REVIEW

Mechanisms of calcification in Fahr disease and exposure of potential therapeutic targets

Melissa E.M. Peters, MSc, Esther J.M. de Brouwer, MD, Jonas W. Bartstra, MD, Willem P.Th.M. Mali, MD, PhD, Huiberdina L. Koek, MD, PhD, Annemieke J.M. Rozemuller, MD, PhD, Annette F. Baas, MD, PhD, and Pim A. de Jong, MD, PhD

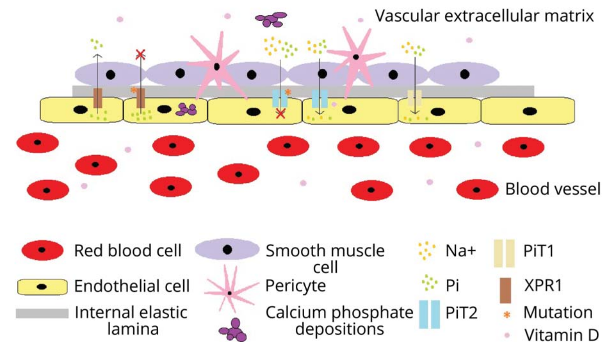
Correspondence
Dr. de Brouwer
E.J.M.deBrouwer-8@umcutrecht.nl

Neurology: Clinical Practice October 2020 vol. 10 no. 5 449-457 doi:10.1212/CPJ.0000000000000782

Abstract

Purpose of review

There is growing interest in disorders involved in ectopic mineralization. Fahr disease or idiopathic basal ganglia calcification can serve as a model for ectopic mineralization in the basal ganglia, which is fairly common in the general population. In this review, we will focus on causative gene mutations and corresponding pathophysiologic pathways in Fahr disease.



Recent findings

Patients with Fahr disease have a variability of symptoms, such as movement disorders, psychiatric signs, and cognitive impairment, but can also be asymptomatic. Fahr disease is mostly autosomal dominant inherited, and there are mutations found in 4 causative genes. Mutations in *SLC20A2* and *XPR1* lead to a disrupted phosphate metabolism involving brain-specific inorganic phosphate transporters. Mutations in *PDGFB* and *PDGFRB* are associated with disrupted blood-brain barrier integrity and dysfunctional pericyte maintenance. In addition, the *MYORG* gene has recently been discovered to be involved in the autosomal recessive inheritance of Fahr.

Summary

Knowledge about the mutations and corresponding pathways may expose therapeutic opportunities for patients with Fahr disease and vascular calcifications in the brain in general.

Fahr disease, also known as idiopathic basal ganglia calcification, is a rare autosomal dominant neurodegenerative disorder,[1,2] characterized by bilateral calcifications in multiple basal ganglia.[3–7] Its prevalence is estimated to be 4.5 per 10.000 persons.[8] Fahr disease has several synonyms, and bilateral striopallidodentate calcinosis or idiopathic basal ganglia calcifications may be preferable, as these terms give an accurate description of the anatomic location of the calcifications.[9] A distinction is made between Fahr disease and Fahr syndrome. Fahr disease is of idiopathic or genetic origin, whereas in Fahr syndrome, the calcifications are secondary to disorders of calcium metabolism. The most common causes of Fahr syndrome are hypoparathyroidism, pseudo-hypoparathyroidism, and pseudo-pseudo-hypoparathyroidism, but other states of chronic hypocalcaemia, e.g., vitamin D deficiency and renal failure, are associated with intracranial calcifications as well.[3,4,10]

Patients with Fahr disease may be asymptomatic or present with movement disorders such as parkinsonism, psychiatric disorders such as psychosis and depression, cognitive impairment, dementia, and a variety of other symptoms.[3,5,11–14] This variability in symptoms can partly be

Departments of Radiology (MEMP, JWB, WPTMM, PAdJ), Geriatrics (EJMdB, HLK), Pathology (AJMR), and Genetics (AFB), University Medical Center Utrecht, The Netherlands.

Funding information and disclosures are provided at the end of the article. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/cp.

Copyright © 2019 American Academy of Neurology

Copyright © 2020 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

explained by the division of the basal ganglia into dorsal and ventral systems. The dorsal striatum plays a role in motor and cognitive functions, whereas the ventral striatum plays a role in motivational functions.[3]

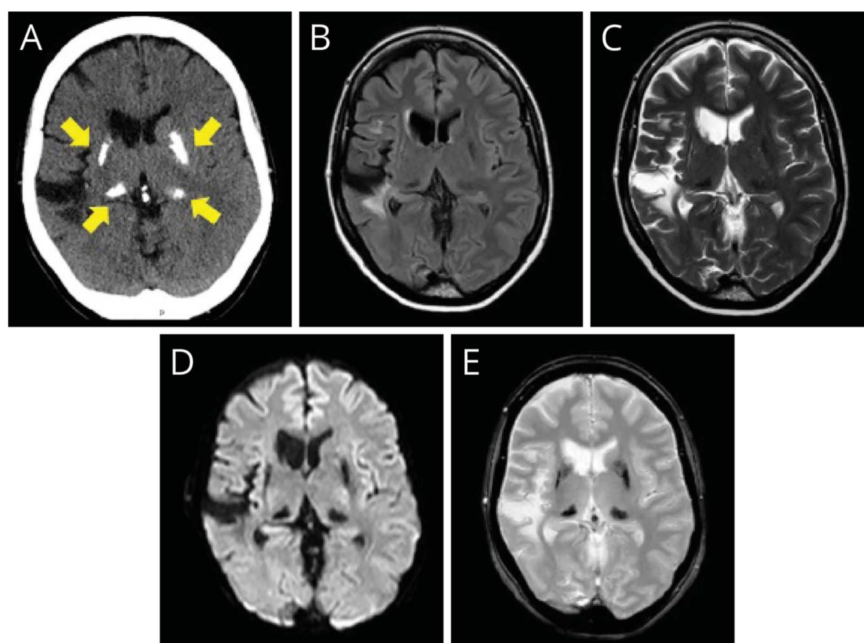
In patients with Fahr disease, normal serum levels of calcium, phosphate, alkaline phosphatase, and parathyroid hormone are measured.[9] The age at onset is mostly between 40 and 50 years,[4] but the symptoms can manifest at any age.[15,16] Patients who become symptomatic early in adulthood mostly develop psychiatric or cognitive disorders,[16] like psychosis,[17] whereas patients who become symptomatic later in life develop mainly movement disorders in combination with other clinical features.[16]

