Original Article

Clinicoradiological and Genetic Analyses of Three Thai Families with Hereditary Hemorrhagic Telangiectasia in Ramathibodi Hospital

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Objective: To study the clinical manifestations, radiological imaging, and genetic mutations of Hereditary Hemorrhagic Telangiectasia [HHT] patients in Ramathibodi Hospital.

Materials and Methods: Clinical phenotypes and radiological imaging of patients and family members were reviewed. Mutation analyses were initially performed in the probands by next generation sequencing, focusing on four known genes causing HHT, endoglin (*ENG*), activin A receptor type II like 1 (*ACVRL1*; *ALK1*), SMAD family member 4 (*SMAD4*), and growth/differentiation factor 2 (*GDF2*). Pathogenic variants were confirmed by conventional sequencing.

Results: Seven symptomatic HHT patients in three families were studied. Spontaneous recurrent epistaxis was noted in all patients with an average age of onset of about 13 years. Mucocutaneous telangiectasia was observed in six patients. Pulmonary arteriovenous malformations [AVMs] were noted in five patients. One symptomatic case had a large pulmonary AVM that needed embolization. Brain AVMs were detected in four patients. All types of HHT associated brain AVMs were observed and included capillary vascular malformations/micro-AVMs in two cases, nidal type brain AVMs in two cases, and pial arteriovenous fistula [AVF] in one case. Osteodural AVF at clivus was detected in one patient. Spinal cord AVM at thoracic level was found in one patient during screening for pulmonary AVMs. The pial AVF, osteodural AVF, and spinal cord AVM were successfully treated by endovascular approaches without complications. All three families were found to have mutations in *ENG* gene and included one known mutation (c.1311+5G>A) and two noval mutations (c.1429-5T>G and c.1533_1534 delGGinsC).

Conclusion: This presented study is the first HHT series in Thailand with comprehensive clinicoradiological and genetic analyses. Various clinical manifestations among family members were observed. Pulmonary AVMs and central nervous system [CNS] AVMs are the major causes of morbidity in these patients. Only *ENG* gene was found to be a cause of HHT in the presented study.

Keywords: Hereditary hemorrhagic telangiectasia, HHT, *ENG* gene, Mutation

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Hereditary hemorrhagic telangiectasia [HHT] or Rendu-Osler-Weber disease [ROW] (OMIM# 187300) is an autosomal dominantly inherited vascular disorder. The prevalence of this disease is estimated to be 1:5,000 to 1:8,000 with some regional variations[1,2].

Clinical diagnosis of HHT is mostly based on the Curaçao criteria[3] including 1) spontaneous recurrent epistaxis, 2) mucocutaneous telangiectases (lips, oral cavity, fingers, or nose), 3) visceral arteriovenous malformations [AVMs] (lung, liver, brain, or spinal cord), or 4) affected first degree relative by the same criteria. The clinical diagnosis of HHT is considered as definite when three or more findings are present; possible or suspected when two findings are present; and unlikely when fewer than two findings are present.

Genes encoding transforming growth factor-beta [TGF-β] signaling pathway are known as the causes of HHT including endoglin (*ENG*), activin A receptor type II like 1 (*ACVRL1*; *ALK1*), SMAD family member 4 (*SMAD4*), and growth/differentiation factor 2 (*GDF2*) genes[4]. Sequence analysis of *ENG* and *ACVRL1* genes has identified pathogenic variants in 87% of individuals

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who met all four Curaçao criteria in North America[5], while a recent report in Japan found mutations in these two genes in 87.5% of patients with definite HHT[6].

Since clinical diagnosis for HHT has many limitations due to age-related penetrance and variability of disease expressions, genetic diagnosis is useful to confirm the clinical diagnosis and to identify asymptomatic cases among an HHT family[7].

Until now, there have been only two case reports of Thai HHT patients and no data of genetic mutations in these groups[8,9]. Therefore, the authors report the clinical features and extensive radiological findings, and genetic analyses in three Thai HHT families diagnosed by using the Curaçao criteria.

Materials and Methods

Patients

Three patients were clinically diagnosed with definite HHT based on the Curaçao criteria in database of interventional neuroradiology unit Ramathibodi Hospital between January 2004 and February 2015. They all have positive family history in first degree relatives. The patients and their family members were contacted and informed about the research information. After written informed consents had been obtained, clinical information and available imaging of these patients and their affected family members were reviewed retrospectively. The three probands and their family members were recalled for additional clinical evaluation and molecular genetic analysis. Clinical evaluation included medical, personal, and familial history, epistaxis severity scores [ESS][10], and physical examination. Neurological examination was performed by a pediatric neurologist.

This descriptive case series was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University.

Genetic analysis

Venous blood samples were collected from the patients and family members for Deoxyriboneucleic Acid [DNA] extraction. Mutation analyses were initially performed in the probands of each family by next generation sequencing, using SureSelect V5 and Illumina HiSeq4000 platform. Variant callings were filtered to focus on the four known HHT causing genes including *ENG*, *ACVRL1*, *SMAD4*, and *GDF2*. Pathogenic variant in each proband was verified by conventional (Sanger) sequencing, and subsequently in affected family members.

