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TGF-β シグナルに注目した CARASIL の画期的治療方法の開発

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TGF-β シグナルに注目した CARASIL の画期的治療方法の開発

研究代表者；野崎 洋明　新潟大学医学部保健学科・助教

研究要旨

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) は常染色体劣性遺伝性の脳小血管病であり，重度の認知症と歩行障害を呈する．CARASIL は *HTRA1* (*high temperature requirement serine peptidase A1*) の変異に起因する transforming growth factor β（TGF-β）シグナルの亢進によっておこる．治療法はまだ発見されていない．

CARASIL と同様に TGF-β シグナルの亢進によっておこる，Marfan 症候群に合併する大動脈瘤には，TGF-β シグナルを抑制する AT1 受容体拮抗薬が奏功する．本研究では，CARASIL のモデル動物である *Prss11 欠損マウス*を用いて，脳内移行が良好な AT1 受容体拮抗薬 candesartan の治療効果を検討した．その結果，16 ヵ月齢から 24 ヵ月齢まで 8 ヵ月間の内服投与により，同マウスにおける脳小血管病変の進行が有意に抑制されることが明らかになった．

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研究目的

CARASIL（cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy）は *HTRA1*（*high temperature requirement serine peptidase A1*）遺伝子の変異によって発症する，常染色体劣性遺伝性の脳小血管病である（Hara K et al. N Eng J Med 2009）．CARASIL 患者の脳小血管では強い壁細胞の変性がおこり，若年成人期に進行性の白質病変に伴う脳症を発症する．本邦で発見された希少疾患であるが，創始者効果を認めず，近年，欧米や中国からも症例が報告されている（Nozaki H, et al. Stroke 2014）．さらに，申請者は，同遺伝子変異のヘテロ接体でも脳小血管病を呈することを見出しており（投稿準備中），従来の想定より多くの CARASIL 患者がいる可能性がある．しかし，有効な治療方法は開発されていない．

CARASIL では，HTRA1 蛋白の機能低下によって生じる TGF-β の過剰分泌と TGF-β シグナルの慢性的な亢進が脳小血管の変性を引き起こすと考えられている．そのため，TGF-β シグナルを抑制する作用を持つ薬剤

が治療薬になる可能性がある. すでに降圧薬として臨床応用されている AT1 受容体拮抗薬は TGF-β シグナルを抑制する作用があり, TGF-β シグナルの亢進によって引き起こされる Marfan 症候群に合併する大動脈瘤に奏功する(Habashi JP, et al. Science 2006). 本研究では, *HTRA1* オルソログである *Prss11* を欠損した CARASIL モデルマウスを用いて, 脳内移行が良好で TGF-β シグナルの阻害作用を有する AT1 受容体拮抗薬 candesartan(Lanz TV, et al. J Clin Invest. 2010)の脳小血管における壁細胞変性に対する治療効果を検討した. 本モデルマウスは 16 ヵ月齢以降に CARASIL 患者と同様の脳小血管の壁細胞変性をおこす, 理想的な疾患モデル動物である. 本研究は分子病態から脳小血管病の治療に迫る物であり, 新規性と国際的な優位性がある. また, candesartan は高血圧患者に対して頻繁に臨床使用されており, 有効性が確認できれば, 速やかな臨床応用が期待できる.

研究方法

Candesartan は TGF-β シグナルの抑制だけではなく, 血圧降下作用も有している. そのため, candesartan の効果が TGF-β シグナルを介したものであるかどうかを検討するためには, TGF-β シグナルを抑制する作用を持たず, candesartan と同等の血圧低下作用を有する薬剤を投与した群を対照にする必要がある. そこで, CARASIL のモデル動物である *Prss11* 欠損マウスを用いて, 16 ヵ月齢から 24 ヵ月齢まで, candesartan 3.0 mg/kg/day, amlodipine 10.0 mg/kg/day をそれぞれ内服した群を用意した(15 – 17 mmHg の血圧降下作用). さらに 24ヵ月齢の野生型マウスと *Prss11* 欠損マウスの非内服群も用意した. これら 4 群のマウスについて, 脳小血管の壁細胞変性を表す定量的指標を使用して, 薬剤の治療効果を検討した. また, 分子病態を評価するために, TGF-β シグナルの second messenger であるリン酸化 smad2/3, およびリガンドである TGF-β の半定量的, あるいは定量的評価を行った. 分担研究者の佐藤俊哉はマウスの管理と解析を, 小野寺理は分子生物学的解析を担当した.

·個々の研究方法

①CARASIL モデルマウスの脳小血管病理と candesartan の治療効果に関する研究 (佐藤俊哉)

1) マウスの処理

月齢16ヵ月の *Prss11* 欠損マウスに対し, 内服投与を開始した. マウスの体重を 30g, 1 日飲水量を 5ml として, 飲水に candesartan を溶解して, 3 mg/kg/day に調節した. 非内服群と, amlodipine を 10mg/kg/day で投与した群を対照にした. 投与開始 8 ヶ月後に, マウスから固定脳を取り出し, 矢状断方向に半割して, floating 切片とパラフィン切片を作製した. 野生型マウスについては, 24 ヵ月齢の固定脳を取り出し, 矢状断方向に半割して, floating 切片とパラフィン切片を作製した.

2) 血管平滑筋細胞面積の評価

血管平滑筋細胞マーカーの α-smooth muscle actin と血管内皮細胞マーカーの lectin を用いて, パラフィン切片に対して 2 重免疫染色を施した. 蛍光顕微鏡を用いて, 脳軟膜動脈を撮影した. 画像解析ソフト Imaris を用いて, 個々の血管平滑筋細胞の面積を定量的に解析した. (野生型 n = 8, *Prss11* 欠損マウス非内服群 n = 7, *Prss11* 欠損マウス candesartan 内服群 n = 4, *Prss11* 欠損マウス amlodipine 内服群 n = 4)

3) ペリサイト被覆率の評価

ペリサイトマーカーとして CD13, 血管内皮細胞マーカーとして lectin を使用し, floating 切片に対して 2 重免疫染色を施した. 共焦点顕微鏡で大脳皮質の毛細血管を撮影し, 画像解析ソフト Imaris で解析を行った. 血管内皮細胞の体積を分母, それを取り巻く周皮細胞の体積を分子とし, その比をペリサイト被覆率として算出した. (野生型 n = 4, *Prss11* 欠損マウス非内服群 n = 4, *Prss11* 欠損マウス candesartan 内服群 n = 2, *Prss11* 欠損マウス amlodipine 内服群 n = 1)

②CARASIL モデルマウスにおける TGF-β シグナルの評価方法と分子病態に関する研究 (小野寺理)

1) 免疫組織化学染色によるリン酸化 smad2/3 の検出

Prss11 欠損マウス, 野生型マウス脳のパラフィン切片を用いて, リン酸化 smad2/3 の免疫組織染色を行った. それぞれのリン酸化 smad2/3 陽性細胞数を比較し, *Prss11* 欠損マウス脳内における TGF-β シグナルレベルの変動を検討した.

2) イムノブロッティングによるマウス脳組織におけるリン酸化 smad2/3 の検出

24ヵ月齢の *Prss11* 欠損マウス, 野生型マウスの大脳皮質, 線条体, 海馬を解剖し, サンプルとした. TGF-β シグナルのセカンドメッセンジャーであるリン酸化 smad2/3 をイムノブロッティングにより検出し, 量について比較検討を行った.

3) マウス脳脊髄液, 血漿のサンプリングと TGF-β の定量

マウス脳脊髄液中, または血中の TGF-β の定量のため *Prss11 欠損マウス*, 野生型マウスよりサンプルを回収した. 脳脊髄液はガラスキャピラリーを用いて大槽腔よりサンプリングを行った. 血漿は心採血より回収した血液に EDTA を加え, 遠心により調整した(*Prss11* 欠損マウス n=5, 野生型マウス n=4). ルミネックス法によって, サンプル中に含まれる TGF-β の定量を行った.

3) マウス血管内皮細胞, アストロサイト初代培養の確立と TGF-β の定量

2～4 ヶ月齢のマウス脳よりマウス脳毛細血管を調整し, puromycin による脳血管内皮細胞選択培養により, 純正血管内皮培養を行った. アストロサイトは生後 3 日齢の新生仔マウス大脳皮質から trypsin 細胞分散によって調整した. 両細胞とも 80～90 コンフルエントの時点で順化培地を回収した. ELISA によって, サンプル中に含まれる TGF-β の定量を行った.

(倫理面への配慮)

動物の愛護及び管理に関する法律に基づいて行うとともに, 新潟大学の動物実験規則および組換え DNA 実験安全管理規則に従い, 学長許可を受けて実施した.

研究結果と考察

・研究班全体としての研究成果

本年度の研究結果から, candesartan の長期経口投与が *Prss11 欠損マウス*における脳小血管の壁細胞変性を抑制することが明らかになった. これは, 脳小血管の変性を治療するという新たなアプローチによって, 初めて効果が実証されたケースである. 同薬は降圧薬としてすでに臨床現場で頻用されている薬剤であり, CARASIL においても速やかな臨床応用が期待できる.