As Fahr disease is clinically heterogeneous, the diagnosis is based on neuroimaging in the absence of another explanation for calcification.[17] Nicolas et al.[18] developed a rating scale in which calcifications are scored from 0 (no calcification) to 5 (severe and confluent) for numerous locations in the brain (lenticular, caudate, thalamus nuclei, subcortical white matter, cortex, cerebellar hemispheres, vermis, pons, and medulla), which gives a total calcification score of 80 maximum. Calcifications are present as high-density areas on CT scans and can be more difficult to detect with conventional MRI sequences in early stages, although dedicated sequences are increasingly able to detect intracranial calcifications[19] (figure 1). The severity of calcifications on CT is correlated with an increase in age, and more calcifications were found in symptomatic patients in comparison with asymptomatic patients.[7,20] The calcifications seem to progress in a typical pattern from mild calcification of only the lentiform nucleus and caudate nucleus to severe and confluent calcification of all basal ganglia except the midbrain.[16]

On MRI, calcium deposition in Fahr disease is hard to distinguish from iron deposition in neurodegeneration with brain iron accumulations (NBIAs).[19,21] NBIA is a neurodegenerative disease characterized by the accumulation of iron in the basal ganglia and to a lesser extend in the substantia nigra and proximity. Patients with NBIA also present movement disorders and cognitive impairment.[21] CT imaging can be used to distinguish calcium and iron depositions, as calcium has a very high density on CT and iron depositions do not.[19] It is interesting that depositions in Fahr disease also contain iron according to histopathologic studies, and calcium deposition may be found on CT in patients with NBIA.[22,23]

There is currently no proven therapy for Fahr disease. Outside the field of neurology and neuroscience, there is growing interest in the etiology and treatment of ectopic mineralization disorders, especially in the cardiovascular system.[24–27] Much knowledge has been gained on several monogenetic disorders,[28] such as arterial calcification due to deficiency of CD73 (ACDC), and some suggestions for therapies are available.[25,29,30] Fahr disease may serve as a model for neurodegenerative diseases with basal ganglia calcifications. The pathophysiologic mechanisms may, to some extent, explain such disease in the general population. Data suggest that the prevalence of symmetrical calcification of the basal ganglia observed in neuroimaging is around 1% in young patients and >20% in elderly.[31,32] In autopsy, brain calcifications in the globus pallidus and dentate nucleus were seen in up to 70% because also microscopic calcifications are detectable in

Figure 1 CT and MRI of a patient with Fahr disease



(A) CT of a patient with Fahr disease, with clear calcifications in the basal ganglia seen as high dense white areas (indicated by yellow arrows). (B) Conventional T1-weighted fluid-attenuated inversion recovery MRI has lower sensitivity for calcifications than CT. (TR/TE 10000/140, flip angle 90). (C) T2-weighted turbo spin echo high-resolution MRI has lower sensitivity for calcifications than CT. (TR/TE 4748/100, flip angle 90). (D) Diffusion-weighted imaging MRI shows already better sensitivity for basal ganglia calcifications than the before-mentioned MRI protocols. (TR/TE 3413/99.4, flip angle 90, b value 1000). (E) Dedicated T2-weighed fast field echo MRI shows already better sensitivity for basal ganglia calcifications than the MRI protocols mentioned in B and C. However, CT is the most appropriate high sensitive neuroimaging method to demonstrate calcifications in the brain. (TR/TE 732/23, flip angle 18). (All above CT and MRI scans have a slice thickness of 5 mm). In addition, the presented MRI sequences are nonspecific in the differentiation between calcium and iron as it is seen in neurodegeneration with brain iron accumulations.

Copyright © 2020 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

histology.[33] Calcium deposits were found in the walls of arterioles and small veins and along pericapillaries and capillaries[3,9,14,34] (figure 2). We have observed similar deposits in the tail of the hippocampus in autopsy samples of patients without Fahr disease.[35]

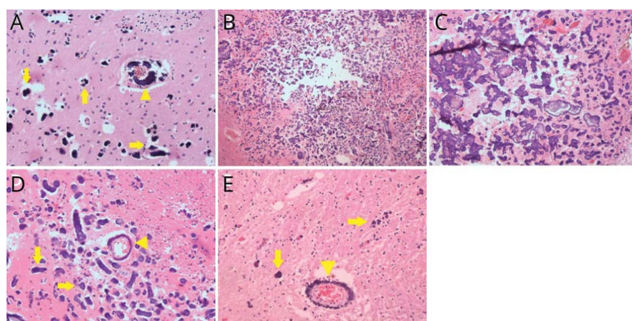
Genetics of Fahr disease

Fahr disease is autosomal dominant inherited. Since 2011, 4 causative genes have been identified. The first gene, Solute Carrier family 20 (Phosphate Transporter), Member 2 (*SLC20A2*), accounts for approximately 40% of the patients with Fahr disease.[14] Mutations in platelet-derived growth factor subunit B (*PDGFB*) and platelet-derived growth factor subunit receptor B (*PDGFRB*) account for 11% and 2% of the patients with Fahr disease, respectively.[6,7] Recently, mutations in xenotropic and polytropic retrovirus receptor (*XPR1*) were found[5] in approximately 2% of patients with Fahr disease. In half of Fahr cases, no mutations are found in one of these genes, and the cause is unknown.

In patients with a gene mutation, the penetrance is almost complete for calcium deposits.[36] However, the clinical penetrance is incomplete and may be around 70%. The penetrance can vary between families and within families. There are no precise numbers available for the different gene mutations.[18]

In this review, we will focus on the physiologic function of these 4 genes, the pathophysiologic pathways in Fahr disease, and summarize evidence from animal models. This might give more insights into its pathophysiology and might expose therapeutic opportunities for select patients with Fahr disease and patients with vascular calcifications in the brain in general.

