild motor features and impaired tandem gait. Patient III:2 has a history of mixed substance abuse; he did not progress radiologically but has progressive chorea. Patient III:3 has mild postural tremor, chorea, and significant cognitive impairment (anomia, visuospatial deficits, reduced working memory and information processing speed). The main phenotype features are displayed in videos 1–4 and summarized in tables e-1 to e-4.

Imaging. Patient III:3 has calcifications in the lenticuliform nucleus and in the white matter. The 3 patients in generation III have widespread calcifications in the basal ganglia (lenticuliform and caudate nuclei) along with varying degrees of calcifications in the thalamus, dentate nucleus, and white matter (figure 1, figures e-1 to e-3, tables e-5 to e-9). Two patients have cortical calcifications (III:1 and III:3). Using the TCS, we detected progression in 2 cases (III:1 and III:3), whereas the coregistration method detected progression in 3 patients (II:3, III:1, and III:3). An estimation of hydroxyapatite concentration in calcified areas is displayed in figure e-4.

Biochemistry. We obtained CSF from 3 patients with patient III:2 having an elevated albumin CSF/serum ratio and patient III:3 elevated levels of neurofilament light chain (NFL) in the CSF. Oxysterol levels were nevertheless normal in all the examined participants (table e-10).

Discussion. Brain calcifications are progressive in 3 individuals from the L9R family. All the 4 participants display some degree of subtle but progressive motor features and mild eye movement abnormalities. Chorea emerged in 2 patients of generation III (III:1 and III:3) and was progressive in another (III:2). Besides chorea, patient III:1 had a mild language impairment and reduced working memory. Patient III:3 has greater cognitive deficits than the index case (III:1).

Progressive calcifications are mentioned in only one previous PDGFB mutation7; however, TCS was not provided. The progressive features and elevated CSF-NfL level in one of the cases support the notion

Supplemental data at Neurology.org/ng

Neurology.org/ng © 2016 American Academy of Neurology

© 2016 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.
that PDGFB-associated PFBC is a neurodegenerative disease. Unexpectedly, only 1 of the 3 patients had signs of an affected BBB. Likewise, the CSF level of the steroid acid 7α-hydroxyl-3-oxo-4-cholestenoic acid, a putative marker of increased BBB permeability, was normal in all the 3 patients. This is in contrast with the results in the hypomorphic PDGFB<ret/ret> mouse. Recent work in this model has demonstrated a more intact BBB in areas prone to calcifications.

The coregistration method we tested here found evidence of progression in 3 patients and the TCS method in 2. Overcoming the ceiling effect of TCS is the main advantage of coregistration. Small sample size is the main limitation of our longitudinal follow-up.

Two levels of penetrance exist in PFBC: one radiologic and one clinical. The radiologic penetrance in PDGFB mutations is high, but, despite calcifications, some individuals are asymptomatic. Reduced clinical
penetrance has been reported in 3 other PDGFB mutations despite the presence of brain calcifications (table e-11).

When brain calcifications will appear and whether their progression will plateau is unknown. Future work has to determine whether our findings can be generalized to other cases of PDGFB-associated PFBC or other forms of PFBC.

From the Department of Neurology (M.P., P.S.), Department of Neuroradiology (H.A., S.H.), Karolinska University Hospital; Department of Clinical Neuroscience (M.P., H.A., J.Y., S.H., P.S.), Division of Clinical Chemistry, Department of Laboratory Medicine (A.S., I.B.), Department of Neuromaging (G.B.), Karolinska Institute, Stockholm, Sweden; Department of Biochemistry (A.S.), Faculty of Medicine, University of Khartoum, Sudan; and St. Erik Eye Hospital (J.Y.), Stockholm, Sweden.

Author contributions: Dr. Paucar, Dr. Almqvist, Prof. Holmin, Prof. Ygge, and Med Dr. Bergendahl: study concept, data collection, and writing of the manuscript. Dr. Paucar wrote the first draft. Dr. Almqvist performed the radiologic evaluation. Prof. Björkhem and Dr. Saeed performed the oxysterol analyses and edited the manuscript. Prof. Svenningsson: study concept and editing of the manuscript.

Acknowledgment: The authors thank all participants, Dr. Ruth H. Walker, Dr. Erik Jensen, and speech therapist Liv Thalén.

Study funding: Stockholm County Council. Martin Paucar was supported by the Stockholm County Council (combined clinical residency and PhD training programs).

Disclosure: Dr. Paucar has received research support from the Stockholm County Council. Dr. Almqvist, Dr. Saeed, and Dr. Bergendahl report no disclosures. Dr. Ygge has served on the editorial board of Acta Ophthalmologica. Dr. Holmin has served on the scientific advisory boards of 2 companies not related to this study, holds a patent for Endolumina medical access device, has received research support from Astra Zeneca, holds a stock option for Smartwise Sweden AB, and has received royalty payments for potential future sales of products from Smartwise Sweden AB. Dr. Björkhem reports no disclosures. Dr. Svenningsson has served on the scientific advisory board of CBD Solutions AB, has served on the editorial board of PLoS One, and has received research support from the Swedish Research Council and ALF Stockholm. Go to Neurology.org/ng for full disclosure forms. The Article Processing Charge was paid by the authors. This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially.

Received May 3, 2016. Accepted in final form June 2, 2016.

Correspondence to Dr. Paucar: martin.paucar-arce@karolinska.se

1. Keller A, Westenberger A, Sobrido MJ, et al. Mutations in the gene encoding PDGF-B cause brain calcifications in humans and mice. Nat Genet 2013;45:1077–1082.
2. Nicolas G, Pottier C, Charbonnier C, et al. Phenotypic spectrum of probable and genetically-confirmed idiopathic basal ganglia calcification. Brain J Neurol 2013;136:3395–3407.
3. Nicolas G, Rovelet-Lecrux A, Pottier C, et al. PDGFB partial deletion: a new, rare mechanism causing brain calcification with leukoencephalopathy. J Mol Neurosci MN 2014;53:171–175.
4. Hayashi T, Legati A, Nishikawa T, Coppola G. First Japanese family with primary familial brain calcification due to a mutation in the PDGFB gene: an exome analysis study. Psychiatry Clin Neurosci 2015;69:77–83.
5. Keogh MJ, Pyle A, Daud D, et al. Clinical heterogeneity of primary familial brain calcification due to a novel mutation in PDGFB. Neurology 2015;84:1818–1820.
6. Saeed AA, Genové G, Li T, et al. Effects of a disrupted blood-brain barrier on cholesterol homeostasis in the brain. J Biol Chem 2014;289:23712–23722.
7. Vanlandewijck M, Lebouvier T, Andaloussi Mæ M, et al. Functional characterization of germline mutations in PDGFB and PDGFRB in primary familial brain calcification. PLoS One 2015;10:e0143407.