申請者は, candesartan が TGF-β シグナルの亢進を抑制することによって, 脳小血管の病理変化を軽減することを想定していた. しかし, 本研究では, candesartan 群だけでなく, 対照

薬剤の amlodipine 投与群でも *Prss11* 欠損マウスにおける脳小血管変性の抑制効果を認めた. また, *Prss11* 欠損マウスの生体内における TGF-β シグナルは, 野生型マウスと *Prss11* 欠損マウスの間に有意差を認めなかった. このことは, candesartan が TGF-β シグナルを抑制することによってではなく, 降圧作用によって脳小血管変性を抑制した可能性を示唆している.

·個々の研究成果

①CARASIL モデルマウスの脳小血管病理と candesartan の治療効果に関する研究 (佐藤俊哉)

1) 壁細胞変性に対する薬剤の効果

血管平滑筋細胞面積については, 非内服群に比して, candesartan 投与群, amlodipine 投与群ともに, 有意に高値であった. Candesartan 投与群と amlopidine 投与群の間には, 平滑筋細胞面積に有意差は認めなかった. ペリサイト被覆率については, 非内服群に比して, candesartan 投与群, アムロジピン投与群のいずれにおいても, 高値を示す傾向があった.

②CARASIL モデルマウスにおける TGF-β シグナルの評価方法と分子病態に関する研究 (小野寺)

1) マウス脳組織のリン酸化 smad2/3

免疫組織化学染色, イムノブロッティングのいずれの評価方法においても, *Prss11 欠損*マウス, 野生型マウス間の脳内リン酸化 smad2/3 レベルに有意差は見られなかった.

2) マウス脳脊髄液, 血漿に含まれる TGF-β

脳脊髄液, 血漿のいずれのサンプルにおいても, *Prss11 欠損*マウス, 野生型マウス間で有意差は見られなかった.

3) マウス血管内皮細胞, アストロサイト初代培養から分泌される TGF-β

血管内皮細胞の初代培養では, *Prss11 欠損*マウス, 野生型マウスの間に有意差は見られなかった. アストロサイト初代培養では, ELISA による検出感度未満であった.

結論

Candesartan は CARASIL モデルマウスにおける脳小血管変性を抑制する.

研究発表

1. 論文発表

1) Onodera O, Sekine Y, Kato T, Koyama A, Nozaki H, Nishizawa M. Emerging molecular mechanism for cerebral small vessel disease: Lessons from hereditary small vessel disease. Neurol Clin Neurosci 2015;3:7-13.

2) Nozaki H, Nishizawa M, Onodera O. Features of cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy. Stroke 2014;45:3447-3453.

3) 上村昌寛, 野崎洋明, 西澤正豊, 小野寺理. CADASIL と CARASIL について. 日本臨床 2014;72:619-623.

4) 野崎洋明, 西澤正豊, 小野寺理. CARASIL の新しいトピックス. 分子脳血管病 2014;13:179-181.

5) 関根有美, 野崎洋明, 西澤正豊, 小野寺理. CADASIL, CARASIL の分子病態機序. 日本臨床 2014;72:148-151.

6) 手塚敏之, 西澤正豊, 野崎洋明, 小野寺理. CADASIL, CARASIL の病態機序. 血管医学 2014;15:51-58.

7) Konno T, Tada M, Tada M, Koyama A, Nozaki H, et al. Haploinsufficiency of CSF-1R and clinicopathologic characterization in patients with HDLS. Neurology 2014; 82:139-148.

8) 関根有美, 野崎洋明, 西澤正豊, 小野寺理. COL4A1-related disorder 日本臨床 2013, 別冊 140-144.

9) 野崎洋明, 西澤正豊, 小野寺理. 遺伝性脳小血管病研究の現況と展望. 日本臨床 2013;71:545-554.

10) 野崎洋明 Consideration of the pathogenesis of CARASIL. 臨床神経学 2012;7:1360-1362.

11) 関根有美, 野崎洋明, 西澤正豊, 小野寺理. 脳小血管病と遺伝性脳小血管病. 循環器内科 2012;71:140-144.

2. 学会発表

1) 野崎洋明, 加藤泰介, 斎藤洋兵, 小山哲秀, 西澤正豊, 小野寺理. HTRA1 遺伝子変異のヘテロ接合体は, 優性阻害効果によって脳小血管病を引き起こす. 2014 年 11 月, 第 33 回日本認知症学会学術集会

2) Yumi Sekine, Taisuke Kato, Hiroaki Nozaki, Sachiko Hirokawa, Toshiya Sato, Atsushi Shiga, Toshikuni Sasaoka, Masatoyo Nishizawa, Osamu Onodera. Excess TGF-β1 secreted from astrocytes impair mural cells in cerebral small arteries. 2014, 55th Annual Meeting of the Japanese Societry of Neurology.

3) Hiroaki Nozaki, Yumi Sekine, Yoshinori Nishimoto, Yutaka Shimoe, Akiko Shirata, Sohei Yanagawa, Mikio Hirayama, Imaharu Nakano, Masatoyo Nishizawa, Osamu Onodera. MRI features of cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy. 2013, the 8th International Congress on Vascular Dementia.

4) 野崎洋明, 斎藤洋兵, 二本松萌, 小山哲秀, 加藤泰介, 西澤正豊, 小野寺理. Dominant negative 効果をもつ変異型 HTRA1 はヘテロ接合体でも脳小血管病を引き起こす. 2013 年, 第 54 回日本神経学会学術大会

5) Taisuke Kato, Yumi Sekine, Atsushi Shiga, Megumi Nihonmatsu, Hiroaki Nozaki, Sachiko Hirokawa, Toshiya Sato, Yasuko Toyoshima, Minesuke Yokoyama, Shoji Tsuji, Masatoyo Nishizawa, Osamu Onodera. High-temperature requirement A serine peptidase 1, the causative gene for hereditary cerebral small vessel disease is expressed only in astrocyte. 2012, the 50th Society for Neuroscience Annual Meeting.

6) Taisuke Kato, Yumi Sekine, Hiroaki Nozaki, Sachiko Hirokawa , Toshiya Sato, Atsushi Shiga, Yasuko Toyoshima, Hitoshi Takahashi, Masatoyo Nishizawa, Osamu Onodera. Histopathological characterization of CARASIL responsible gene; HTRA1 deficient mouse. 2012 年, 第 2 回新潟大学脳研究所共同研究拠点国際シンポジウム

知的所有権の取得状況

なし

特許取得

なし

実用新案登録

なし

その他

なし

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Onodera O, Sekine Y, Kato T, Koyama A, Nozaki H, Nishizawa M	Emerging molecular mechanism for cerebral small vessel disease: Lessons from hereditary small vessel disease	Neurol Clin Neurosci	3	7-13	2015
Nozaki H, Nishizawa M, Onodera O	Features of cerebralautosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy	Stroke	45	3447-3453	2014
上村昌寛, 野崎洋明, 西澤正豊, 小野寺理	CADASILとCARASILについて	日本臨床	72	619-623	2014
野崎洋明, 西澤正豊, 小野寺理	CARASILの新しいトピックス	分子脳血管病	13	179-181	2014
関根有美, 野崎洋明, 西澤正豊, 小野寺理	CADASIL, CARASILの分子病態機序	日本臨床	72	148-151	2014
手塚敏之, 西澤正豊, 野崎洋明, 小野寺理	CADASIL, CARASILの病態機序	血管医学	15	51-58	2014
Konno T, Tada M, Tada M, Koyama A, Nozaki H, Onodera O, et al.	Haploinsufficiency of CSF-1R and clinicopathologic characterization in patients with HDLS	Neurology	82	139-148	2014
関根有美, 野崎洋明, 西澤正豊, 小野寺理	COL4A1-related disorder	日本臨牀	別冊	314-318	2013
野崎洋明, 西澤正豊, 小野寺理	遺伝性脳小血管病研究の現況と展望	日本臨牀	71	545-554	2013
野崎洋明	Consideration of the pathogenesis of CARASIL	臨床神経学	7	1360-1362	2012

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REVIEW ARTICLE

Emerging molecular mechanism for cerebral small vessel disease: Lessons from hereditary small vessel disease

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Abstract

Cerebral small vessel disease is a common disorder in the elderly. The findings of hereditary small vessel disease studies clearly show that small vessel diseases have a distinct molecular pathway that is different from that in large vessels. However, the anatomical and functional heterogeneity of the cerebral small vessel system makes it difficult to understand the concept and molecular mechanism for small vessel disease. The purpose of this review is to explain the heterogeneity of small vessels and the importance of the components of the capillary system in the pathogenesis of cerebral small vessel disease. Although traditional investigations have focused more attention on the arteriole, the most functional part of small arteries is the capillary. Therefore, the capillary might play an important role in the pathogenesis of small vessel disease. In the capillary, pericytes and astrocytes are unique components with marked diversity. However, the molecular signature and function of pericytes remain unknown. Furthermore, the morphology and molecular signature of astrocytes in the cortex and white matter are quite different. Therefore, the mechanism of small vessel disease is not simple, and must be investigated considering the diversities of small vessels. In the capillary, cross-talk between cell components exists. Among these cell signaling pathways, recent findings on the gene responsible for hereditary small vessel disease show that transforming growth factor-β and platelet-derived growth factor-β could contribute to the molecular pathogenesis of small vessel disease. These findings provide useful information for the development of a new therapeutic strategy for small vessel disease.