Figure 2 Histology of calcification of different brain structures, which belong to the basal ganglia, of a patient with Fahr disease



(A) HE staining of calcification of the capillaries (arrows) and an artery (arrowhead) in the caudate nucleus-putamen, ×20 magnification. (B) HE staining overview of calcifications in capillaries and arteries in the globus pallidum. The dark purple staining indicates calcium, ×4 magnification. (C) ×10 magnification of the capillaries and arteries calcified in the globus pallidum; the dark purple staining indicates calcium. (D) Capillaries (arrows) and arteries (arrowhead) in the putamen, pallidum, and insula are calcified (HE staining), ×20 magnification. (E) Calcified artery (arrowhead) and capillaries (arrows) in the thalamus and subthalamicus (HE staining), ×20 magnification. HE = hematoxylin and eosin.

Solute carrier family 20 (phosphate transporter), member 2

SLC20A2 is localized to chromosome 8p11.21[37] and encodes for the type III sodium-dependent inorganic phosphate (Pi) transporter 2 (PiT2).[14] PiT2 is a transmembrane Na+/Pi cotransporter that plays an important role in the maintenance of Pi homeostasis, which is essential for adenosine triphosphate synthesis.[38–40] This transporter provides the transport of Pi from the CSF into the blood.[40] *SLC20A2* is expressed in many tissues. High expression levels were found in the brain, especially in the neurons, astrocytes, vascular smooth muscle cells, and vascular endothelial cells in the brain.[38,39] PiT2 is located in the cortex, basal ganglia (especially in the globus pallidus), and substantia nigra.[e1] This indicates that these brain tissues are more sensitive to an imbalance in Pi homeostasis.[14] Pi has essential functions in storage and release of metabolic energy, (bone) mineralization, nucleic acid synthesis, electrolyte transporter, and neurologic functions.[39] PiT2 contains 2 copies of the protein homology domain (PD001131), 1 in the amino-terminal (N-terminus) and 1 in the carboxy-terminal (C-terminus). These domains are highly conserved in plants and animals, which indicates that the role of these 2 domains is important in different species.[e2]

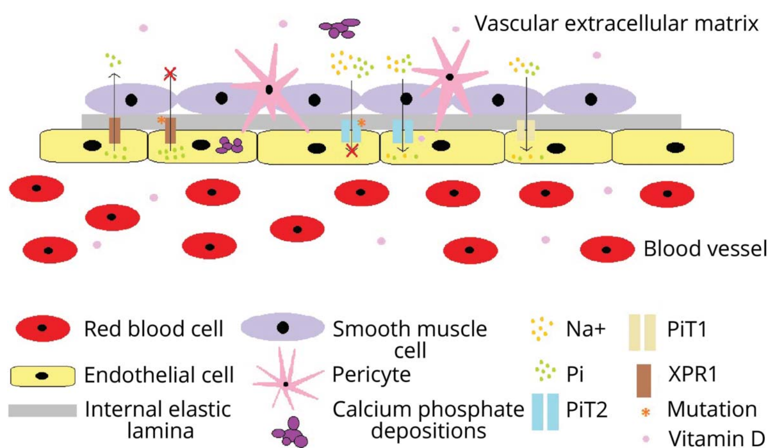
Gene mutations

Wang et al.[14] were the first to describe various missense mutations in the *SLC20A2* gene associated with Fahr disease. Thereafter, different other genetic studies were performed, which found mutations that mostly lead to a dysfunctional PiT2 protein.[14,e2–e7] Loss-of-function mutation in the *SLC20A2* gene is found in approximately 40% of patients with Fahr and causes the accumulation of Pi and formation of calcium phosphate depositions in the form of hydroxyapatite in the vascular extracellular matrix (figure 3). This occurs on tissue-specific locations where the PiT2 transporter is located.[e8] Genotype-phenotype correlation studies have shown that mutations in *SLC20A2* are significantly more associated with parkinsonism (in 21% of the cases in the study of Batla et al.)[e8] than with mutations in the other genes associated with Fahr disease.[e8] Higher levels of Pi were observed in CSF of especially *SLC20A2*-associated patients with Fahr disease, which suggest the possibility of Pi as biomarker for the diagnosis of this type of Fahr in patients.[e9]

Analysis of the role of several *SLC20A2* mutations showed the critical role in Na/Pi transport in Chinese hamster ovary (CHO) cells. When the amino acids Glu55 and Glu575, located in the transmembrane domains were exchanged by a glutamate or lysine, the phosphate transport was extremely reduced or stopped completely in CHO cells.[e4] When the amino acid Ser113, located in the protein homology domain of the N-terminus, and Ser593, located in the protein homology domain of the C-terminus, were exchanged by alanine, it led to a decrease in the phosphate transport in CHO cells by 10-fold.[e10] These results showed that *SLC20A2* is important for phosphate transport, and mutations associated with Fahr disease will lead to an extreme reduction of

Copyright © 2020 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

Figure 3 Mutations in the genes *SLC20A2* and *XPR1* are associated with a disrupted phosphate metabolism in patients with Fahr disease



On the left side of the figure, 2 *XPR1* transporters were illustrated. The left *XPR1* transporter shows the normal situation, whereby secretion of Pi from intracellular to extracellular is mediated. The right *XPR1* transporter shows a mutation (orange asterisk), and because of this mutation, the Pi efflux is disturbed. This leads to calcium depositions in endothelial cells. In the middle part of the figure, two PiT2 transporters were illustrated. The right PiT2 transporter shows the normal situation, whereby an influx of Pi and Na+ is shown. It is suggested that vitamin D could bind to the promoter of the *SLC20A2* gene in the nucleus[38] of endothelial cells and increase the PiT2 expression. The left PiT2 transporter shows a mutation (orange asterisk), which leads to a disturbed Pi influx. In some mutations, vitamin D cannot bind to the promoter of *SLC20A2*, which leads to less PiT2 expression. These mutations cause calcium depositions in the vascular extracellular matrix. The right part of the figure shows the function of a PiT1 transporter; it ensures Pi influx into the endothelial cell, just like *SLC20A2*. However, the *SLC20A1* gene is not directly associated with calcifications in Fahr disease. The transporters XPR1, PiT2, and PiT1 are not only located on the vascular endothelial cells as in this figure is shown. XPR1 is also located at vascular smooth muscle cells; PiT2 and PiT1 are also located at neurons, astrocytes, and vascular smooth muscle cells. For this figure, it has been chosen to show the transporters only on the endothelial cells.

