

Case Report

A novel germline mutation in SMAD4 gene in a hereditary hemorrhagic telangiectasia family

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Abstract: Hereditary hemorrhagic telangiectasia (HHT) is a rare autosomal dominant disorder characterized by telangiectases, epistaxis and visceral arteriovenous malformations. Recently, germline mutations, including mutations in the *ENG*, *GDF2*, *ACVRL1* and *SMAD4* genes, have been clarified to be involved in HHT patients. Here, we report a HHT family with a novel germline mutation in the *SMAD4* gene. A 45-year-old man manifested massive hemoptysis and was angiographically proven to have multiple pulmonary arteriovenous malformations (PAVM). His mother and elder system also had histories of massive hemoptysis due to PAVM rupture. They also manifested mucocutaneous telangiectases and greater frequency of epistaxis. Subsequently, genetic screening in venous blood samples revealed a series of mutations in the 3'UTR of *SMAD4* gene, which including 5 site mutations, 1 insertion and 2 deletions. To our knowledge, this mutation has not been previously described. Careful family history collection and genetic screening in suspicious patients are needed to identify HHT, and regular surveillance is recommended.

Keywords: Hereditary hemorrhagic telangiectasia, mutation, *SMAD4*

Introduction

Hereditary hemorrhagic telangiectasia (HHT) or Rendu-Osler-Weber syndrome is a rare autosomal dominant disorder characterized by hamartomatous vascular development that leads to abnormal blood vessel formation in the skin, mucous membranes, and often occur in the organs such as lungs, liver, and brain. Patients manifest systemic mucocutaneous telangiectases, epistaxis and visceral arteriovenous malformations (AVMs) [1]. The etiology of the HHT is not yet understood. Recent data indicated that HHT might be associated with germline mutations of several genes, including *ENG*, *GDF2*, *ACVRL1* and *SMAD4* [1-3]. *SMAD4*, as a transcription factor, crucially regulates vascular-generating genes expression [2, 3]. Mutation of *SMAD4* is a rare condition that contributes approximate 2% of HHT [2]. Here, we report a novel series of *SMAD4* gene mutations in 3' UTR in a HHT family. To the best of our knowledge, this certain mutation pattern has not been described previously.

Case report

A 45-year-old male was admitted to ER due to a sudden onset of massive hemoptysis 1 day ago. He is a cocaine-abuser and consumed a pack of cigarettes per day. On collection of family history, his mother died from hemorrhagic shock caused by massive hemoptysis and elder sister also had a history of hemoptysis and accepted pulmonary lobectomy 8 years ago. He discharged more than 350 ml of blood during his stay in ER and had a poor response to hemostatic treatment. His hemoglobin level kept descending from 88 g/L to 68 g/L within 24 hours. Chest computer tomography angiogram (CTA) indicated multiple pulmonary arteriovenous malformations (PAVMs) located in whole right lobe and left inferior lobe (**Figure 1A, 1B**). Digital subtraction angiograph (DSA) was then performed to locate ruptured PAVM. Unfortunately, angiogram indicated that the hemorrhagic PAVM, which located in the middle right lobe, was unsuitable to be embolized (**Figure 1C, 1D**). The patient was then trans-

