

# Hemorrhagic cerebral small vessel disease caused by a novel mutation in 3' UTR of collagen type IV alpha 1

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Collagen type IV alpha 1 (COL4A1) is a major basement membrane component. Haploinsufficiency mutations in *COL4A1* interrupt the basement membrane integrity, causing hemorrhagic stroke.<sup>1</sup> Recently, mutations causing overproduction of *COL4A1* mRNA have been identified in patients with cerebral small vessel disease (CSVD), including in members of families with pontine autosomal dominant microangiopathy with leukoencephalopathy<sup>2</sup> and multi-infarct dementia of Swedish type.<sup>3</sup> These mutations are located in the 3' untranslated region (UTR) of *COL4A1* and disrupt the binding of miR-29 to the 3'UTR of *COL4A1*.<sup>2</sup> Both diseases are characterized by leukoencephalopathy, multiple microbleeds (MBs), and multiple lacunar infarctions (LIs) with pontine involvements.<sup>2–4</sup> However, among these patients, only case of hemorrhagic stroke was reported.<sup>5</sup> Thus, it is unclear whether these mutations, which increase the amount of normal COL4A1, cause hemorrhagic stroke. Here, we report a patient with hemorrhagic stroke with a novel mutation in the 3'UTR of *COL4A1*.

## Case report

The patient in this case report had no vascular risk factors, such as hypertension, diabetes, and smoking. Detailed family history was unavailable because his parents died in his childhood. He developed a hemorrhagic stroke at age 30 years. Although detailed information on the episode was unavailable, he had no deficits except for cognitive impairment. However, he had progressive gait disturbance and cognitive impairment. At age 42 years, he was admitted to another hospital, following which, he moved to our hospital at age 44 years. On admission, his blood pressure was 114/74 mm Hg, and laboratory examination showed no renal dysfunction (serum Cr, 0.42 mg/dL). Neurologic examination revealed adiadochokinesis and spasticity in both the lower limbs; he was unable to stand up without support. The Mini-Mental State Examination score in Japanese was 11 of 30. Gastrostomy was performed due to progressive dysphagia at age 46 years. At age 54 years, brain MRI using the 3 T system showed severe white matter lesions and multiple LIs with pontine involvement. Multiple MBs were found in the subcortical regions, deep structures, brainstem, and cerebellum. In addition, superficial cortical siderosis at the right temporal lobe and hemorrhagic changes at the splenium of the left corpus callosum were observed (figure, A). He died at age 56 years because of pneumonia. An autopsy was not performed.

This study was approved by the ethical board of Niigata University. We extracted genomic DNA from the blood sample. Sequence analysis of exons 2–24 in *NOTCH3* and exons 1–9 in high-temperature requirement A serine peptidase 1 was negative for pathogenic mutations.

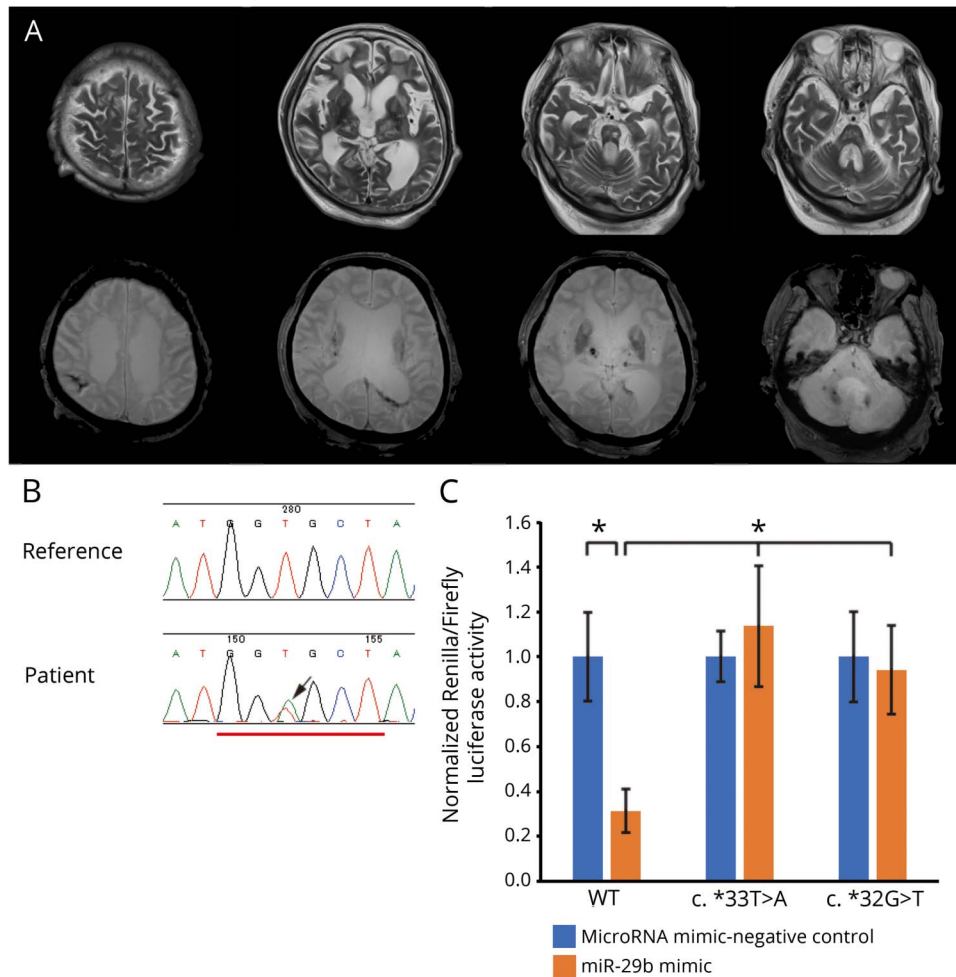
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(A) Brain MRIs using the 3 T system (Skyra, Siemens, Germany) at age 54 years. Upper panel: T2-weighted images of brain MRI show severe white matter lesions (WMLs) and multiple lacunar infarctions including the pons. In addition, severe diffuse brain atrophy is also observed. WMLs are not found in the anterior temporal lobe. Lower panel: T2\*-weighted images (T2\*WI) of brain MRI show multiple microbleeds. In addition, superficial cortical siderosis in the right parietal lobe and hemorrhagic findings in the left splenium of the corpus callosum are seen. (B) Sequence analysis of *COL4A1* shows a heterozygous mutation of c.\*33T>A in the 3' untranslated region (UTR) (arrow). The redline indicates the seed sequence of miR-29. (C) The amplicons with WT, c.\*33T>A or c.\*32G>T of *COL4A1* 3'UTR were subcloned into psiCHECK-2 Dual-Luciferase Reporter plasmid (Promega, Madison, WI; Cat. #C8021). WT was used as a negative control of disruption of the miR-29 binding to the seed sequence, and c.\*32G>T was used as a positive control. These plasmids were cotransfected into the HEK293T cells with 10 pmol of either miRIDIAN hsa-miR-29b-3p or Mimic Negative Control #1 (Dharmacon, Lafayette, CO; Cat. # C-300521-05-0005 and Cat. # CN-001000-01-05, respectively), using Dharma FECT Duo (Dharmacon, Cat. # T-2010-01). After 48 hours of incubation, Renilla/Firefly luciferase activities in the lysed cells were measured using the Dual-Luciferase Reporter Assay System (Promega, Cat. #E1910) in a FilterMax F5 Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, CA). Renilla/Firefly luciferase activities were measured twice. Renilla luciferase activity was normalized to the background Firefly luciferase activity produced from the same psiCHECK-2 vector for each condition. Statistical analysis was performed using RStudio (version 1.0.153). Each group was compared by a 2-sided Welch test. The graph shows the ratio of normalized Renilla/Firefly luciferase activities of each construct. In WT, the normalized Renilla/Firefly luciferase activity was significantly decreased in miR-29b than in the mimic negative control. In c.\*33T>A, the normalized Renilla/Firefly luciferase activity did not decrease in miR-29b. \* $p < 0.001$ . I bars indicate SD. *COL4A1* = collagen type IV alpha 1; WT = wild type.