phosphate import into the endothelial cells. High concentrations of phosphate remain in the vascular extracellular matrix and will cause vascular calcifications.[e8]

It has been suggested that *SLC20A2* might be regulated by vitamin D because the promotor contains a predicted vitamin D receptor binding site.[38] The role of vitamin D (calcitriol) in the suppression of calcification has been studied in vitro.[38] Calcitriol could upregulate *SLC20A2* mRNA expression but not *SLC20A1* or *XPR1* mRNA expression. High extracellular Pi concentration led to a strong decrease in *SLC20A2* and *SLC20A1* mRNA expression and a small decrease in *XPR1* mRNA expression in calcified cells. The reduction of the Pi transporters shows that cells anticipate the situation to maintain Pi homeostasis. In vitro treatment with calcitriol resulted in reduced calcification. To prove the hypothesis that *SLC20A2* is regulated by vitamin D, the gene *SLC20A2* was knocked down in SaOs2 (sarcoma osteogenic) cells, and subsequently, the cells were no longer protected for the effects of calcification by calcitriol, and the vitamin D–mediated inhibition of calcification was reduced[38] (figure 3).

Animal models

With biochemical and immunohistochemical analyses, it was found that PiT2 is broadly expressed in the mouse brain.[39] Expression was found in neurons, astrocytes, and vascular endothelial cells.[e11] Inden et al.[e11] investigated the locations of PiT1 and PiT2 in the mouse brain. *SLC20A1* and *SLC20A2* mRNA expression was found in the cortex and striatum, and the highest expression of both genes was found in the cerebellum of the mice brains.[e11]

Jensen et al.[e6] investigated the role of PiT2 in mice, using *SLC20A2* homozygous knockout mice. These mice produced a truncated *SLC20A2* mRNA, which lack 8 downstream coding exons, leading to extracellular phosphate accumulation. In *SLC20A2* knockout mice, higher levels of Pi were found in the CSF compared with wild-type mice. This supports the findings that PiT2 also plays a role in Pi export from CSF and maintaining low Pi levels in the CSF.[e12] Deficiency of *SLC20A2* in homozygous knockout mice lead to brain calcifications in the thalamus, basal ganglia, and cortex,[e6,e13] emphasizing the role of PiT2 in brain calcification. However, because Fahr concerns a heterozygous autosomal dominant disease, these results might not represent the actual pathophysiology of Fahr disease.

Xenotropic and polytropic retrovirus receptor

XPR1 is localized to chromosome 1q25.3, and it encodes for the XPR1 with Pi exporter function.[5,e14] It is suggested that mutations in *XPR1* are associated with parkinsonism[5] and cognitive dysfunction (66.7%).[e8] Beside calcifications in the basal ganglia and cerebellum, the cortical areas also contain heavy calcifications in patients with mutations in this gene.[e8] *XPR1* contains an intracellular cytoplasmic N-terminal SPX domain (named by the genes *SYG1*, *PHO81*, and *XPR1*), a transmembrane domain and an intracellular C-terminal domain.[e14] *XPR1* regulates the phosphate export, which is seen in animals, plants, and fungi.[e15] It is thought to be a Pi transporter, which ensures an efflux of Pi from intracellular toward the vascular extracellular matrix[38] and thereby has an opposite function compared with PiT2. Another study into the function of XPR1, however, found it to be a G protein–coupled receptor involved in cell apoptosis via inactivation of the adenylyl cyclase pathway without Pi transporter function.[e16]

Copyright © 2020 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

Gene mutations

Legati et al.[e14] first discovered that mutations in the *XPR1* gene are associated with Fahr disease. After that, other studies found various mutations in the *XPR1* gene resulting in a decrease in phosphate efflux and subsequently calcium depositions in endothelial cells[5,e14,e15,e17] (figure 3).

Mutations in *XPR1* are associated with different neurodegenerative diseases. Xu et al.[e18] found a relation between a frameshift mutation in *XPR1* and schizophrenia. Fujioka et al.[e19] found an alternative splicing variant of *XPR1*, which is related to amyotrophic lateral sclerosis and frontotemporal lobar degeneration. Manavalan et al.[e20] found that altered protein expression of XPR1 in the hippocampus is linked to aging-related dementia (Alzheimer disease).

Animal models

XPR1 is expressed in different brain regions in the mouse[e14]; however, deletion of the *XPR1* gene in mice is embryonic lethal. It shows that this gene has an crucial function in early development, but its exact role is still unknown.[e16]

Platelet-derived growth factor subunit B

PDGFB is localized to chromosome 22q13.1 and encodes a disulfide-linked dimer.[19,e21] It is a paracrine growth factor important in mesenchymal cells, neurons, smooth muscle cells, and endothelial cells and has a function in the recruitment of pericytes during angiogenesis[e22,e23] (figure 4A). PDGFB signaling has different effects on these different cell types, but in every cell type, a mutation in this gene leads to calcification in the vascular extracellular matrix.[e23] Endothelial cells produce and secrete PDGFB, which binds the receptor PDGF-Rβ on pericytes and vascular smooth muscle cells. Progenitor PDGFB and its receptor PDGFRβ are important for the regulation of pericyte formation and migration around blood vessels during angiogenesis.[e24]

