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Case Report

Identification of a Novel *ACVRL1* Gene Mutation (c.100T>A, p.Cys34Ser) in a Japanese Patient with Possible Hereditary Hemorrhagic Telangiectasia (Osler-Weber-Rendu Disease)

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Hereditary hemorrhagic telangiectasia (HHT; also known as Osler-Weber-Rendu disease) is an autosomal dominant genetic disorder that causes frequent epistaxis, mucocutaneous telangiectasia, and visceral arteriovenous malformations. Four genes (*ENG*, *ACVRL1*, *SMAD4*, and *GDF2*) have been identified as pathogenic in HHT. We describe the case of a 50-year-old Japanese man highly suspected of having HHT due to recurrent epistaxis, mucocutaneous telangiectasia, and a family history. Genomic analysis revealed a novel missense mutation of c.100T>A, p.Cys34Ser in the patient's *ACVRL1* gene. We used 6 freeware programs to perform an *in silico* analysis of this mutation. The results demonstrated the mutation's high pathogenicity.

Key words: *ACVRL1*, hereditary hemorrhagic telangiectasia, *in silico* analysis, missense mutation, Osler-Weber-Rendu disease

Hereditary hemorrhagic telangiectasia (HHT) (Osler-Weber-Rendu disease) is an autosomal dominant genetic disorder characterized by multiple arteriovenous malformations (AVMs). The common manifestations of this disease are frequent epistaxis, mucocutaneous telangiectasia on typical locations (lips, oral mucosa, and hands), and visceral AVM in the gastrointestinal tract, liver, lung, and/or central nervous system [1,2]. Frequent and recalcitrant bleeding from the nose or mucocutaneous lesions can worsen the patients' quality of life, and major bleeding from a visceral AVM is sometimes lethal. It is thus very important to diagnose HHT at the earliest possible time point.

Four genes (*ENG*, *ACVRL1*, *SMAD4*, and *GDF2*) have been identified as pathogenic in HHT [3]. Among these, the mutation of the 2 major genes, *ENG* and *ACVRL1*, account for about 80% of all HHT cases [3,4]. HHT type 1 (OMIM #187300) is associated with a mutation of *ENG* with a chromosomal location of 9q34.11. *ENG* encodes endoglin, which is a component protein of the transforming growth factor beta (TGF-β) receptor complex [5-7]. HHT type 2 (OMIM #600736) is associated with *ACVRL1* (*ALK1*) mutation, which is located in 12q13.13. *ACVRL1* encodes a serine/threonine-protein kinase receptor R3, *i.e.*, ACVRL1, which is also known as activin A receptor-like kinase 1 (ALK1). ACVRL1 protein acts as a cell-surface receptor for the TGF-β signaling pathway [6,7]. Herein, we describe a case of possible HHT in which a novel missense mutation of *ACVRL1* without mutation in *ENG* was detected.

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Case Report

A 50-year-old Japanese man (III-1 in Fig. 1) visited our hospital due to spontaneous and recurrent epistaxis. He had experienced epistaxis since childhood. Over the 3 years prior to his admission, the frequency of the patient's epistaxis increased to almost daily occurrences upon waking and at night. On visual inspection, the patient exhibited multiple red or pink spots reflecting telangiectasias on the lips, tongue, and palate (Fig. 2). Whole-body computed tomography, magnetic resonance imaging of the brain, and abdominal echography did not show any visceral AVM. The laboratory results were as follows: white blood cell count, 5,600/μL; red blood cell count, 4,760,000/μL; hemoglobin concentration, 14.4 g/dL; platelet count, 253,000/μL; prothrombin time (international normalized ratio), 0.90; and activated partial thromboplastin time, 32.0 sec. These findings indicated that there was no anemia or thrombocytopenia, or any coagulation system disorder.

