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

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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.^{1,3-6} 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 II:3 has calcifications in the lentiform nucleus and in the white matter. The 3 patients in generation III have widespread calcifications in the basal ganglia (lentiform 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.

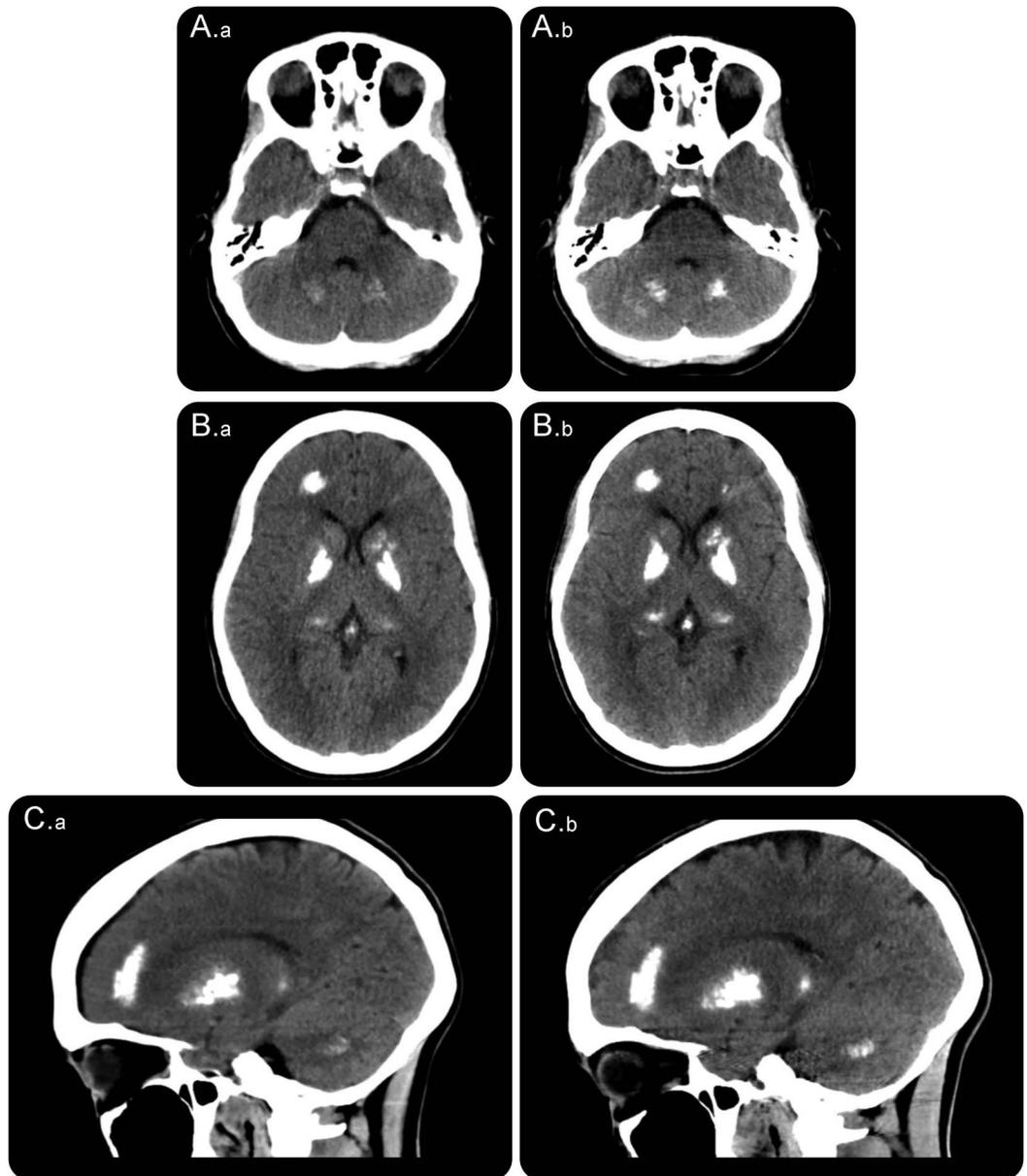
Biochemistry. We obtained CSF from 3 patients with patient III:2 having an elevated albumin CSF/serum ratio and patient III:3 elevated levels of neurofilament light chain (NFL) in the CSF. Oxysterol levels were nevertheless normal in all the examined participants (table e-10).

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* mutation⁵; however, TCS was not provided. The progressive features and elevated CSF-NFL level in one of the cases support the notion

Supplemental data
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Figure 1 Progressive calcifications in a family with the L9R mutation in the *PDGFB* gene



Density progression, as measured by change in Hounsfield units (HU), of calcifications in cerebellum, thalamus, basal ganglia, and frontal white matter in patient III:1. To the left axial (A.a and B.a) and sagittal sections (C.a) from the first CT scan done in 2009 and to the right sections performed in 2014 (A.b, B.b, and C.b). The sagittal sections display the right hemispheres of the brain and cerebellum (C.a and C.b). According to the TCS method, using the axial image could score the white matter changes as “moderate” but seem to be “severe” in the sagittal image. In the white matter (B.a and B.b), the HU increased by 142% or 124 HU, but the TCS remained unchanged at 3 points. In the lentiform nucleus (B.a and B.b), the HU increased by 29% or 70 HU, but the TCS remained also unchanged at 5 points. In the thalamus (B.a and B.b), the HU increased by 22% or 11 HU, but the TCS increased from 2 to 3 points. In the cerebellum (A.a and A.b), the HU increased by 44% or 17 HU and also increased in TCS from 2 to 3 points (for more details see table e-8).

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*^{het/het} mouse.² Recent work in this model has demonstrated a more intact BBB in areas prone to calcifications.⁷

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.

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