Introduction

The vessel system in the brain is fundamental for maintaining brain function. Among the components of the vessel system, the small vessels play an important role in maintaining the function. Diseases that mainly involve small vessels are known as cerebral small vessel disease (SVD). SVD is a common disorder in elderly populations, and contributes to dementia, gait disturbance and stroke.[1] The concept of SVD changes our understanding of cerebral vascular disease in that the molecular pathogenesis of SVD is different from that of large vessel disease, which is mainly caused by arteriosclerosis. With advances in molecular genetics, the genes that cause cerebral SVD have been identified. Study findings have clearly shown that SVD has a distinct molecular pathway that does not involve large vessels. However, little is known about the molecular basis of SVD.[2]

The concept of cerebral SVD is still obscure. Although small vessels are anatomically and functionally different, we are still not sure which type of small vessel contributes to the pathogenesis of cerebral SVD. Furthermore, although a blood–brain barrier and perivascular drainage of interstitial fluid from the brain parenchyma have been proposed as a function in the small vessels in the brain, the precise functions and involved components of small vessels are not fully understood.[3,4] To understand cerebral SVD, the function and precise structure of the small vessels in the brain must be clarified. Capillaries and surrounding astrocytes are unique, and play an important role in the function of small vessels. However, previous studies on cerebral small vessels have mainly focused on the arterioles, which are larger than capillaries.[1] The purpose of the present review is to explain the heterogeneity of the cerebral small vessel system, and the importance of mural cells in the pathogenesis of cerebral SVD, based on the

findings from studies about the molecular pathogenesis of hereditary SVD.

Heterogeneity of small vessels

A characteristic feature of the brain arteries is that they mainly lose their external elastic membrane.[5] This feature of the cerebral arteries might contribute to the high frequency of brain vessel aneurysms.[6] Among the small vessels in the brain, there are at least three different types of vessels: pial artery, arteriole and capillary.[7] The pial artery is composed of the following cells and connective tissue layers (starting from the luminal side): endothelial cells, basement membrane, internal elastic membrane, smooth muscle cells, basement membrane, leptomeningeal cells and connective tissue.[7] These small arteries have anastomoses.[8] The anatomical differences among these three vessels include the absence or presence of the internal elastic membrane, smooth muscle cells and perivascular space. The elastic membrane is absent in the arterioles, and smooth muscle cells and the perivascular space are both absent in the capillaries.

There are two types of arterioles, according to their anatomical position in the cerebrum. One is the superficial perforating artery arising from the pial artery (smaller than large arteries), and the other is the deep perforating artery arising from the anterior, middle and posterior cerebral arteries (large arteries). Superficial perforating arteries are further divided into four types by the depth of the vessels from the cerebral surface. Two types of superficial perforating arteries irrigate the different layers in the cortex, and the others irrigate the corticomedullary junction and white matter.[9,10] In the cortex, small arteries are more abundant than in white matter, and make anastomoses.

The superficial perforating artery that branches into the periventricular area and irrigates the white matter is known as the medullary artery. The medullary artery is divided into two types by its shape after penetrating the cortex. After penetrating the cortex, one artery extends straightly through the white matter and the other bends at a right angle at the subcortical area to access the deep white matter. The arteries make anastomoses around the ventricular wall.[11] The superficial perforating arteries coil, loop and spiral within wide adventitial spaces at the corticomedullary junction; the function of these structures is unknown.[11] These arteries have thick adventitial sheaths and large perivascular spaces in the white matter not in the cortex.[11] Meanwhile, the deep perforating artery branches out directly from the large artery, and most of the arteries reach the basal ganglia and thalamus.[12] In addition, some of these arteries have a dual leptomeningeal cell layer, resulting in a relatively large perivascular space.[7] This unique structure might appear as a relatively large perivascular space on T2-weighted magnetic resonance imaging (MRI).

Another important characteristic of the small vessels is their regulation by the neuron.[13,14] The pial artery is densely innervated by the peripheral nervous system. In contrast, small cortical arteries (arteriole or capillary) are innervated by interneurons in the cortex or subcortical pathway neurons. It is not known if these neuronal regulations also exist in small arteries in the subcortical area; however, the small vessels in the white matter might be less tightly regulated by the nervous system.

Diversities of capillaries

The most important function of capillaries in the small vessel system is as a blood–brain barrier.[4] The non-fenestrated endothelial cells and tight junction compose this system. The endothelial cells are enveloped with pericytes and astrocyte end-feet. This structure is sometimes called a neurovascular unit; however, the contribution of the nervous system to capillaries in the subcortical area is not clear.[13] In the present review, these components will be described as a capillary unit, including capillaries, astrocytes and pericytes. In a capillary unit, the endothelial cell plays several important roles for barrier function: forming a tight junction, selective transport system and endocytosis.[4,15] To maintain these characteristic features, the endothelial cells in capillary express several unique molecules, which are not observed in the endothelial cells in arterioles; for example, claudin and occludin in tight junctions, glucose transporter 1 for selective transportation and caveolin 1 for selective transcytosis.[4] In addition, a recent study showed extravasation of clots in the capillary to the brain parenchyma by the endothelial cells, suggesting that the function and characteristics of endothelial cells in the capillary might be different from those in the arteriole.[16] Furthermore, it is not known if all the endothelial cells in the central nervous system are identical regardless of their location. Endothelial cells are tightly associated with pericytes, which are mural cells in the capillary, by autocrine and paracrine signaling.[17,18] If the characteristics and function of pericytes vary according to their location, the same might also be true for endothelial cells.[19]

In capillaries, smooth muscle cells are absent, and pericytes cover some extent of the abluminal side of endothelial cells. Compared with other species, the small vessels in the human cortex are covered by a larger number of pericytes.[20] Recent findings show that pericytes play an important role in maintaining the blood–brain barrier function.[15,17,20–23] In addition, a decrease in the number of pericytes causes neurodegeneration through a non-ischemic or hypo-oxygenic pathway.[24] Pericytes are cells attached to the abluminal side of endothelial cells in the capillary and covering the basement membrane along with endothelial cells. There are several molecular signatures that can distinguish most of the pericytes form other cells; however, none of the single molecular markers can distinguish all of the pericytes from other brain cells.[20,21] The lineage of the pericytes in the central nervous system is different in each part of brain.[17,20,25] The embryonic sources of pericytes include neuroectoderm-derived neural crest cells, which give rise to pericytes in the forebrain, and mesoderm-derived mesenchymal stem cells, which give rise to pericytes in the midbrain, brain stem and spinal cord.

This complex is enveloped by astrocytes. Although the contribution of the astrocytes to maintain the barrier function is not fully understood, the astrocytes might contribute to the direction of the selective transportation between the

luminal side and the brain parenchyma.[26,27] The astrocyte also has marked heterogeneities, including protoplasmic and fibrous astrocytes.[26,28–31] The protoplasmic astrocyte is predominantly found in the cortex, and has many branching processes with end-feet that envelop the synapse and capillaries. Furthermore, some of the branches extend to the surface of the cerebrum. In contrast, the fibrous astrocyte is found in white matter, and has a few unbranched processes with end-feet that envelop Ranvier nodes and capillaries. Although the lineage differences of each astrocyte in the cerebrum is still obscure, the astrocytes in the spinal cord have a distinct lineage depending on the anatomical position in the spinal cord.[28,32]

Small arteries have marked anatomical and functional diversities. Most prominently, small vessels in the cortex and white matter are different in many aspects. The difference is not simply explained by the difference of the circulation dynamics or number of capillaries. The regulations by the nervous system and the type of cells that compose the capillaries are fundamentally different between small vessels in the cortex and white matter. These results indicate that small vessels do not have a single architecture. We should to pay more attention to the heterogeneities of cerebral small vessels when we study the molecular pathogenesis of SVD.

Which type of small vessel is responsible for the clinical features of SVD?

MRI has shown several aspects of SVD, white matter hyperintensity, lacunar infarction, microbleeds, cortical subarachnoid hemorrhage, cortical microinfarction and cortical thinning.[2,33] Among these features, which feature is mostly responsible for the clinical symptoms of SVD? The most prominent feature of SVD on MRI is white matter hyperintensity (WMHI). Indeed, several hereditary SVD show diffuse WMHI; thus, there is no doubt that WMHI is a result of small vessel alterations. The lower density of capillaries in white matter might explain the vulnerability of the white matter in SVD.[11] As the medullary artery is severely affected in sporadic SVD, Okeda *et al.* proposed the earthen pipe hypothesis for the molecular pathogenesis of SVD.[34,35] They speculated that the hypoperfusion resulting from a loss of autoregulation of small vessels contributes to the white matter pathology in SVD.

Although the autoregulation disturbance hypothesis might explain a part of the molecular pathogenesis of white matter injury, the loss of the smooth muscle cell layer cannot simply explain the entire feature of SVD. The pathological findings of idiopathic basal ganglia calcification do not support this hypothesis. Patients with idiopathic basal ganglia calcification present massive calcifications in the perforating arteriole, specifically, in a portion of the media intima.[36–38] In the small vessels of patients with this disease, the contracting property of the arteriole should be completely diminished. However, WMHI is not an early finding in these patients.[39–41] The difference between this small vessel pathology and the other SVD is that the affected area is calcified and protected from the bloodstream. Therefore, an additional mechanism should exist to explain the molecular pathogenesis of WMHI. In addition, accumulating evidence shows that WMHI is not strongly correlated with the clinical symptoms in hereditary SVD.[42–46] This feature markedly precedes the onset of the neurological symptoms. Thus, the significance of WMHI on development of clinical manifestations in SVD should be carefully assessed.[47,48]

The capillary, which plays the most important role in the small vessel system, could contribute to the clinical manifestations in SVD.[49] Capillary alterations can cause cognitive impairment and movement dysfunction through different mechanisms: (i) dysfunction of the barrier function; (ii) dysregulation of microcirculation dependent on neuronal activity; and (iii) failure of interstitial fluid draining.[17,50,51] Previous studies have attempted to explain the selective vulnerability for white matter, as changes in the white matter are mostly prominent in SVD. However, if WMHI is just a consequence of the dysfunction of the small vessel system, we might lose track of the true pathogenesis that contributes to the neurological manifestations of SVD. Further studies should focus on the alteration of the microcirculation system, including the capillary and surrounding cells, to understand the pathogenesis of SVD.