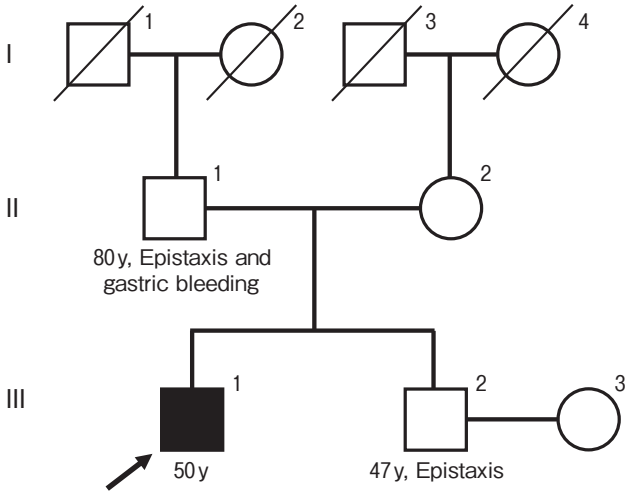

Fig. 1 The pedigree of the patient's family. The proband patient is indicated by a *black-filled square* and *arrow*. *Squares* denote men and *circles* represent women. The patient's father also had frequent epistaxis and underwent 2 surgeries due to recalcitrant gastric bleeding, and the patient's brother had epistaxis. Although the father and brother were suspected to have HHT, they could not be diagnosed with HHT due to insufficient clinical information.

The patient's father (II-1 in Fig. 1) and brother (III-2 in Fig. 1) also had frequent epistaxis. His father had undergone a surgical operation for recalcitrant gastric bleeding twice. The patient had no spouse or children. It was not clear whether his grandfathers and grandmothers had symptoms of HHT (Fig. 1). Based on these findings, the patient was suspected of having possible HHT in accordance with Curaçao's diagnostic criteria [8].

Blood samples were collected from the patient after genetic counseling by a clinical geneticist. Written informed consent was obtained prior to the sample collections, which were analyzed and used for the publication of this case report. Both the genetic testing and overall study protocol were approved by the Ethics Committee of Nihon University School of Medicine (nos. 149-0 and 149-1). Genomic DNA was isolated from the patient's peripheral blood lymphocytes using an established procedure. Unfortunately, the patient's

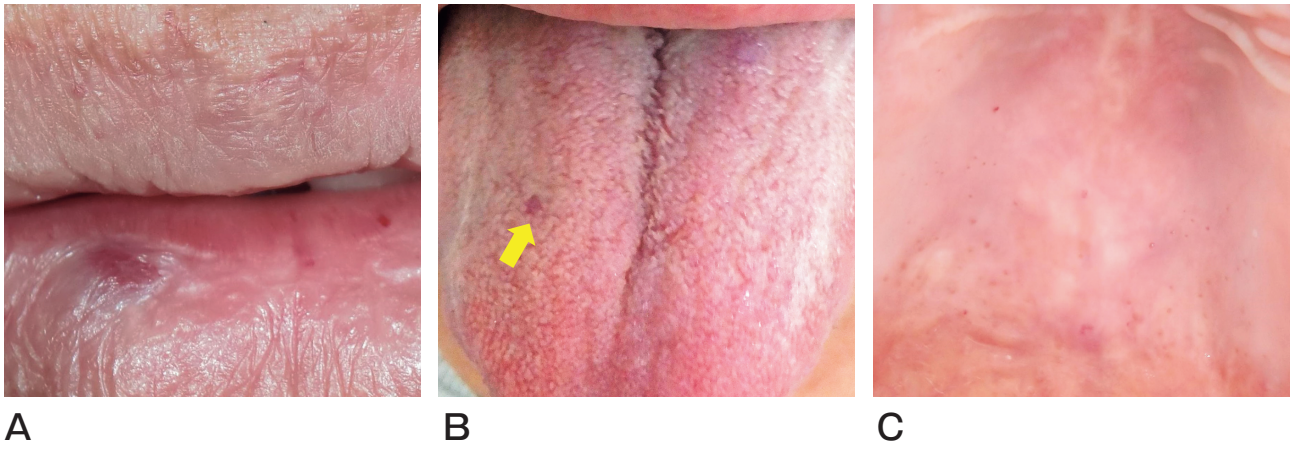

Fig. 2 Typical images of the patient's telangiectasias. **A**, Some large or small red spots were observed on the lips; **B**, A red spot was also observed on the tongue (*yellow arrow*); **C**, Several small red or pink spots were observed on the palate.

father, mother, and brother lived far away, and they did not agree to undergo the genetic testing.