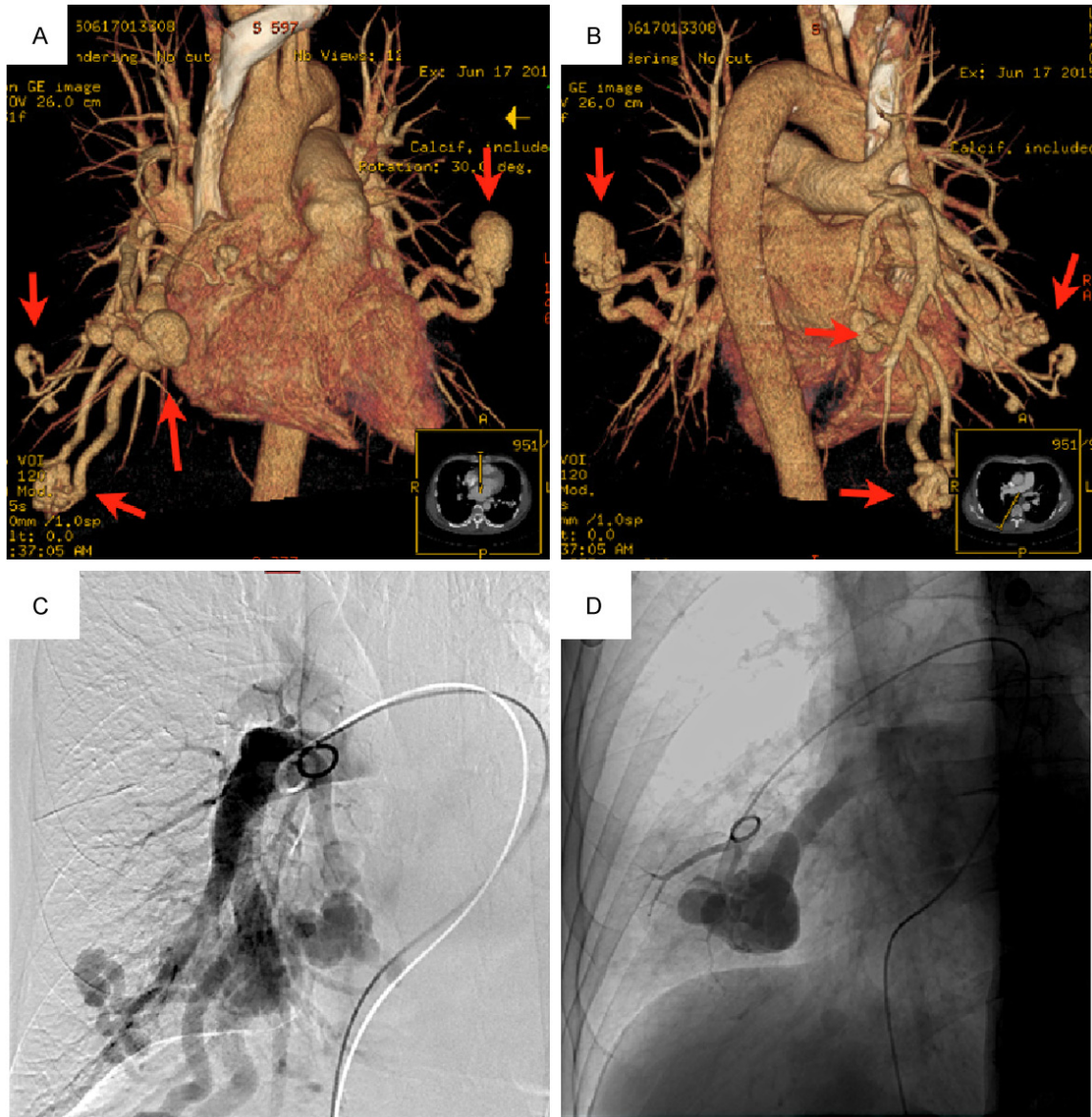


Figure 1. Radiological images of the HHT patient. Computer tomography angiogram (CTA) reconstruction of chest scan (A, B) and catheter-directed angiogram (C, D) revealed multiple pulmonary arteriovenous malformations (PAVM) in patient's lung.

ferred to thoracic surgery department and accepted emergency right lobectomy. After hospitalized for additional 10 days, he was discharged in good condition.

A few days after discharge, the patient together with his elder sister, came to out-patient clinic for further consultation. On reviewing the intact medical history, we were aware of that patient's nephew was also found an un-ruptured PAVM in routine health check-up 2 years ago and under regular follow-up (**Figure 2A**). His elder sister,

who underwent lobectomy 10 years ago, manifested several clusters of angioectasis and increasing frequency of epistaxis (**Figure 2B**).

Based on the family history, signs and symptoms, we diagnosed them HHT. In order to confirm our diagnosis, we collected venous blood sample and performed polymerase chain reaction (PCR) to directly sequence the 4 HHT-related genes: *ENG*, *ACVRL1*, *GDF2* and *SMAD4* [2]. Sequenced genes were mapping with GRCh37/hg19 database by applying the Needle