Which component of small vessels is important for the pathogenesis of SVD?

The degeneration of the smooth muscle cells and the splitting of the internal elastic membrane are characteristic features in sporadic and some hereditary small vessels. The splitting of the internal elastic fiber might cause the transition of smooth muscle cells and their migration and proliferation to the media intima, not apoptosis.[52] Furthermore, the disturbance of elastic fiber by reducing the amounts of component protein, elastin, does not cause SVD.[52–55] In contrast, patients with the mutation in actin, which is mainly expressed in the smooth muscle cells, resemble those of sporadic SVD.[56] Therefore, the degeneration of smooth muscle cells might contribute to the pathogenesis of SVD.

However, the capillary, a functional small vessel, does not have smooth muscle cells. The capillary has several unique structures that distinguish it from other vessels and small vessels in other organs. These unique structures might explain why these disorders specifically affect the brain. Thus, whether the components of the capillary unit are important for pathogenesis of SVD will be addressed in the present review.

One of the components of the cells in the capillary unit is the pericyte. Pericytes are cells that share the basement membrane with endothelial cells. However, the lack of markers to identify pericytes makes it difficult to investigate the involvement of these cells in the human brain.[15,17,20] The involvement of pericytes in SVD is well characterized in diabetic retinopathy. In this disorder, pericyte apoptosis is an early manifestation. The absence of pericytes is recognized as a "pericyte ghost", which represents the trace of the pericyte as a space between the basement membrane.[57] In contrast, in brain parenchyma, it would be difficult to recognize these traces. Therefore, there is a limitation to recognizing

pericyte alterations in the human brain. However, the importance of pericytes for maintaining the neuron and the blood–brain barrier has recently been recognized.[15,17,20] The involvement of pericytes has been observed in patients and a mouse model of cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy, a hereditary SVD,[58,59] or idiopathic basal ganglia calcification.[22,23,36,39,60] Thus, it would be interesting to investigate the contribution of pericytes in cerebral SVD.

The other unique component in the capillary unit is the astrocyte. The morphology and molecular signature of astrocytes is different between those in the cortex and white matter.[26,28–31] Fibrous astrocytes are plentiful in the white matter, and have cylindrical processes with dense glial filaments stained with glial fibrillary acidic protein. Protoplasmic astrocytes are popular in the gray matter, and have more irregular processes and few glial filaments. Protoplasmic astrocytes contact and sheathe synapses and blood vessels. Therefore, there is a possibility that an alteration on a specific type of astrocyte results in the vulnerability of specific areas in the brain. For example, the mutation of the glial fibrillary acidic protein, which is a fundamental skeletal protein in the astrocyte and more popular in the fibrous astrocytes than protoplasmic astrocytes, causes demyelination in the white matter. Although in the patients with a mutation in the *GFAP* gene, the astrocytes in white matter are predominantly affected, the clinical manifestations of the patients are quite different from the SVD. Thus, it might be difficult to consider that the astrocyte takes a primary role in the pathogenesis of SVD.

Finally, the perivascular space, known as the Virchow–Robin space, is a unique structure in the small vessel systems in the brain. Several hypotheses for the significance of the space between the adventia and parenchyma (glia limitans) in the brain have been provided.[61] One of the hypotheses is that the space functions as a pathway for drainage of fluid or proteins from the brain parenchyma.[3,62] Weller *et al.* use the term, "protein elimination-failure angiopathy," for the disorder caused by the impairment of drainage pathway by small vessels.[3] Cerebral amyloid angiopathy, which predominantly involves the cortical and pial arteries, has been considered part of the elimination failure disorders.[3] The disease has been classified into two types depending on the presence or absence of amyloid accumulation in capillaries.[7,63,64] In cerebral amyloid angiopathy, amyloid deposit in the internal space of the mural cells results in the disappearance of smooth muscle cells. In addition, WMHI is well observed in patients with Alzheimer's disease. Although the elimination failure hypothesis is promising, more evidence should be accumulated to prove that the perivascular space functions as a drainage system in the brain.

Alteration of the signaling pathway between the cell components of the microcirculation system causes SVD

The identification of the gene responsible for hereditary SVD provides the molecular pathway for SVD. Several molecular mechanisms have been identified in SVD: (i) the alteration of structural proteins in the small vessel system;[65] (ii) accumulation of the abnormal proteins or dysfunctional metabolism in the small vessel system;[66,67] and (iii) alteration of the cell signaling pathway in the small vessel system. The present review will focus on the contribution of the cell signaling pathway on the pathogenesis of SVD.

We recently identified the causative genes for hereditary SVD, cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL).[68] Mutations in the high-temperature requirement A (HTRA) serine peptidase 1 (HTRA1) gene cause the disease. Disorganization of the internal elastic membrane and loss of vascular smooth muscle cells were observed in small cerebral arteries in CARASIL.[69] These pathological findings resemble those observed in patients with non-hereditary cerebral SVD.[34,35] HTRA1 has a serine protease activity, and decreases transforming growth factor-β (TGF-β) family signaling.[70] CARASIL-associated mutant HTRA1 show decreased protease activity and fail to decrease TGF-β family signaling.[68,71] Furthermore, the fibronectin containing extra type III domain A and versican, which are induced by increased TGF-β signaling, accumulate and TGF-β1 is increased in the media intima of small cerebral arteries of patients with CARASIL.[68,71] These findings show that increased TGF-β signaling plays a pivotal role in the pathogenesis of SVD in CARASIL. HTRA1 decreases TGF-β1 signaling by interfering with the maturation of proTGF-β1 in the intracellular space. HTRA1 cleaves the pro-domain of proTGF-β1, and cleaved proTGF-β1 is degraded.[71] Consequently, the amount of mature TGF-β1 is reduced. The intracellular cleavage of proTGF-β1 is a novel mechanism to regulate the amount of TGF-β1.[71,72] The relationship between the dysregulation of TGF-β signaling and the loss of smooth muscle cells in small cerebral vessels might show an emerging molecular mechanism for cerebral SVD. TGF-β is a well-known cytokine that is secreted from endothelial cells, pericytes and astrocytes.[15,17] The receptors for TGF-β are also expressed in these cells. Therefore, TGF-β signaling could affect autocrine or paracrine signaling. Although it is still not clear in which cell HTRA1 is expressed, the endothelial cell is a possible candidate and regulates TGF-β signaling.[73,74]

Another component of the cell signaling pathway, which functions between endothelial cells and pericytes, is the platelet-derived growth factor-β (PDGFβ).[15,17] The decreased PDGFβ or receptor for PDGFβ decreases the number of pericytes, and results in the dysfunction of the blood–brain barrier accompanied with neurodegeneration.[22–24] Mutations in PDGFβ or receptor for PRGFβ cause idiopathic basal ganglia calcification.[39,60] Although the neuropathological findings with these mutations have not been reported, neuropathological findings in patients with idiopathic basal ganglia calcification showed calcium deposition in pericytes.[38] The mural cells have the capacity to transition into several characteristic states. For example, smooth muscle cells transition from the contracting type to the non-contracting type as well as the osteogenic type, depending on the balance of the signaling pathway.[74,75] It would be interesting to investigate the transition of pericytes to osteogenic pericyte as a result of decreased PDGF-β signaling.

Conclusion

Small cerebral vessels are a lost world in the brain architecture. In the pharmacological field, the role of the small vessels in the brain in relation to the function of the blood–brain barrier and the molecules in the tight junction has been investigated. Indeed, the tight junction plays an important role in maintaining the barrier function; however, recent advances in small vessel research show that selective endocytosis in the capillary also plays an important role in the barrier function.[15] Furthermore, the capillary unit also functions in the draining of interstitial fluid.[3] The fine regulation of microcirculation in the cortex might also be important to maintain brain function.[13,14,51] The pericytes, astrocytes and neuronal regulation could take an important role for these functions. To clarify the pathogenesis of SVD, further research on the anatomical and functional heterogeneity in the small vessels and surrounding cells is required. Furthermore, additional insight on how the cell signaling pathway maintains the small vessel units will provide useful information for the development of a new therapeutic strategy to prevent the progression of SVD.