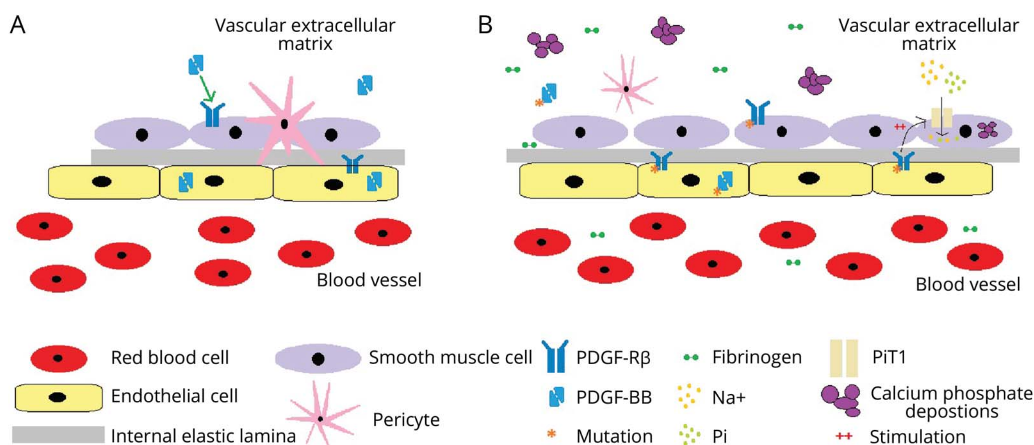
Gene mutations

Loss-of-function mutations in both genes, *PDGFB* and *PDGFRβ*, cause a vasculature lacking pericytes and leads to Fahr disease[e25] (figure 4B). The pericytes play a crucial role in the blood-brain barrier. It is therefore likely that dysfunction of the blood-brain barrier plays an essential role in Fahr disease. The finding of glycoprotein fibrinogen, which is important for the formation of blood clots, in the perivascular environment in a patient with Fahr disease also implies that dysfunction of the blood-brain barrier plays an important role in disease development.[e26] In addition, in mice with a dysfunction of the blood-brain barrier caused by a deficiency of the occludin protein, calcifications of the brain were observed.[e27] Moreover, in humans, calcifications occur by loss-of-function mutations in the occludin gene,[e28] which supports the involvement of the blood-brain barrier in the formation of calcifications.[6]

Animal models

Homozygous *PDGFB* deletion in mice led to a severe lack of vascular smooth muscle cells and pericytes, which was lethal.[e22,e29] As well as mutated *PDGFB*, mutations in *PDGFRB* resulted in blood-brain barrier abnormalities and a lack of pericyte recruitment, which led to an increased permeability.[6] Keller et al.[6] investigated the role of *PDGFB* in mice, using hypomorphic *PDGFB* alleles. In this study, *PDGFB* knockout mice developed calcifications in the brain. In early stages (2 months), matrix deposits were found in

Figure 4 Mutations in the genes *PDGFB* and *PDGFRB* are associated with dysfunction in blood-brain barrier integrity and pericyte maintenance in patients with Fahr disease



(A) This figure shows the normal situation, whereby the ligand PDGF-BB binds to its receptor PDGF-Rβ (green arrow). This ensures the blood-brain barrier integrity and pericyte maintenance. (B) This figure shows a mutated situation, whereby both *PDGFB* and *PDGFRB* could be mutated (orange asterisk). In this situation the ligand cannot bind to its receptor, and the underlying pathway will be activated only partly or not at all. This leads to dysfunction of the blood-brain barrier and pericyte recruitment. The glycoprotein fibrinogen is found in the vascular extracellular matrix, which indicates that the blood-brain barrier is disrupted, and plasma proteins can pass this barrier and may form locations for mineral depositions in the vascular extracellular matrix. Activating mutation in *PDGFRB* could also stimulate the expression of the Na+/Pi transporter PiT1, which leads to a phosphate influx into the smooth muscle cells; this can cause calcium phosphate depositions in this cell type.

Copyright © 2020 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

the midbrain and thalamus; these expanded in size and number in later stages (4 months). One-year old mice showed severe calcifications in the basal ganglia and thalamus. With energy-dispersive X-ray spectroscopy analysis, it was found that the calcifications consist of calcium and phosphate. This indicates that the process is comparable between *PDGFB* knockout mice and humans with Fahr disease.[6]

Because we know that *PDGFB* in the human brain is expressed in endothelial cells and neurons, the researchers investigated in which cell type *PDGFB* is relevant in Fahr disease. They used *PDGFB*-null mice with transgenic re-expression of *PDGFB* in endothelial cells by the rescue allele R26P. No expression of PDGFB in neurons was found, but in the endothelial cells, the expression depended on the copy number of the rescue allele. Not neuronal but only endothelial levels of PDGFB were correlated with calcification in the brain in mice. When the mouse had 2 copies of the R26P allele, no brain calcifications were seen, but when the mouse had only 1 copy of this allele, only 50% PDGFB expression was seen in the endothelial cells. They compared this with total loss of function, retention motif knockout ($PDGFB^{ret/ret}$) mouse in which the retention motif on the C-terminal is deleted. The dimer PDGF-BB could not bind to the receptor anymore. The researchers found that mice that did have only 1 R26P copy allele developed smaller calcifications, but on the same locations and composition as the $PDGFB^{ret/ret}$ mice. A correlation between the severity of calcification and the degree of pericyte deficiency and blood-brain barrier dysfunction was found.[6] In $PDGFB^{ret/ret}$ mice, a decrease in pericytes around the blood vessels was seen, and the pericytes that were present were not tightly clamped to the endothelial cells. It is suggested from these results that the retention motif of *PDGFB* is needed for the presentation of endothelial PDGF-BB to the adjacent pericytes.[e25]

Platelet-derived growth factor subunit receptor B

The genes *PDGFRA* and *PDGFRB* encode for 2 related tyrosine kinase receptors PDGF-Rα and PDGF-Rβ. PDGFA and B are ligands that bind these receptors, respectively.[6,7,13] The gene *PDGFRB* is localized to chromosome 5q33.1.[e21,e30] Expression of *PDGFRB* is especially found in the neurons, endothelial cells, vascular smooth muscle cells, and pericytes of the basal ganglia and dentate nucleus of the cerebellum.[19] *PDGFRB* has an important role in maintaining the blood-brain barrier by pericyte recruitment and angiogenesis as described above.[e28,e31]

After activation, the receptor dimerizes, autophosphorylation takes place, and the downstream signaling pathway of mitogen-activated protein kinases, phosphatidylinositol-3 kinase, phospholipase Cγ, signal transducers, and activators of transcription is activated. This activation of the pathway stimulates cell proliferation, differentiation, survival, and migration and has different functions in vascular smooth muscle cells.[7,e32] When the ligand is absent, the kinase

A first potential treatment for patients with Fahr disease is bisphosphonate therapy. Newer nitrogen-containing bisphosphonates, such as alendronate, predominantly inhibit osteoclasts.

receptor is inactive.[7] The most important function of this pathway is the proliferation and migration of vascular smooth muscle cells and pericytes.