Entire coding exons of *ENG* and *ACVRL1*, which included intronic boundaries of the genes, were amplified by polymerase chain reaction using established protocols. The amplicons were examined using the direct sequencing apparatus of Sanger's method (3130 DNA Analyzer; Thermo Fisher Scientific, Waltham, MA). Sequence data were processed using the GeneMapper™ ID-X software ver. 1.3 and mapped to the human genome sequence.

The analysis of the *ENG* gene revealed the presence of a wild-type sequence in all exons. The *ACVRL1* gene exhibited a novel heterozygous mutation (c.100T>A) in exon 3, indicating an amino acid substitution of p.Cys34Ser (Fig. 3). There were no other mutations detected in other exons.

To predict the pathogenicity of the p.Cys34Ser missense mutation, we performed an *in silico* analysis using 6 software programs. During the first part of the analysis, MutationTaster (http://www.mutationtaster.org/; accessed July 4, 2018), SIFT (http://sift.jcvi.org/; accessed July 4, 2018), PROVEAN (http://provean.jcvi.org/index.php; accessed July 4, 2018), Align GVGD (http://agvgd.hci.utah.edu/; accessed July 4, 2018), and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/; accessed July 4, 2018) were used to evaluate the mutation as "disease causing," "damaging," "deleterious," "most likely deleterious," and "probably damaging," respectively, as these could be used to demonstrate the high pathogenicity of the mutation. In addition, the PANTHER-PSEP program (http://www.pantherdb.org/; accessed July 5, 2018), which uses an algorithm to calculate the length of time a given amino acid has been preserved in the evolutionary lineage, showed that the Cys34 amino acid residue was highly preserved for a period of 1,037 million years (Table 1).

Discussion

We identified a novel *ACVRL1* mutation (c.100T>A, p.Cys34Ser) in a Japanese patient with possible HHT. To the best of our knowledge, there have been no reports that have identified the missense mutation of

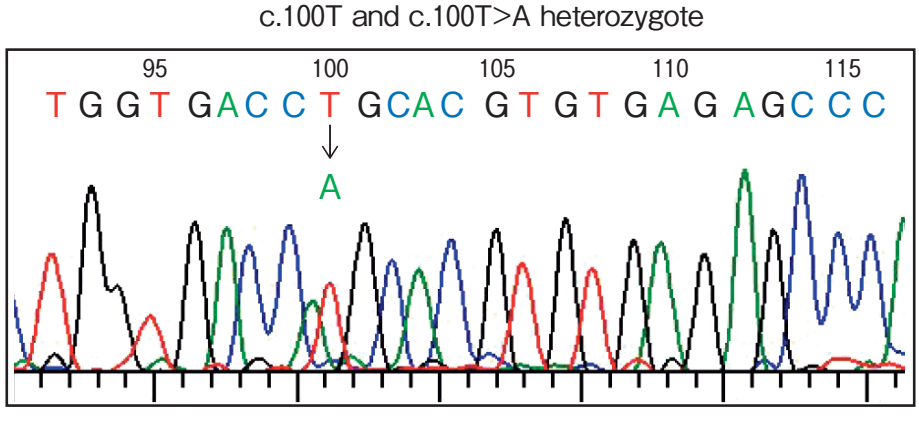

Fig. 3 Direct sequencing of the *ACVRL1* gene using Sanger's method showed an overlapping of thymine and adenine in the codon residue no. 100, which indicated a heterozygous mutation of c.100T and c.100T>A.