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References

1 Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol.* 2010; **9**: 689–701.
2 Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. *Lancet Neurol.* 2013; **12**: 483–97.
3 Carare RO, Hawkes CA, Jeffrey M, Kalaria RN, Weller RO. Cerebral amyloid angiopathy, prion angiopathy, CADASIL and the spectrum of protein elimination failure angiopathies (PEFA) in neurodegenerative disease with a focus on therapy. *Neuropathol. Appl. Neurobiol.* 2013; **39**: 593–611.
4 Daneman R. The blood-brain barrier in health and disease. *Ann. Neurol.* 2012; **72**: 648–72.
5 Wilkinson IM. The vertebral artery. Extracranial and intracranial structure. *Arch. Neurol.* 1972; **27**: 392–6.
6 Fujimoto K. "Medial defects" in the prenatal human cerebral arteries: an electron microscopic study. *Stroke* 1996; **27**: 706–8.
7 Weller RO, Boche D, Nicoll JA. Microvasculature changes and cerebral amyloid angiopathy in Alzheimer's disease and their potential impact on therapy. *Acta Neuropathol.* 2009; **118**: 87–102.
8 Nonaka H, Akima M, Nagayama T, Hatori T, Zhang Z, Ihara F. Microvasculature of the human cerebral meninges. *Neuropathology* 2003; **23**: 129–35.
9 Reina-De La Torre F, Rodriguez-Baeza A, Sahuquillo-Barris J. Morphological characteristics and distribution pattern of the arterial vessels in human cerebral cortex: a scanning electron microscope study. *Anat. Rec.* 1998; **251**: 87–96.
10 Akima M. A morphological study on the microcirculation of the central nervous system. Selective vulnerability to hypoxia. *Neuropathology* 1993; **13**: 99–112.
11 Nonaka H, Akima M, Hatori T, Nagayama T, Zhang Z, Ihara F. Microvasculature of the human cerebral white matter: arteries of the deep white matter. *Neuropathology* 2003; **23**: 111–8.
12 Nonaka H, Akima M, Nagayama T, Hatori T, Zhang Z. The fundamental architecture of the microvasculature of the basal ganglia and changes in senility. *Neuropathology* 1998; **18**: 47–54.
13 Hamel E. Perivascular nerves and the regulation of cerebrovascular tone. *J. Appl. Physiol.* 2006; **100**: 1059–64.
14 Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat. Rev. Neurosci.* 2004; **5**: 347–60.
15 Armulik A, Genové G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev. Cell* 2011; **21**: 193–215.
16 Lam CK, Yoo T, Hiner B, Liu Z, Grutzendler J. Embolus extravasation is an alternative mechanism for cerebral microvascular recanalization. *Nature* 2010; **465**: 478–82.
17 Winkler EA, Bell RD, Zlokovic BV. Central nervous system pericytes in health and disease. *Nat. Neurosci.* 2011; **14**: 1398–405.
18 Gaengel K, Genove G, Armulik A, Betsholtz C. Endothelial-mural cell signaling in vascular development and angiogenesis. *Arterioscler. Thromb. Vasc. Biol.* 2009; **29**: 630–8.
19 Cleaver O, Melton DA. Endothelial signaling during development. *Nat. Med.* 2003; **9**: 661–8.
20 Sá-Pereira I, Brites D, Brito MA. Neurovascular unit: a focus on pericytes. *Mol. Neurobiol.* 2012; **45**: 327–47.
21 Krueger M, Bechmann I. CNS pericytes: concepts, misconceptions, and a way out. *Glia* 2010; **58**: 1–10.
22 Daneman R, Zhou L, Kebede AA, Barres BA. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* 2010; **468**: 562–6.
23 Armulik A, Genové G, Mäe M *et al.* Pericytes regulate the blood-brain barrier. *Nature* 2010; **468**: 557–61.
24 Bell RD, Winkler EA, Sagare AP *et al.* Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. *Neuron* 2010; **68**: 409–27.
25 Korn J, Christ B, Kurz H. Neuroectodermal origin of brain pericytes and vascular smooth muscle cells. *J. Comp. Neurol.* 2002; **442**: 78–88.
26 Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol.* 2010; **119**: 7–35.
27 Wolburg H, Noell S, Mack A, Wolburg-Buchholz K, Fallier-Becker P. Brain endothelial cells and the glio-vascular complex. *Cell Tissue Res.* 2009; **335**: 75–96.
28 Molofsky AV, Krenick R, Ullian E *et al.* Astrocytes and disease: a neurodevelopmental perspective. *Genes Dev.* 2012; **26**: 891–907.
29 Oberheim NA, Goldman SA, Nedergaard M. Heterogeneity of astrocytic form and function. *Methods Mol. Biol.* 2012; **814**: 23–45.
30 Zhang Y, Ben A. Barres. Astrocyte heterogeneity: an underappreciated topic in neurobiology. *Curr. Opin. Neurobiol.* 2010; **20**: 588–94.
31 Matyash V, Kettenmann H. Heterogeneity in astrocyte morphology and physiology. *Brain Res. Rev.* 2010; **63**: 2–10.
32 Hochstim C, Deneen B, Lukaszewicz A, Zhou Q, Anderson DJ. Identification of positionally distinct astrocyte subtypes whose

identities are specified by a homeodomain code. *Cell* 2008; **133**: 510–22.
33 Gouw AA, Seewann A, van der Flier WM *et al.* Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. *J. Neurol. Neurosurg. Psychiatr.* 2010; **82**: 126–35.
34 Okeda R, Murayama S, Sawabe M, Kuroiwa T. Pathology of the cerebral artery in Binswanger's disease in the aged: observation by serial sections and morphometry of the cerebral arteries. *Neuropathology* 2004; **24**: 21–9.
35 Tanoi Y, Okeda R, Budka H. Binswanger's encephalopathy: serial sections and morphometry of the cerebral arteries. *Acta Neuropathol.* 2000; **100**: 347–55.
36 Kobayashi S, Yamadori I, Miki H, Ohmori M. Idiopathic nonarteriosclerotic cerebral calcification (Fahr's disease): an electron microscopic study. *Acta Neuropathol.* 1987; **73**: 62–6.
37 Kozik M, Kulczycki J. Laser-spectrographic analysis of the cation content in Fahr's syndrome. *Arch. Psychiat. Nervenkr.* 1978; **225**: 135–42.
38 Duckett S, Galle P, Escourolle R, Poirier J, Hauw JJ. Presence of zinc, aluminum, magnesium in striopalledodentate (SPD) calcifications (Fahr's disease): electron probe study. *Acta Neuropathol.* 1977; **38**: 7–10.
39 Westenberger A, a-Murias MGI, Domingo A *et al.* Mutations in the gene encoding PDGF-B cause brain calcifications in humans and mice. *Nat. Genet.* 2013; **45**: 1077–82.
40 Wang C, Li Y, Shi L *et al.* Mutations in SLC20A2 link familial idiopathic basal ganglia calcification with phosphate homeostasis. *Nat. Genet.* 2012; **44**: 254–6.
41 Manyam BV. What is and what is not "Fahr's disease". *Parkinsonism Relat. Disord.* 2005; **11**: 73–80.
42 Viswanathan A, Godin O, Jouvent E *et al.* Impact of MRI markers in subcortical vascular dementia: a multi-modal analysis in CADASIL. *Neurobiol. Aging* 2010; **31**: 1629–36.
43 Liem MK, van der Grond J, Haan J *et al.* Lacunar infarcts are the main correlate with cognitive dysfunction in CADASIL. *Stroke* 2007; **38**: 923–8.
44 Fukutake T. Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL): from discovery to gene identification. *J. Stroke Cerebrovasc. Dis.* 2011; **20**: 85–93.
45 Nishimoto Y, Shibata M, Nihonmatsu M *et al.* A novel mutation in the HTRA1 gene causes CARASIL without alopecia. *Neurology* 2011; **76**: 1353–5.
46 Yanagawa S, Ito N, Arima K, Ikeda S-I. Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy. *Neurology* 2002; **58**: 817–20.
47 Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ* 2010; **341**: c3666.
48 Sonnen JA, Larson EB, Crane PK *et al.* Pathological correlates of dementia in a longitudinal, population-based sample of aging. *Ann. Neurol.* 2007; **62**: 406–13.
49 Brown WR, Moody DM, Thore CR, Challa VR, Anstrom JA. Vascular dementia in leukoaraiosis may be a consequence of capillary loss not only in the lesions, but in normal-appearing white matter and cortex as well. *J. Neurol. Sci.* 2007; **257**: 62–6.
50 Iadecola C. The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia. *Acta Neuropathol.* 2010; **120**: 287–96.
51 Itoh Y, Suzuki N. Control of brain capillary blood flow. *J. Cereb. Blood Flow Metab.* 2012; **32**: 1167–76.
52 Brooke BS, Bayes-Genis A, Li DY. New insights into elastin and vascular disease. *Trends Cardiovasc. Med.* 2003; **13**: 176–81.
53 Wint DP, Butman JA, Masdeu JC *et al.* Intracranial arteries in individuals with the elastin gene hemideletion of williams syndrome. *Am. J. Neuroradiol.* 2014; **35**: 90–94.
54 Faury G, Pezet M, Knutsen RH *et al.* Developmental adaptation of the mouse cardiovascular system to elastin haploinsufficiency. *J. Clin. Invest.* 2003; **112**: 1419–28.
55 Li DY, Brooke B, Davis EC *et al.* Elastin is an essential determinant of arterial morphogenesis. *Nature* 1998; **393**: 276–80.
56 Munot P, Saunders DE, Milewicz DM *et al.* A novel distinctive cerebrovascular phenotype is associated with heterozygous Arg179 ACTA2 mutations. *Brain* 2012; **135**: 2506–14.
57 Engerman RL, Kern TS. Progression of incipient diabetic retinopathy during good glycemic control. *Diabetes* 1987; **36**: 808–12.
58 Joutel A, Monet-Lepretre M, Gosele C *et al.* Cerebrovascular dysfunction and microcirculation rarefaction precede white matter lesions in a mouse genetic model of cerebral ischemic small vessel disease. *J. Clin. Invest.* 2010; **120**: 433–45.
59 Dziewulska D, Lewandowska E. Pericytes as a new target for pathological processes in CADASIL. *Neuropathology* 2012; **32**: 515–21.
60 Nicolas G, Pottier C, Maltête D *et al.* Mutation of the PDGFRB gene as a cause of idiopathic basal ganglia calcification. *Neurology* 2013; **80**: 181–7.
61 Owens T, Bechmann I, Engelhardt B. Perivascular spaces and the two steps to neuroinflammation. *J. Neuropathol. Exp. Neurol.* 2008; **67**: 1113–21.
62 Weller RO, Galea I, Carare RO, Minagar A. Pathophysiology of the lymphatic drainage of the central nervous system: implications for pathogenesis and therapy of multiple sclerosis. *Pathophysiology* 2010; **17**: 295–306.
63 Thal DR, Capetillo-Zarate E, Larionov S, Staufenbiel M, Zurbruegg S, Beckmann N. Capillary cerebral amyloid angiopathy is associated with vessel occlusion and cerebral blood flow disturbances. *Neurobiol. Aging* 2009; **30**: 1936–48.
64 Thal DR, Griffin WS, de Vos RA, Ghebremedhin E. Cerebral amyloid angiopathy and its relationship to Alzheimer's disease. *Acta Neuropathol.* 2008; **115**: 599–609.
65 Kuo DS, Labelle-Dumais C, Gould DB. COL4A1 and COL4A2 mutations and disease: insights into pathogenic mechanisms and potential therapeutic targets. *Hum. Mol. Genet.* 2012; **21**: R97–110.
66 Zarate YA, Hopkin RJ. Fabry's disease. *Lancet* 2008; **372**: 1427–35.
67 Monet-Lepretre M, Haddad I, Baron-Menguy C *et al.* Abnormal recruitment of extracellular matrix proteins by excess Notch3ECD: a new pathomechanism in CADASIL. *Brain* 2013; **136**: 1830–45.
68 Hara K, Shiga A, Fukutake T *et al.* Association of HTRA1 mutations and familial ischemic cerebral small-vessel disease. *N. Engl. J. Med.* 2009; **360**: 1729–39.
69 Oide T, Nakayama H, Yanagawa S, Ito N, Ikeda S-I, Arima K. Extensive loss of arterial medial smooth muscle cells and mural extracellular matrix in cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL). *Neuropathology* 2008; **28**: 132–42.
70 Oka C, Tsujimoto R, Kajikawa M *et al.* HtrA1 serine protease inhibits signaling mediated by TGF-β family proteins. *Development* 2004; **131**: 1041–53.
71 Shiga A, Nozaki H, Yokoseki A *et al.* Cerebral small-vessel disease protein HTRA1 controls the amount of TGF-β1 via cleavage of proTGF-beta1. *Hum. Mol. Genet.* 2011; **20**: 1800–10.