Gene mutations

Different loss-of-function mutations in *PDGFRB* in patients with Fahr disease were described in various studies,[7,e33,e34] which lead to permeability of pericytes around vessels and calcium deposits in the vessel wall and perivascular space.[6,7] Patients with mutations in the *PDGFRB* gene had an early age at onset of disease symptoms in comparison with mutations in the other genes (*SLC20A2*, *PDGFB*, and *XPR1*).[e8] Presumably, headache and depression are the most common features for mutations in this gene, but evidence is limited.[e8]

Mutations in *PDGFRB* are not only involved in the formation of calcification due to loss of blood-brain barrier integrity and pericyte maintenance but also cause dysregulation of Pi transport in vascular smooth muscle cells. Different studies show that an activating mutation in *PDGFRB* is involved in vascular calcifications by regulating the phosphate transporter PiT1, encoded by *SLC20A1*.[7,e35,e36] PiT1 is located on the cell membrane and in the endoplasmic reticulum of vascular smooth muscle cells and endothelial cells.[7,e36] Activating mutation of *PDGFB* increases the expression of PiT1 causing a Pi influx from vascular extracellular matrix into the vascular smooth muscle cells. This results in the formation of calcium phosphate depositions in the smooth muscle cells[e36] (figure 4B). It appears that *PDGFRB* has different functions, and mutations in this gene can lead to different types of calcifications in the brain. A recent study demonstrated that *PDGFRB* colocalizes with *XPR1*, and these genes regulate each other.[e37] It is not yet known whether *PDGFRB* also regulates the PiT2 transporter in the brain.[6,7]

The above-mentioned genes account for approximately 55% of the patients with Fahr disease. For the other 45% of the cases, it is unclear why these patients have Fahr disease, but until recently, no causative genes for the other 45% of the cases were found. A recent study (June 2019) found another gene, the *MYORG* gene, which causes Fahr with an autosomal recessive pattern of inheritance. Sixteen patients with *MYORG* mutations, with a median age at onset of 52 years,

Copyright © 2020 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

showed a high clinical penetrance and motor impairment, which mainly manifested in dysarthria. In addition to extensive calcifications in the basal ganglia, *MYORG* patients also exhibit calcifications in the brainstem.[e38] The prevalence of these mutations in patients with Fahr disease and the pathophysiologic mechanism behind the calcifications remain to be established.

Recap

The above-mentioned genes encode for proteins that are located at different cell types in the brain; this may explain the different locations of calcification in the brain. Mutations in *SLC20A2* and *PDGFB* lead to calcifications in the vascular extracellular matrix,[e8,e23] mutations in *XPR1* lead to calcifications in the endothelial cells,[5] and mutations in *PDGFRB* lead to calcifications in smooth muscle cells.[e36] It is unknown why these mutations in these various genes are limited to the basal ganglia. The different mechanisms behind brain calcifications in patients with Fahr are unknown yet; future research is warranted to understand why calcium depositions occur in these different cell types. The wide spectrum of symptoms makes the diagnosis difficult. However, a slight separation can be made between the symptoms in comparison to the location of calcification. As mentioned earlier, patients with mutations in *PDGFRB* have an early age at onset, resulting mostly in headache and depression.[e8] This might be explained by the effect of the mutations in this gene, loss of blood-brain barrier integrity, and pericyte maintenance. The blood-brain barrier is important for the whole brain, and it is to imagine that damage of the blood-brain barrier will express at an early age at onset. Patients who become symptomatic later in life, mostly have mutations in *SLC20A2* and *XPR1* and mainly develop movement disorders in combination with other clinical features.[16]

Potential treatment options

Because no treatment or therapy to reduce the progression of calcification currently exists,[3,e39] treatment focuses on symptom management with, for example, anti-Parkinson, antidepressant, and antipsychotic medication.[e39] Deeper understanding of the genetics and the pathophysiologic mechanism behind this disease can provide new insight into treatment of patients with Fahr disease and of ectopic (brain) calcification beyond Fahr.

A first potential treatment for patients with Fahr disease is bisphosphonate therapy. Newer nitrogen-containing bisphosphonates, such as alendronate, predominantly inhibit osteoclasts. Although used for the treatment of osteoporosis, older non–nitrogen-containing bisphosphonates were initially shown to prevent heterotopic mineralization.[e40] The bisphosphonate etidronate is a molecular homologue of the circulating calcification inhibitor inorganic pyrophosphate (PPi). The potential of etidronate for the treatment of vascular calcification is convincingly shown in patients with calcification disorders, such as generalized arterial calcifications in infancy and pseudoxanthoma elasticum.[25,e41] A clinical trial into the effect of etidronate in patients with ACDC is currently running (clinicaltrials.gov: NCT01585402).[26] Of interest, possible beneficial effects of non–nitrogen-containing bisphosphonates on arterial calcifications in the population were also demonstrated in a recent meta-analysis.[29] The authors concluded that these bisphosphonates reduce all-cause mortality in various noncardiovascular patient groups.[29] Non–nitrogen-containing bisphosphonates were also tested on patients with Fahr disease where they were shown to pass the blood-brain barrier and improve symptoms, but not visual assessment of calcification burden.[e42,e43]

