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MICROANGIOPATHY IN PRIMARY FAMILIAL BRAIN CALCIFICATION: EVIDENCE FROM SKIN BIOPSIES

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Autosomal dominant primary familial brain calcification (PFBC) is a rare cerebral microvascular calcifying disorder defined by the presence of calcifications affecting at least the basal ganglia with no secondary cause. It is associated with diverse symptoms including movement disorders, psychiatric disturbances, and cognitive impairment.[1] PFBC is caused by loss-of-function mutations in 2 groups of genes: (1) *PDGFB*, which encodes the platelet-derived growth factor B[2] and *PDGFRB*, which encodes its main receptor platelet-derived growth factor receptor–beta (PDGFR-β)[3] and (2) *SLC20A2* and *XPR1* encoding inorganic phosphate transporters.[4,5] Mice carrying *Pdgfb* hypomorphic alleles exhibit lower pericyte coverage in cerebral microvessels, blood-brain barrier (BBB) impairment, and cerebral microvascular calcifications.[2] Recently, a novel *PDGFB* mutation was reported in an Italian family with PFBC and white-matter hyperintensities (WMH).[6] Although brain calcification is a mandatory criterion for diagnosing PFBC, WMH were also reported as a major neuroimaging feature in the first described families with a *PDGFRB* or a *PDGFB* mutation[2,3] (table e-1 at Neurology.org/ng). To date, the precise nature of WMH remains unknown but may be regarded as resulting from microangiopathy. This led to the hypothesis that in mice, alterations of the microvessels leading to BBB impairment may be a causal mechanism between microangiopathy and vascular calcifications.[2] Transmission electron microscopy analysis of a skin biopsy from a patient belonging to the above-mentioned *PDGFB* family revealed thickened and fragmented areas in the basal lamina, consistent with microangiopathy.[6] We report here the results of skin biopsies performed in 2 patients carrying a *PDGFRB* and an *XPR1* mutation, respectively.

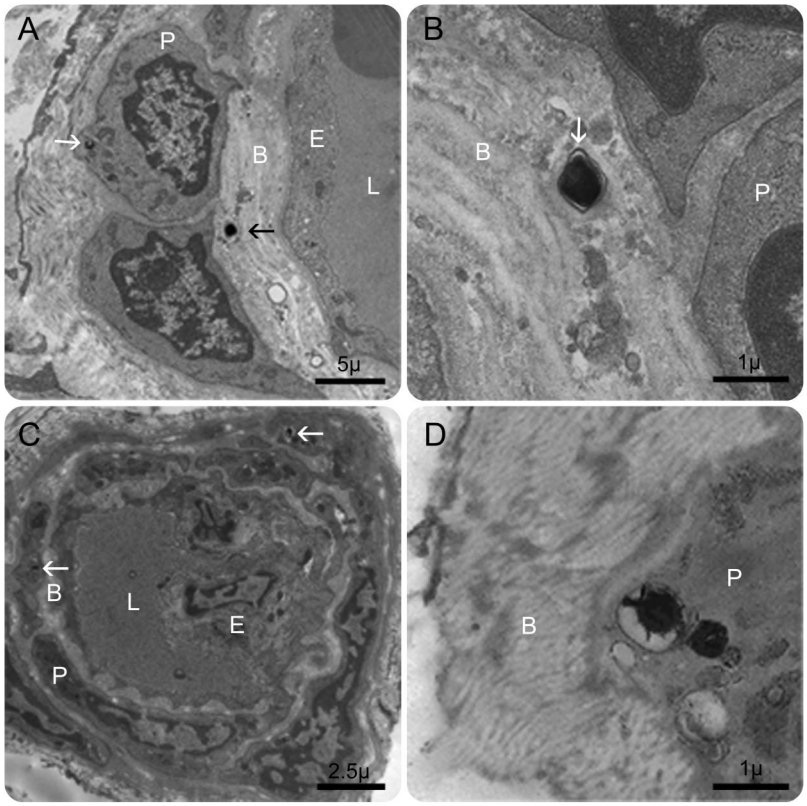
Methods. This study was approved by our institution's ethics committee. After having obtained written informed consent from the patients, punch biopsy was performed. Ultrastructural studies were conducted according to standardized protocols. Briefly, tissue samples were fixed in a 2% glutaraldehyde fixative solution, postfixed with osmium tetroxide, and embedded in resin epoxy. Semithin sections were stained with toluidine blue. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a Philips CM10 electron microscope.

Results. In the proband of the family with the p.Leu658Pro *PDGFRB* mutation,[3] biopsy analysis revealed no lesions of endothelial cells, whereas the basal lamina was thickened, and with microcalcifications within and around the pericytes and in the basal lamina (figure, A). These microcalcifications were sometimes included in double- or single-layered membranes in the vicinity of the basal lamina (figure, B). In another patient with a recently described p.Ser136Asn *XPR1* mutation,[5] microcalcifications were also observed within pericytes of the capillaries (figure, C) but remained located in the cytoplasm of the pericytes, laying under the plasma membrane (figure, D).