72 Shi M, Zhu J, Wang R *et al.* Latent TGF-β structure and activation. *Nature* 2011; **474**: 343–9.
73 Grau S, Baldi A, Bussani R *et al.* Implications of the serine protease HtrA1 in amyloid precursor protein processing. *Proc. Natl Acad. Sci. U. S. A.* 2005; **102**: 6021–6.
74 Olson LE, Soriano P. PDGFRβ signaling regulates mural cell plasticity and inhibits fat development. *Dev. Cell* 2011; **20**: 815–26.
75 Giachelli CM. Regulation of vascular calcification: roles of phosphate and osteopontin. *Circ. Res.* 2005; **96**: 717–22.

Topical Review

Section Editors: James Meschia, MD, and Elisabeth Tournier-Lasserve, MD

Features of Cerebral Autosomal Recessive Arteriopathy With Subcortical Infarcts and Leukoencephalopathy

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The cerebral small vessel system plays a fundamental role in maintaining higher brain function.[1] Although lacunar stroke has been recognized as a disease in which small vessels are mainly affected, advances in neuroradiological examination extend our knowledge of small vessel disease to white matter lesions, microbleeds, and cortical microinfarction.[2] Accumulating evidence indicates that the risk factors and the therapeutic strategies are different for large vessel disease and small vessel disease.[3] Moreover, the recent discoveries on monogenic disorders, which mainly affect small vessels, clearly indicate that the human cerebral small vessels have distinct molecular characteristics of cerebral large vessels.[4] However, little is known about the molecular pathogenesis of small vessel disease and how it is different from that of large vessel disease. The investigation of hereditary small vessel disease is necessary to clarify the molecular pathogenesis of cerebral small vessel disease (CSVD).

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy is the most common dominant inherited CSVD, whereas cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) is a rare form of inherited CSVD.[5,6] Fukutake[6,7] has proposed the clinical triad for CARASIL, leukoencephalopathy, alopecia, and lumbago and has summarized the clinical findings of CARASIL in these patients. The identification of the causative gene for CARASIL allows a new understanding of the molecular pathogenesis of CSVD.[5] Although CARASIL has been considered to be restricted to Japan, we now know that CARASIL exists in other populations. In this review, we update the clinical findings of CARASIL confirmed by genetic analysis and molecular pathogenesis of CARASIL.

What Is CARASIL?

In 1976, Maeda et al[8] reported familial unusual encephalopathy of the Binswanger's type without hypertension in siblings whose parents were consanguineous. They showed early adult-onset dementia, pseudobulbar palsy, and pyramidal and extrapyramidal symptoms. Postmortem studies revealed diffuse and focal demyelination with sparing of U-fibers, multiple small foci of perivascular softening in the cerebral white matter and the basal ganglia, and severe arteriosclerotic changes in the meningeal small arteries and long arteries in the cerebral white matter. The other characteristic features were severe lumbago and alopecia during the teenage years. In 1995, Fukutake and Hirayama[6] studied the reported cases, including their own cases of juvenile-onset Binswanger-type encephalopathy accompanied by alopecia and lumbago in an autosomal recessive form and proposed new disease criteria for CARASIL.

In 2009, Hara et al[5] identified that the mutation in the high-temperature requirement serine peptidase A1 (*HTRA1*) gene codes a protease in patients with CARASIL. To date, 10 mutations in the *HTRA1* gene have been identified in 12 families (Figure 1A; Table).[5,6,9–19] Most patients with CARASIL have been reported in Japan; however, in families with CARASIL, we cannot find any founder haplotype that explains this regional accumulation. Moreover, 2 Chinese families,[15,16] 2 white families,[17,18] and 1 Turkish family[19] have been identified as having CARASIL. As described later in this review, clinical heterogeneity has been recognized in CARASIL. These findings indicate that CARASIL is not unique to the Japanese population and might be underdiagnosed.

Clinical Features of CARASIL

We have obtained and summarized clinical features of patients with genetically proven CARASIL from the literature or medical records (Table).[5,6,9–19] Patients with CARASIL present with early adult-onset dementia, gait disturbance, alopecia, and low back pain.[5,6] Motor and mental abnormalities develop at the age of ≈30 years (dementia: mean age of onset, 35.1 years [range, 24–50 years]; gait disturbance: mean age of onset, 30.7 years [range, 23–39 years]). Then, a diffuse symmetrical white matter lesion is noticed on neuroradiological

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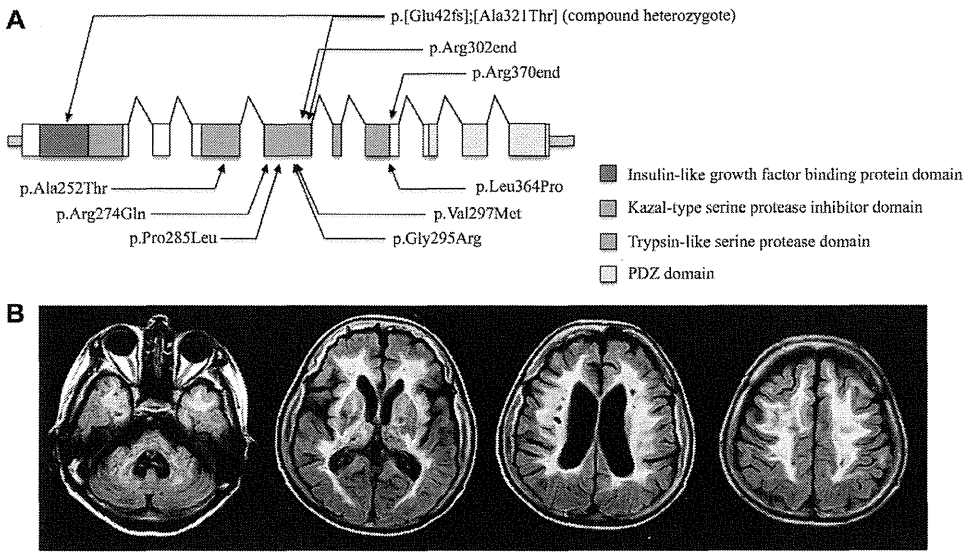