A second candidate therapy could be vitamin D. Keasey et al.[38] suggested that the gene *SLC20A2* is regulated by vitamin D and that vitamin D reduced calcifications in the brain. They found vitamin D deficiency in patients with Fahr disease. It is suggested that in a normal situation, vitamin D can bind to a promoter region of the *SLC20A2* gene and upregulate *SLC20A2* mRNA. This mechanism could therefore be a possibility for a treatment for patients with Fahr disease. First, it should be considered whether patients with Fahr disease often have a vitamin D deficiency in combination with *SLC20A2* mutations. If this mutation is not located in the vitamin D binding site, vitamin D therapy could be considered. Taking into account that vitamin D can pass the blood-brain barrier to a limited amount and high levels of vitamin D could be harmful, further research is certainly needed.[40,e44]

Third, some mutations found in Fahr disease resemble those in a specific subgroup of patients with cystic fibrosis who have a nonsense mutation leading to a truncated nonfunctional protein. Treatment with ataluren or PTC124 ensures that an early stop codon will not be read and that translation occurs to the normal stop codon. This results in a full-length cystic fibrosis transmembrane conductance regulator product, with normal function. A similar treatment is also used for patients with Duchenne muscular dystrophy.[e45] In patients with Fahr disease, truncating mutations are also found in the *SLC20A2* gene[e2] and the *PDGFB* gene.[e46] Similar treatments could therefore be an option for patients with Fahr disease with nonsense mutations.

Pi/PPi imbalance is known to be involved in genetic syndromes that lead to ectopic mineralization outside the brain.

A second candidate therapy could be vitamin D.

Copyright © 2020 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

TAKE-HOME POINTS

- Fahr disease, also known as idiopathic basal ganglia calcification, is mostly a rare autosomal dominant neurodegenerative disorder, characterized by bilateral calcifications in multiple basal ganglia.[19]
- Mutations in *SLC20A2*, the first gene linked to Fahr disease, lead to inhibition of phosphate uptake in the cell and thereby to deposition of calcium phosphate in the extracellular matrix.[14]
- Mutation in the gene *XPR1*, encoding a retroviral receptor with phosphate export function, leads to intracellular calcium deposition.[e14]
- Mutations in PDGFB, a growth factor with an important role in the recruitment of pericytes during angiogenesis, and *PDGFRB*, the receptor for the growth factor, lead to pericyte deficiency and thereby to disruption of the blood-brain barrier, which can lead to calcium deposition in the vascular extracellular matrix.[6,7,e25]
- There are currently no therapies available for Fahr disease; however, treatment with bisphosphonates seems promising, as it is well tolerated, and a case series shows improvement of symptoms by some patients.[e43]

Bisphosphonates, vitamin D, and drugs that target nonsense mutations are of interest for further investigation in Fahr disease and ectopic mineralization in the brain in the population. Outside neurodegenerative disease interest in the treatment of arterial calcifications is growing, and several food supplements and drugs, such as vitamins, antibodies, and proteins, are at various stages of (clinical) testing as recently reviewed; however, still novel therapeutics will be discovered and investigated. It is important that these drugs inhibit the calcification growth and avoid reacting on bones or interfering with physiologic mineralization.[e47] Incomplete understanding and the complexities about Fahr disease and vascular calcification in general impede the development of specifically targeted medicines. However, those drugs could eventually play a role in the treatment of Fahr disease and ectopic brain mineralization in the population.

Study funding
No targeted funding reported.

Disclosure
The authors report no disclosures relevant to the manuscript. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/cp.

Publication history
Received by *Neurology: Clinical Practice* June 11, 2019. Accepted in final form October 10, 2019.

Appendix Authors

Name	Location	Contribution
Melissa E.M. Peters, MSc	University Medical Center Utrecht	Designed and conceptualized the study; analyzed the data; and drafted the manuscript for intellectual content
Esther J.M. de Brouwer, MD	University Medical Center Utrecht	Analyzed the data and drafted the manuscript for intellectual content
Jonas W. Bartstra, MD	University Medical Center Utrecht	Analyzed the data and drafted the manuscript for intellectual content
Willem P.Th.M. Mali, MD, PhD	University Medical Center Utrecht	Revised the manuscript for intellectual content
Huiberdina L. Koek, MD, PhD	University Medical Center Utrecht	Revised the manuscript for intellectual content
Annemieke J.M. Rozemuller, MD, PhD	University Medical Center Utrecht	Revised the manuscript for intellectual content
Annette F. Baas, MD, PhD	University Medical Center Utrecht	Revised the manuscript for intellectual content
Pim A. de Jong, MD, PhD	University Medical Center Utrecht	Designed and conceptualized the study and revised the manuscript for intellectual content

References

1. Delacour A. Ossification des capillaires du cerveau. Ann Med Psychol 1850;2: 458–461.
2. Fahr T. Idiopathische verkalkung der hirngefasse. Zentralbl Allg Pathol 1930;50: 129–133.
3. Mufaddel AA, Al-Hassani GA. Familial idiopathic basal ganglia calcification (Fahr's disease). Neurosciences (Riyadh) 2014;19:171–177.
4. Bonazza S, La Morgia C, Martinelli P, Capellari S. Strio-pallido-dentate calcinosis: a diagnostic approach in adult patients. Neurol Sci 2011;32:537–545.
5. Anheim M, López-Sánchez U, Giovannini D, et al. XPR1 mutations are a rare cause of primary familial brain calcification. J Neurol 2016;263:1559–1564.
6. 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.
7. Nicolas G, Pottier C, Maltête D, et al. Mutation of the PDGFRB gene as a cause of idiopathic basal ganglia calcification. Neurology 2013;80:181–187.
8. Nicolas G, Charbonnier C, Campion D, Veltman JA. Estimation of minimal disease prevalence from population genomic data: application to primary familial brain calcification. Am J Med Genet B Neuropsychiatr Genet 2018;177:68–74.
9. Manyam BV. What is and what is not "Fahr's disease". Parkinsonism Relat Disord 2005;11:73–80.
10. Burke SL, Maramaldi P. An evaluative review of the evidence supporting idiopathic basal ganglia calcification for inclusion in the compassionate allowances intitiative. Basal Ganglia 2017;7:1–8.
11. Nicolas G, Richard AC, Pottier C, et al. Overall mutational spectrum of SLC20A2, PDGFB and PDGFRB in idiopathic basal ganglia calcification. Neurogenetics 2014; 15:215–216.
12. Ferreira JB, Pimentel L, Keasey MP, et al. First report of a de novo mutation at SLC20A2 in a patient with brain calcification. J Mol Neurosci 2014;54:748–751.
13. Arts FA, Velghe AI, Stevens M, Renauld JC, Essaghir A, Demoulin JB. Idiopathic basal ganglia calcification-associated PDGFRB mutations impair the receptor signalling. J Cell Mol Med 2015;19:239–248.
14. 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.
15. Ramos EM, Carecchio M, Lemos R, et al. Primary brain calcification: an international study reporting novel variants and associated phenotypes. Eur J Hum Genet 2018;26: 1462–1477.
16. 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.