Whole-exome sequencing was performed by Macrogen (South Korea). After excluding the intronic, synonymous variants or variants with more than 1% of allele frequency in the database of 1000 Genome phase 3, we investigated the causative genes of CSVD.<sup>6</sup> Furthermore, we removed the nonpathogenic mutations as referenced by the database of ExAC (exac.broad-institute.org) and ClinVar (ncbi.nlm.nih.gov/clinvar/). A novel mutation in the 3'UTR of *COL4A1* (c.\*33T>A) was identified and confirmed by the Sanger method (figure, B). This mutation is located within the binding region of miR-29 as identified from the TargetScan database (targetscan.org/vert\_72/). Dual-Luciferase reporter assay was performed to investigate

whether this mutation disrupts the binding of miR-29 to the 3'UTR of *COL4A1* as described previously.<sup>2</sup> Normalized Renilla/Firefly luciferase activities were significantly higher in c.33\*T>A than in WT (figure, C), indicating that c.33\*T>A disrupts the miR-29 binding to the 3'UTR of *COL4A1*.

## Discussion

Herein, we report a patient with a novel mutation in 3'UTR of *COL4A1*. The mutation increased the amount of *COL4A1* mRNA, as the previously reported mutations in 3'UTR. Our case

resembles multi-infarct dementia of Swedish type with respect to several clinical features, such as multiple LIs and MBs, cortical atrophy, and absence of anterior temporal lesions.<sup>3</sup> Cortical atrophy, which is frequently observed in advanced cases with hereditary CSVD, might be caused by secondary degeneration of the cerebral cortex.<sup>7</sup> However, some clinical features were different from those of previous reports, such as longer disease duration or absence of ischemic stroke. The most characteristic finding in this patient is a hemorrhagic lesion in the splenium of the corpus callosum. In families with multi-infarct dementia of Swedish type, 1 patient had a hemorrhagic stroke in the thalamus and lentiform nucleus during anticoagulation treatment, which is a typical lesion. In our patient, the atypical hemorrhagic lesions in the absence of risk factors stressed the association between hemorrhagic CSVD and the mutation. Our case also suggests that the increased expression of COL4A1 may sufficiently interrupt the integrity of the basement membrane causing hemorrhagic stroke.

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### Disclosure

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Name	Location	Role	Contribution
<b>Naoko Sakai, MD</b>	Niigata University, Japan	Author	Drafted the manuscript, acquired the data, and conducted statistical analysis

### Appendix (continued)

Name	Location	Role	Contribution
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<b>Taisuke Kato, PhD</b>	Niigata University, Japan	Author	Acquired the data and revised the draft
<b>Hiroaki Nozaki, MD, PhD</b>	Niigata University, Japan	Author	Acquired the data and revised the draft
<b>Akihide Koyama, PhD</b>	Niigata University, Japan	Author	Acquired the data and revised the draft
<b>Shouichirou Ando, MD</b>	Niigata University, Japan	Author	Revised the draft
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<b>Motohiro Kato, MD, PhD</b>	Fukuoka Mirai Hospital, Japan	Author	Obtained clinical data and revised the draft
<b>Osamu Onodera, MD, PhD</b>	Niigata University, Japan	Author	Critically revised the draft, designed the study, and acquired funding

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