Discussion. We herein report hypodermal microvessel calcifications in skin biopsies from patients with PFBC. To our knowledge, only one skin biopsy analysis was previously reported in a patient with PFBC, who carried a *PDGFB* mutation.[6] As for the latter patient, the basal lamina appeared thickened in our patients, particularly in the *PDGFRB* mutation carrier, although no fragmentation was observed, indicating that *PDGFRB* and *XPR1* mutation carriers also exhibit microangiopathy. WMH on fluid-attenuated inversion recovery or T2-weighted MRI have also been observed in *SLC20A2* and *XPR1* mutation carriers (table e-1). These lesions are therefore not specific to *PDGFB* or *PDGFRB* mutation carriers in which BBB alteration was thought to be a prominent disease mechanism. Furthermore, similar thickening of the microvessel basal lamina on skin biopsies has also been observed in other leukoencephalopathies such as *COL4A1*-related disorders,[7] where different patterns of calcifications might be encountered in some cases. A summary of the literature review of vascular, clinical, imaging, and microvessel examination of skin biopsies found in PFBC and 2 other leukoencephalopathies (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy and *COL4A1*-related disorders) is provided in table e-1.

Supplemental data at Neurology.org/ng

Figure **Ultrastructural hallmarks of small capillary lesions in the 2 patients**



p.Leu658Pro *PDGFRB* mutation carrier (A, B): presence of microcalcifications in a pericyte (A, white arrow) and in the basal lamina (A, black arrow) (OM ×5,200), appearing to be membrane bound at a higher magnification (OM ×28,500) (B). p.Ser136Asn *XPR1* mutation carrier (C, D): small calcifications in the pericytes (C, white arrows) (OM ×2,650), sometimes located under the pericyte plasma membrane (OM ×28,500) (D). L = lumen; E = endothelial cell; B = basal lamina; P = pericyte; OM = original magnification.

Microangiopathy likely results from different mechanisms in the 2 groups of patients with PFBC and in other cerebral microangiopathies.

Whether BBB alteration is the cause of calcification in *PDGFB* or *PDGFRB* mutation carriers or not is currently debated. The original group who linked BBB deficiency in *PDGFB*-deficient mice and in humans with mutations in the same gene has recently shown that calcification-prone regions in *Pdgfb*$^{ret/ret}$ mice had a more intact BBB and higher pericyte coverage compared with calcification-nonprone brain regions.[8] Additional studies in *Slc20a2* knockout mice suggest that brain calcifications are found even with normal BBB structure and function, through a 2-hit mechanism whereby increased CSF inorganic phosphate leads to calcification in arteriolar smooth muscle cells due to an enhanced vulnerability caused by *Slc20a2* deficiency.[9] Although the existence of microangiopathy itself in the context of *PDGFB* or *PDGFRB* haploinsufficiency is not challenged by the recent mouse model report,[8] a putative direct causal link between microangiopathy and brain calcification is highly questioned. Additional studies on mice models might also evaluate the existence of microangiopathy outside the brain and if these models reproduce properly the phenotype variability found in patients.

Microvascular changes on skin biopsy are not specific to *PDGFB* mutation carriers. Systematic examination of skin biopsies of other patients with PFBC or differential diagnoses is warranted to replicate and explore these observations in depth. The significance of microangiopathy in both groups of patients and the mechanisms leading to microvascular changes in *XPR1* or *SLC20A2* mutation carriers remain to be determined, but further encourage to search for the potential pathways connecting the PDGFB/PDGFR-β response to the inorganic phosphate transporters SLC20A2 and XPR1.

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1. Nicolas G, Charbonnier C, de Lemos RR, et al. Brain calcification process and phenotypes according to age and sex: lessons from SLC20A2, PDGFB, and PDGFRB mutation carriers. Am J Med Genet B Neuropsychiatr Genet 2015;168:586–594.
2. 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.
3. Nicolas G, Pottier C, Maltete D, et al. Mutation of the PDGFRB gene as a cause of idiopathic basal ganglia calcification. Neurology 2013;80:181–187.

4. Wang C, Li Y, Shi L, et al. Mutations in SLC20A2 link familial idiopathic basal ganglia calcification with phosphate homeostasis. Nat Genet 2012;44:254–256.
5. Legati A, Giovannini D, Nicolas G, et al. Mutations in XPR1 cause primary familial brain calcification associated with altered phosphate export. Nat Genet 2015;47:579–581.
6. Biancheri R, Severino M, Robbiano A, et al. White matter involvement in a family with a novel PDGFB mutation. Neurol Genet 2016;2:e77.
7. Plaisier E, Gribouval O, Alamowitch S, et al. COL4A1 mutations and hereditary angiopathy, nephropathy, aneurysms, and muscle cramps. N Engl J Med 2007; 357:2687–2695.
8. Vanlandewijck M, Lebouvier T, Andaloussi Mae M, et al. Functional characterization of germline mutations in PDGFB and PDGFRB in primary familial brain calcification. PLoS One 2015;10:e0143407.
9. Wallingford MC, Chia J, Leaf EM, et al. SLC20A2 deficiency in mice leads to elevated phosphate levels in cerebrospinal fluid and glymphatic pathway-associated arteriolar calcification, and recapitulates human idiopathic basal ganglia calcification. Brain Pathol 2017;27:64–76.

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