SMAD4 mutation in HHT

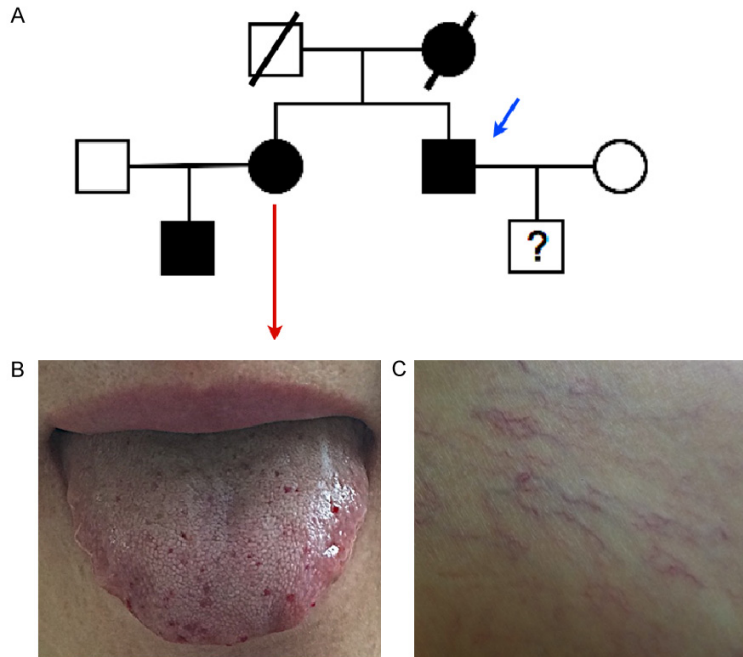


Figure 2. (A) Family tree of the present case. Blue arrow points at the patient, his mother, elder sister and nephew had been diagnosed with HHT. His elder sister, for instance, manifests systemic mucocutaneous telangiectases in tongue (B) and skin (C).

Alignment software (CBI, Peking University). The phenotypes of *ENG* and *ACVRL1* genes, which are much more common to cause HHT, were identical to wild type. Only 1 single point-mutation of *GDF2* was detected in the 3' UTR (pG>A(1775), **Figure 3A**). On the other hand, 3 kinds of mutation patterns were found in the 3' UTR of *SMAD4*, including a 5 site mutations, 1 insertion and 2 deletions (**Figure 3B**). This finding refers to the mechanism of *SMAD4* dysfunction mainly building on the transcription or translation obstacle and much likely to cause the vascular malformation in this family. The accurate location is listed in **Table 1** and **Figure 3**.

Discussion

Hereditary hemorrhagic telangiectasia (HHT) or Rendu-Osler-Weber syndrome is an autosomal dominant disorder characterized by aberrant vascular development affecting approximate 1/10,000 in general populations [2]. HHT is clinically confirmed when an individual meets 3 or more clinical hallmarks: mucocutaneous telangiectases, epistaxis, visceral AVMs and a first degree HHT relative [1, 2]. With the absence of well-structured intervening capillary, direct

artery-to-vein connection in lungs, spinal cord, brain and other organs would lead to AVM rupture in a certain circumstance. In this case, for instance, PAVM rupture was highly possible to be induced by pulmonary vessel spasm after cocaine inhalation. Moreover, epistaxis is most common feature and exhibits age-related penetrance [3], which is consisted with our observation in this family.

HHT is a genetically heterogeneous disorder caused by a number of genes involved in the transforming growth factor-beta (TGF- β) signaling pathway that regulates endothelial proliferation, differentiation, apoptosis, and migration [4, 5]. Four known genes are currently used for HHT genetic testing: *ENG*, *ACVRL1*, *GDF2* and *SMAD4* [1-3]. Pathogenic

variants of these 4 genes occupies more than 85% of individuals who meet unequivocal clinical diagnostic criteria for HHT. *ENG* and *GDF2* are two key components of the transmembrane glycoproteins serve as TGF- β receptor. Mutations of *ENG* and *ACVRL1* genes account for the majority of HHT patients [2, 3]. According to McDonald and colleagues' study, approximately 80% of individuals with HHT have a mutation in *ENG* and *ACVRL1* genes [4]. Mutations of *SMAD4* and *GDF2*, on the other hand, are much rarer patterns and occupy about 2% in HHT, respectively [2].