Table 1 In silico analysis of Cys34 residue using 6 type of software

Mutation	Algorithm					
	MutationTaster	SIFT	PROVEAN	Align GVGD	PolyPhen-2	PANTHER-PSEP
C.100T>A, p.Cys34Ser (present case)	Disease causing (Score: 0.999999995)	Damaging (Score: 0.00)	Deleterious (Score: −6.430)	Most likely deleterious (Class C65, Score: 111.67)	Probably damaging (Score: 1.000)	Probably damaging (Score: 1,037 my)
C.101G>A, p.Cys34Tyr (described in [18, 19])	Disease causing (Score: 0.999999999)	Damaging (Score: 0.02)	Deleterious (Score: −5.971)	Most likely deleterious (Class C65, Score: 193.72)	Probably damaging (Score: 1.000)	Probably damaging (Score: 1,037 my)

(my: million years)

p.Cys34Ser (ACVRL1 Database in Mutation Databases of ARUP Laboratories, Department of Pathology, University of Utah, Salt Lake City, UT, http://arup.utah.edu/database/ACVRL1/ACVRL1_display.php; accessed June 13, 2019).

Since 2000, HHT diagnoses have generally been made based on Curaçao's clinical diagnostic criteria. These criteria consist of four elements: (i) spontaneous and recurrent epistaxis; (ii) multiple telangiectasias at characteristic sites such as the lips, oral cavity, fingers, and nose; (iii) visceral AVM; and (iv) the presence of a first-degree relative with HHT based on these criteria. If 3 or 4 criteria are met, the patient is diagnosed with "definite" HHT; if 2 criteria are met, patients are considered to have "possible" HHT. Patients meeting only one or none of the criteria are regarded as "unlikely" to have HHT [8]. As the present patient met two of the four criteria at his initial visit, we made the diagnosis of possible HHT but not definite HHT.

Additionally, the patient's father was highly suspected of having HHT due to his episodes of epistaxis and 2 instances of recalcitrant gastric bleeding. Although it appeared that the patient's brother also potentially had HHT, his clinical information did not meet the required diagnostic criteria. If the patient's father or brother were diagnosed with HHT, our patient would be diagnosed as having definite HHT because the presence of a first-degree relative with HHT would be confirmed. Our patient's case was thus possible HHT and quite close to definite HHT.

Bossler *et al.* described an HHT case with a c.101G>A, p.Cys34Tyr mutation, which was a missense mutation affecting the same amino acid residue [9]. They reported that the patient fulfilled all 4 elements of Curaçao's criteria. Al-Saleh *et al.* also described a neonatal case of HHT with c.101G>A, p.Cys34Tyr mutation [10]; although the patient had no history of multiple telangiectasia or epistaxis, he had a large AVM in the liver, plus anemia. That patient's mother also exhibited the same mutation and had recurrent epistaxis, cutaneous telangiectasia, and pulmonary AVM, which met three of the 4 Curaçao criteria. The c.101G>A, p.Cys34Tyr mutation is therefore definitely pathogenic. Our *in silico* analysis using six software programs also showed high pathogenicity for p.Cys34Tyr and p.Cys34Ser mutations (Table 1). Taking the past and present findings together, it appears that the c.100T>A, p.Cys34Ser mutation identified in our patient is highly pathogenic.

ACVRL1 (ALK1) protein is a serine-threonine kinase expressed mainly on the surface of endothelial cells. It acts as a receptor for the TGF-β family of proteins. ACVRL1 includes 503 amino acids, and the extracellular domain (residues 22-118) binds the bone morphogenetic proteins BMP9 and BMP10 [11]. Scotti *et al.* performed a bioinformatics analysis using a structural model, and they analyzed 29 known *ACVRL1* mutations in the extracellular domain. Their results demonstrated that Cys34Tyr mutation has highly destabilizing effects on the protein structure [12]. Ricard et al. generated 19 ACVRL1 protein mutants, including 3 mutants of the extracellular domain (Cys51Tyr, Cys77Trp, and Asn96Asp), and transfected them into cells. They found that all of the ACVRL1 mutants retained their ability to bind BMP9, except for the 3 mutants of the extracellular domain. In addition, the cells transfected with these 3 extracellular mutants had defective BMP9 signaling [13]. A functional analysis of the Cys34 residue has not been published, but mutation of this residue may also induce a defect in BMP9 signaling.

We have thus identified a novel c.100T>A, p.Cys34Ser mutation in *ACVRL1*. It is likely that this mutation is pathogenic, but there is a slight possibility that it is nonpathogenic. Further investigations, including similar case reports, will be needed to confirm our observation.

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