Copyright © 2020 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.

17. Sobrido MJ, Hopfer S., G. D. Familial idiopathic basal ganglia calcification. Seattle, WA: GeneReviews; 2007.
18. Nicolas G, Pottier C, Charbonnier C, et al. Phenotypic spectrum of probable and genetically-confirmed idiopathic basal ganglia calcification. Brain 2013;136:3395–407.
19. Deng H, Zheng W, Jankovic J. Genetics and molecular biology of brain calcification. Ageing Res Rev 2015;22:20–38.
20. Manyam BV, Walters AS, Narla KR. Bilateral Striopallidodentate calcinosis: clinical characteristics of patients seen in a registry. Mov Disord 2001;16:258–264.
21. Li A, Paudel R, Johnson R, et al. Pantothenate kinase-associated neurodegeneration is not a synucleinopathy. Neuropathol Appl Neurobiol 2013;39:121–131.
22. Kobayashi S, Yamadori I, Miki H, Ohmori M. Idiopathic nonarteriosclerotic cerebral calcification (Fahr's disease): an electron microscopic study. Acta Neuropathol 1987; 73:62–66.
23. Wu YW, Hess CP, Singhal NS, Groden C, Toro C. Idiopathic basal ganglia calcifications: an atypical presentation of PKAN. Pediatr Neurol 2013;49:351–354.
24. Demer LL, Tintut Y. Vascular calcification: pathobiology of a multifaceted disease. Circulation 2008;117:2938–2948.
25. Ziegler SG, Ferreira CR, MacFarlane EG, et al. Ectopic calcification in pseudoxanthoma elasticum responds to inhibition of tissue-nonspecific alkaline phosphatase. Sci Transl Med 2017;9:eaal1669.
26. Kranenburg G, de Jong PA, Bartstra JW, et al. Etidronate for prevention of ectopic mineralization in patients with Pseudoxanthoma Elasticum. J Am Coll Cardiol 2018; 71:1117–1126.
27. Uitto J, Li Q. Vascular mineralization in Pseudoxanthoma lasticum: etidronate to the rescue? J Am Coll Cardiol 2018;71:1127–1129.
28. Lanzer P, Boehm M, Sorribas V, et al. Medial vascular calcification revisited: review and perspectives. Eur Heart J 2014;35:1515–1525.
29. Kranenburg G, Bartstra JW, Weijmans M, et al. Bisphosphonates for cardiovascular risk reduction: a systematic review and meta-analysis. Atherosclerosis 2016;252: 106–115.
30. Lin T, Wang XL, Zettervall SL, Cai Y, Guzman RJ. Dorsomorphin homologue 1, a highly selective small-molecule bone morphogenetic protein inhibitor, suppresses medial artery calcification. J Vasc Surg 2017;66:586–593.
31. Yamada M, Asano T, Okamoto K, et al. High frequency of calcification in basal ganglia on brain computed tomography images in Japanese older adults. Geriatr Gerontol Int 2013;13:706–710.
32. Baba Y, Broderick DF, Uitti RJ, Hutton ML, Wszolek ZK. Heredofamilial brain calcinosis syndrome. Mayo Clin Proc 2005;80:641–651.
33. Saleem S, Aslam HM, Anwar M, et al. Fahr's syndrome: literature review of current evidence. Orphanet J Rare Dis 2013;8:156.
34. Sobrido MJ, Coppola G, Oliveira J, Hopfer S, G. D. Primary familial brain calcification. Seattle, WA: GeneReviews; 2004.
35. Peters MEM, Kockelkoren R, de Brouwer EJM, et al. Histological validation of calcifications in the human hippocampus as seen on computed tomography. PLoS One 2018;13:e0197073.
36. Westenberger A, Klein C. The genetics of primary familial brain calcifications. Curr Neurol Neurosci Rep 2014;14:490.
37. Dai X, Gao Y, Xu Z, et al. Identification of a novel genetic locus on chromosome 8p21.1-q11.23 for idiopathic basal ganglia calcification. Am J Med Genet B Neuropsychiatr Genet 2010;153B:1305–1310.
38. Keasey MP, Lemos RR, Hagg T, Oliveira JR. Vitamin-D receptor agonist calcitriol reduces calcification in vitro through selective upregulation of SLC20A2 but not SLC20A1 or XPR1. Sci Rep 2016;6:25802.
39. Inden M, Iriyama M, Takagi M, Kaneko M, Hozumi I. Localization of type-III sodium-dependent phosphate transporter 2 in the mouse brain. Brain Res 2013;1531:75–83.
40. Nitschke Y, Rutsch F. Inherited arterial calcification syndromes: etiologies and treatment concepts. Curr Osteoporos Rep 2017;15:255–270.

References e1-e47 available at: links.lww.com/CPJ/A151

Share Your Insights, Expertise, and Experiences

- How are you employing drugs and devices in your field?
- What ethical challenges do you face?
- Do you have a case report that is illustrative of a clinical challenge?
- What challenges have you faced or successes have you enjoyed in bringing greater efficiency to your practice?

Deliver a high-quality, peer-reviewed message to your colleagues in practice, submit your paper at *NPub.org/NCP/submit*.

Copyright © 2020 American Academy of Neurology. Unauthorized reproduction of this article is prohibited.