SMAD4 gene locates on chromosome 18q21.1 and contains 11 exons. Various mutations that cause *SMAD4* loss-of-function would cause vascular-lineage cell malformation, epithelial hyperplasia and even carcinoma [9]. It has been clarified that the most common mutation of *SMAD4* is a 4-base-deletion in exon 9 [7, 8]. Most reported *SMAD4* mutations are small insertions, deletions or single base substitutions leading to nonsense, splicing or missense mutations [5, 9]. In the present case, the exon and intron region are identical to wild type. However, an unusual mutation pattern was

SMAD4 mutation in HHT

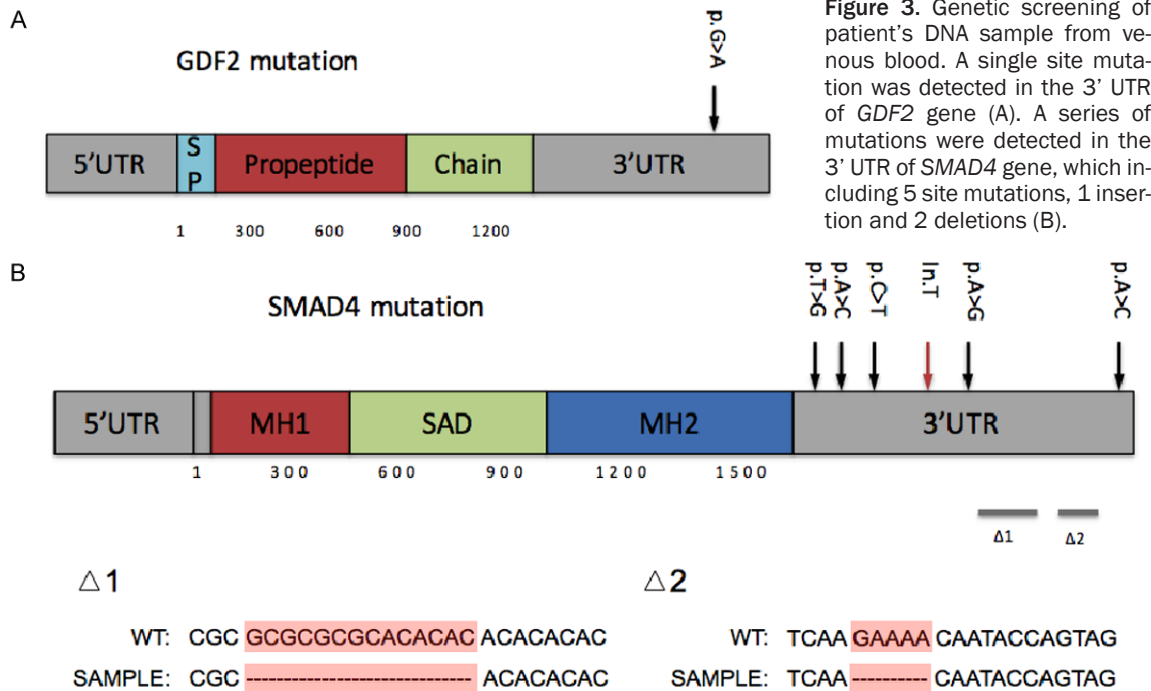


Table 1. Mutation patterns of *SMAD4* genes in current case

Mutation pattern	Accurate location
Site mutation	pT>G(3576), pA>C(3666), pC>T(4248), pA>G(7732), pA>C(8191)
Insertion	InT(7630)
Deletion	$\Delta 1$ (7735-7748), $\Delta 2$ (8058-8062)

found in the 3' UTR of *SMAD4* gene that most likely to cause it loss-of-function.

SMAD4, as a transcription factor participates in TGF- β pathway, serves as a common partner downstream to other SMADs and regulates several gene expressions involved in vascular generation [10, 11]. Some researchers reported HHT caused by *SMAD4* gene mutation would be more often to be associated with juvenile polyposis (JP) [4, 6], but others debated that there also existed JP-free HHT with *SMAD4* mutation [12]. In the present case, we did not find colorectal polyps by enteroscopy (data not shown). Instead, the clinical hallmark of the present case is poor-structured PAVM and systemic mucocutaneous telangiectases. Above mentioned evidence indicates that HHT is inherited autosomal dominant disorder with considerable intrafamilial variability.

In summary, this case report describes a new *SMAD4* gene mutation pattern in a HHT family.

Careful family history collection, familiarity of diagnose criteria, and if necessary, genetic testing are needed to confirm suspicious HHT patients currently. For those diagnosed HHT patients, regular surveillance of potential ruptured AVM and malignancy is recommended.

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Disclosure of conflict of interest

None.

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SMAD4 mutation in HHT

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