Figure 1. *HTRA1* mutations and brain MRI findings in cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy. **A**, Distribution of *HTRA1* mutations. *HTRA1* gene consists of 9 exons (squares): those encoding the insulin-like growth factor–binding protein domain (red; 35–111 aa); the Kazal-type serine protease inhibitor domain (blue; 114–155 aa); the trypsin-like serine protease domain (orange; 204–364 aa); and the PDZ domain (green; 382–473 aa). All individuals are homozygotes for missense or nonsense mutations, except for the patient with p.[Glu42fs];[Ala321Thr].[5,6,9–19] **B**, Brain images of fluid-attenuated inversion recovery of the patient with p.Arg370end. Extensive white matter lesions involving the anterior temporal lobe are seen. These findings are accompanied by multiple lacunes in the periventricular regions and the thalami. The hyperintensities in the external and internal capsules are also observed.

examination, suggesting CARASIL. The patients do not have hypertension or diabetes mellitus, which are the major risk factors for sporadic CSVD. Diffuse thinning of hair without hairline recession beginning during the teenage years or when patients are in their 20s has been recognized in 9 of 12 families (mean age, 16.7 years; range, 0–27 years). Pubic hair loss and body hair loss have not been reported. Acute mid- to lower-back pain has been noticed at a mean age of 24.9 years (range, 14–39 years). Mood changes (apathy and irritability), pseudobulbar palsy, hyper-reflexia, Babinski sign, and urinary incontinence are frequently observed. Motor and cognitive functions slowly decline, and 7 of 13 patients needed wheelchairs by 30 to 40 years of age. An acute ischemic stroke event has been reported in 23.1%, and no hemorrhagic stroke events have been reported. Five of 13 patients (38.5%) have experienced horizontal nystagmus. Two of 13 patients (15.4%) have experienced advanced-stage seizures. Obvious migraines have not been reported in these patients, and there has been no skin color change in the extremities. Retina and kidney involvement have not been reported; however, in other small vessel diseases, involvement of retinal vessels or involvement of renal dysfunction has been reported.

Neuroradiological Findings in CARASIL

We have directly reviewed brain MRI results from 7 patients with CARASIL (Table).[5,6,9,11–14] White matter hyperintensity on T2-weighted or fluid-attenuation inversion recovery images is symmetrically distributed and located periventricular to subcortical white matter (Figure 1B). Abnormalities are observed in the white matter of the anterior temporal lobe, cerebellum, brain stem, and external capsule. Although these findings resemble those of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, it is not clear whether the white matter changes in the anterior temporal poles and external capsule, which are characteristic early signs in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy,[20] are also observed in early stages of CARASIL. The magnetic resonance spectroscopy finding of a patient with dementia and pyramidal signs has shown a normal N-acetyl aspartate peak in the white matter lesion, indicating the absence of neuroaxonal degeneration.[21] In contrast, the choline peak was elevated, which is a finding consistent with ischemia-induced demyelination. Lacunar infarctions are detected in the thalamus, basal ganglia, and deep white matter. At the progressive stage, diffuse brain atrophy and both lobar and nonlobar microbleeds in cerebral cortex, thalamus, and cerebellum are observed. U-fibers are relatively preserved even during the late stage. Brain magnetic resonance angiography and conventional angiography do not show any pathological changes. Single-photon emission computed tomography shows hypoperfusion in the frontal lobe. On spinal MRI, spondylosis deformans and disk degeneration are observed in cervical and lumbar spine at the age of ≈30 years. Interestingly, these findings have not been identified during their early stages. Therefore, it is still unknown why lumbago symptoms occur during the teenage years.

Cerebral Small Vessel Pathology in CARASIL

The autopsy findings of CARASIL have been reported in 3 instances: in a patient with p.Arg302end, a mutation in the *HTRA1* gene; in a sibling with p.Ala252Thr; and in the original patient.[8,12,22,23] In the cerebral small arteries, smooth muscle cells were extensively lost, even in arteries without

Table. Summary of Clinical Features in Patients With Cerebral Autosomal Recessive Arteriopathy With Subcortical Infarcts and Leukoencephalopathy and *HTRA1* Mutations

														Mean Ages at Onset, y (Range)
Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	...
Reference	5, 12	13, 14	14	16	17	5, 10	5, 11	5, 6	5, 9	15	5	19	18	...
Family	I	II	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	...
Consanguinity of family	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	...
Mutation (nucleotide and amino acids)	c.754G>A p.A252T	c.821G>A p.R274Q	c.821G>A p.R274Q	c.854C>T p.P285L	c.883G>A p.G295R	c.889G>A p.V297M	c.889G>A p.V297M	c.904C>T p.R302end	c.904C>T p.R302end	c.1091T>C p.L364P	c.1108C>T p.R370end	c.1108C>T p.R370end	c.[126delG];[961G>A] p.[E42fs];[A321T]	...
Age at time of study, y	48	41	44	26	43	33	50	44	46	27	44	27	29	...
Sex	F	M	F	F	M	M	F	M	F	F	F	F	F	...
Brain MRIs were dierctly reviewed by author	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	No	Yes	No	No	...
Symptoms, y														
Migraine	–	–	–	–	–	–	–	–	–	–	–	–	–	...
Alopecia	–	–	–	0	18	14	16	16	14	27	18	27	–	16.7 (0–27)
Spinal spondylosis	Teens	39	37	26	34	33	39	22	32	26	21	27	29	30.4 (21–39)
Gait disturbance	39	38	37	23	34	29	31	26	29	27	35	27	24	30.7 (23–39)
Acute stroke event	38	–	–	–	–	–	–	31	–	–	–	–	24	31.0 (24–38)
Mood change	–	–	43	24	34	–	–	–	29	–	35	–	–	33.0 (24–43)
Urinary incontinence	–	–	–	24	34	29	50	38	31	–	–	–	–	34.3 (24–50)
Dementia	–	38	43	24	–	33	50	37	29	27	35	–	–	35.1 (24–50)
Seizure	–	–	–	–	–	–	–	42	31	–	–	–	–	36.5 (31–42)
Wheel chair bound	48	41	42	–	43	–	–	37	32	–	39	–	–	40.3 (32–48)
Neurological findings														
Horizontal nystagmus	–	–	+	–	–	+	–	–	+	–	+	–	+	...
Pseudobulbar palsy	+	+	–	+	+	+	+	+	+	–	+	–	–	...
Hyper-reflexia of limbs	+	+	+	+	+	+	+	+	+	+	+	+	–	...
Babinski sign	–	–	–	+	+	+	+	+	+	+	+	+	–	...
Rigidity	–	–	–	–	–	+	–	+	–	–	–	–	–	...
Optic fundi	Slight arteriolosclerosis	Not done	Normal	Not done	Not done	Not done	Normal	Not done	Normal	Not done	Not done	Not done	Not done	...
Risk factors														
Hypertension	–	–	–	–	–	–	–	–	–	–	–	–	–	...
Diabetes mellitus	–	–	–	–	–	–	–	–	–	–	–	–	–	...
Dyslipidemia	–	–	–	–	–	–	–	–	–	–	–	–	–	...
Alcohol	–	+	–	–	+	–	–	–	–	–	–	–	–	...
Smoking	–	+	–	–	+	–	–	–	–	–	–	–	–	...

Information from the patients whose clinical features are available.[5,6,9–19] + indicates present; and –, not available.

sclerotic changes. Sclerotic changes were mild and infrequent; most of the arteries were enlarged rather than exhibiting luminal stenosis. Tunica media of the cerebral small arteries exhibited hyalinosis and were immunopositive for fibrinogen. These pathological findings resemble those observed in nonhereditary ischemic CSVD.[24] In the patients with nonhereditary ischemic CSVD, marked degeneration of vascular smooth muscle cells with collapse and dilatation in the cerebral small arteries, the so-called earthen pipe phenomenon, were observed.[24] These changes might disturb autoregulatory mechanisms for cerebral blood flow, resulting in ischemic changes in the deep white matter.[25]

The internal elastic membrane, which is composed of elastin, is split into multiple layers and fragmented. Some intima is thickened with fibrosis and involves myointimal cells, which were sparsely stained by α-smooth muscle actin antibody. Arterial adventitia was thin and decreased immunoreactivity for type I, type III, and type VI collagens.[23] These changes were relatively limited in cerebral small arteries and were not detected in intracranial large arteries and extracranial arteries. Lysosome-like bodies were found in the cytoplasm of smooth muscle cells in small arteries.[12] No obvious deposit or inclusion, including granular osmiophilic material or amyloid, was observed. Diffuse myelin pallor in the cerebral white matter with sparing U-fibers and multiple small foci of perivascular softening in the cerebral white matter, basal ganglia, and brain stem were observed.

Loss of HTRA1 Protease Function Causes CARASIL

The *HTRA1* gene consists of 9 exons producing HTRA1, a serine protease belonging to the HTRA protein family whose members have dual activities as chaperones and serine proteases (Figure 1A).[26] HTRA1 has an N-terminal insulin-like growth factor–binding protein domain, a Kazal-type serine protease inhibitor domain, a trypsin-like serine protease domain, and a C-terminal PDZ domain.[27] HTRA1 proteases exist as trimers, thus allowing communication between adjacent subunits to regulate protease. The activation cascade is initiated by the ligand-dependent interaction of neighboring HTRA1s in a trimer, thereby inducing the proper adjustment of the activation domain His220, Asp250, and Ser328 in the trypsin-like serine protease domain.[28,29]

To date, 10 mutations in the *HTRA1* gene have been identified in 13 patients from 12 families (Figure 1A).[5,6,9–19] They include 7 missense mutations, 2 nonsense mutations, and 1 deletion mutation. The premature termination codons, which are caused by the nonsense or deletion mutations, fulfill the criteria of the nonsense-mediated mRNA decay, indicating the marked reduction of the amounts of mRNA from these alleles.[5,30] All of the missense mutations were located in or around the protease domain of HTRA1, suggesting the reduction in the protease activity. The disease-associated mutant HTRA1s (p.Ala252Thr, p.Arg274Gln, and p.Val297Met) decrease their protease activity.[5,14] These findings indicate that CARASIL is caused by the loss of HTRA1 or its protease activity. Among the mutations with HTRA1, the residual HTRA1 activity of p.Arg302end, which completely loses its protease domain, should be the lowest. Therefore, we can speculate that patients with p.Arg302end show the most severe phenotype with CARASIL; however, the onset and the clinical severities are similar for the patients with p.Arg302end and other patients (Table).

Dysregulation of Transforming Growth Factor-β Signaling Underlies Molecular Pathogenesis in CARASIL

Studies have shown that HTRA protein decreases transforming growth factor-β (TGF-β) family signaling.[31] TGF-β is a cytokine that promotes cell differentiation and fibrous proliferation in response to tissue damage and has an important role in vascular integrity.[32] Loss of HTRA1 activity leads to an increase in TGF-β signaling. CARASIL-associated mutant HTRA1s fail to decrease TGF-β family signaling.[5] Moreover, the extra domain A of fibronectin and versican, which are induced by increased TGF-β signaling, accumulate in the hypertrophic intima of cerebral small arteries.[5] In addition, hyaluronan, an extracellular matrix protein that is induced by TGF-β1 signaling, also accumulates in the small cerebral arterial walls.[30] In endothelial cells of small cerebral arteries, the expression of phosphorylated Smad2, which is induced by TGF-β1 signaling, increased. Finally, TGF-β1 and latency-associated peptide, which forms a complex with TGF-β1, increase in the cerebral small arteries of patients with CARASIL.[5,30] No expression of extra domain A of fibronectin was detected in arterial walls of coronal tissue, renal arteries, or the aorta from a patient with CARASIL. These findings indicate that the increased TGF-β signaling plays a pivotal role in the pathogenesis of CSVD in CARASIL. Acceleration of TGF-β signaling might cause the degeneration of vascular smooth muscle cells because TGF-β signaling has an important role in the differentiation of vascular smooth muscle cells. In extracentral nervous system symptoms of CARASIL, upregulation of TGF-β family signaling might cause alopecia or spondylosis deformans.[33,34]

How the HTRA1 Inhibits TGF-β Signaling

TGF-β signaling is temporally and spatially regulated by balance among maturation, sequestration, and presentation (Figure 2).[32,35] TGF-β is synthesized as a homodimeric proprotein (proTGF-β) and is subsequently cleaved into an N-terminal latency-associated peptide and a C-terminal mature TGF-β by a proprotein convertase, such as furin, in the trans-Golgi network. Latency-associated peptide forms a noncovalent complex with a dimer of mature TGF-β. This complex binds to a latent TGF-β–binding protein, and the bound complex is then secreted and anchored to the extracellular matrix, resulting in the sequestration of the mature TGF-β in the extracellular space. The sequestered mature TGF-β is activated by serine protease, matrix metalloproteinase, or acidic microenvironments in the extracellular space. The extracellular matrix, which stores TGF-β in a complex with latency-associated peptide and latent TGF-β–binding protein, also regulates the bioavailability of TGF-β. The activation of mature TGF-β is the rate-limiting step for TGF-β signaling.

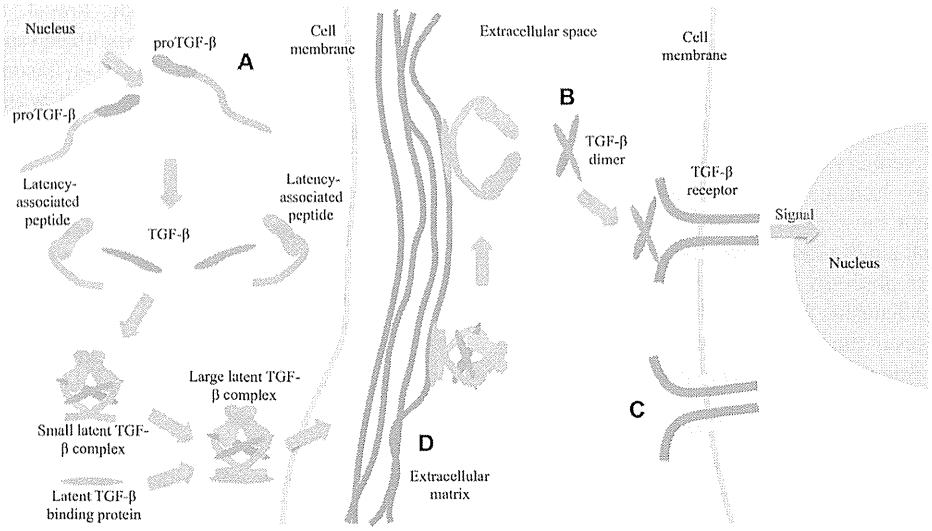

Figure 2. The schema of transforming growth factor-β (TGF-β) processing and possible interactions between TGF-β and HTRA1. **A**, TGF-β is synthesized as a proprotein (proTGF-β), which undergoes proteolytic processing.[32,35] The proTGF-β is then cleaved by furin convertase. The cleaved products yield a small latent TGF-β complex, in which the latency-associated peptides and the TGF-β dimer are connected. The small latent TGF-β complex binds with latent TGF-β binding protein and is secreted into the extracellular space and anchored in the extracellular matrix. Chemical stress or proteases can open small latent TGF-β complex to release the TGF-β dimer. The TGF-β dimer binds to TGF-β receptor. HTRA1 might cleave (**A**) proTGF-β,[30] (**B**) TGF-β dimer,[31,36] (**C**) TGF-β receptor,[37] or (**D**) extracellular matrix proteins.[26] The cleaved products are degradated, resulting in the reduction of TGF-β signaling.

Tighter regulation of bioavailability of TGF-β in intracellular and extracellular spaces is important for regulating its signaling.

For the downregulation mechanism of TGF-β signaling by HTRA1, we have proposed that HTRA1 cleaves the prodomain of proTGF-β1 in the endoplasmic reticulum before furin processes proTGF-β1 in the trans-Golgi network.[30] The aberrant cleaved products of proTGF-β1 are degradated by the endoplasmic reticulum–associated degradation system, leading to a reduced amount of mature TGF-β1. In contrast, it has been reported that HTRA1 cleaves mature TGF-β1 or TGF-β1 receptors in extracellular space.[31,36,37] However, all results in regard to the downregulation of TGF-β signaling by HTRA1 were obtained by the overexpression conditions; thus, the downregulation mechanism under physiological conditions is still unclear.

Why Vascular Pathology Is Predominant in Cerebral Small Vessels

The selectivity of cerebral small vessels in CARASIL is not explained by the expression of HTRA1. Although the specificity of the antibodies has not been evaluated fully, HTRA1 is ubiquitously expressed in various human tissues.[38] Therefore, we have to consider a unique role of HTRA1 or TGF-β family signaling in maintaining the integrity of cerebral small vessels. TGF-β1 is secreted from astrocytes, microglia, smooth muscle cells, and endothelial cells in neurovascular units and plays an important role in maintaining their function and survival.[39] HTRA1 is expressed in endothelial cells and astrocytes in cerebral small vessels.[40] HTRA1 cleaves proTGF-β1 and downregulates TGF-β1 synthesis in these cells.[30] The intracellular cleavage of proTGF-β1 is a unique mechanism for regulating the amount of TGF-β family protein, indicating that this mechanism has some specific role for circumstance-dependent regulation of TGF-β signaling in cerebral small vessels.

The other factors that regulate TGF-β signaling are an activation system and receptors for TGF-β. Fibrinogen-bound latent TGF-β interacts with astrocytes, leading to active TGF-β formation.[39] In CARASIL patients, fibrinogen deposited in tunica media of cerebral small arteries might accelerate the TGF-β signaling in cerebral small vessels. TGF-β1 binds type I and type II receptors on the plasma membrane, and each type involves several different receptors. On ligand-induced heteromeric complex formation, the type I receptor is phosphorylated by the type II receptor. TGF-β signaling is temporally and spatially regulated by the diversity of these receptors and coreceptors in each cell type.[41] Different expressions of the receptors in each type of cell in cerebral small arteries might be associated with the vascular pathology of CARASIL. The profiles of the receptors and coreceptors in the cerebral small arteries should be elucidated.

Clinical Heterogeneity of CARASIL

Although leukoencephalopathy, lumbago, and alopecia are the clinical triad of CARASIL, we have realized that some patients with mutations in the *HTRA1* gene do not show signs of alopecia (Table). The frequency of alopecia in families with genetically proven CARASIL is 72.7%. Moreover, when low back pain begins, sometimes there is no apparent neuroradiological finding in the lumbar spine.

We have to be cautious because most of the reported cases of CARASIL are suspected because of the existence of early-onset leukoencephalopathy. However, there is a possibility that residual protease activity could affect the severity of the disease. Thus, we imagine that some patients show later-onset