Results

Seven symptomatic HHT patients in three families were included in the present study. Other affected family members were not available at the time of the present study.

Clinical information and imaging

Clinical presentations of the probands and their available symptomatic family members are summarized in Table 1. Pedigree and clinical features

Table 1. Clinical presentation and genetic analyses of three probands and their available affected family members

Family	Member ID	Relationship with the proband	Gender	Current age (year)	Clinical presentation (year)					Possible causing mutation in *ENG* gene*,#	Type of mutation
					Epistaxis	Mucocutaneous telangiectasia	Pulmonary AVM	Brain AVM	Spinal cord AVM		
1	III-2	Proband 1	Male	21	+ (13)	+	+ (9)¶	+© (18)§	-	c.1311+5G>A	Splicing
	II-5	Father	Male	53	+ (30)	+	-	-	-		
2	III-3	Proband 2	Male	21	+ (12)	+	+ (13)§	+© (5 days)¶	+ (13)§	c.1429-5T>G	Splicing
	II-7	Father	Male	52	+ (17)	+	+ (44)§	+Ⓝ (44)§	-		
	II-6	Aunt	Female	54	+ (20)	+	-	-	-		
3	III-2	Proband 3	Male	15	+ (11)	-	+ (12)§	+Ⓟ,Ⓝ (10)¶	-	c.1533_1534 delGGinsC (p.A511AfsX7)	Frameshift
	III-3	Younger twin brother	Male	15	+ (11)	+	+ (12)§	-	-		

AVM = arteriovenous malformation

Plus (+) signs indicate the presence of each clinical presentation

¶ Age of onset, § Age of detection

Ⓟ Pial AVF, Ⓝ Nidal type AVM, © Capillary vascular malformation/micro-AVM

* Nucleotide numbers are in reference to GENBANK Accession number NM_001114753

Suspected pathogenic mutation of each proband by next generation sequencing was confirmed by direct sequencing and co-segregation with the disease in each proband and their affected relatives

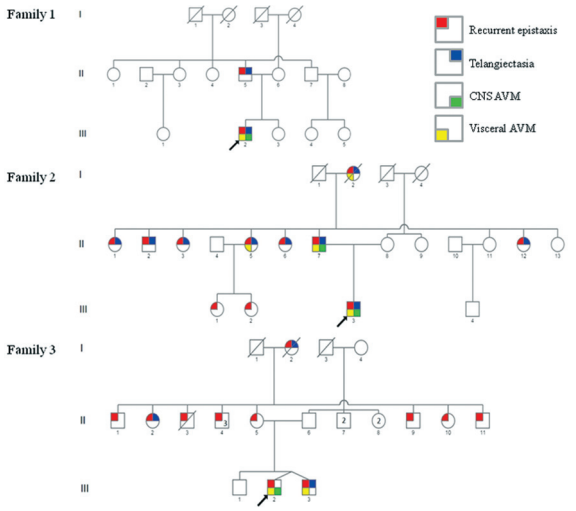

Figure 1. Pedigree of three HHT families in the study. Pedigree symbols: filled symbol, affected individual (4 components; Recurrent epistaxis, Telangiectasia, Central Nervous System [CNS] AVMs, and visceral AVMs); open symbol, unaffected individual. A slashed symbol indicates deceased family member.

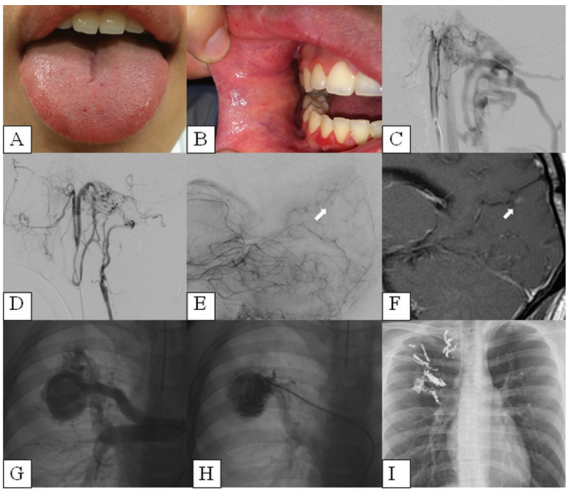

Figure 2. HHT phenotype in family 1: (A, B) Telangiectases on the tongue and buccal mucosa of the proband. (C) Right ascending pharyngeal angiogram of the proband before and (D) after treatment showed a benign osteodural AVF at right-sided clivus and complete obliteration of the AVF. (E) Right internal carotid angiogram in late capillary phase and (F) contrasted brain MRI of the proband demonstrated a capillary vascular malformation at right occipital lobe (arrow). (G) Right pulmonary angiogram of the proband showed a large pulmonary AVM at right upper lobe [RUL] before and (H) during embolization. (I) Frontal chest film of the proband after 3 sessions of pulmonary AVMs embolization showed multiple coil meshs at right upper lobe.

of each probands are shown in Figure 1.

Family 1

The proband (family 1, III-2) was a 21-year-old man, who was clinically diagnosed with HHT due to recurrent spontaneous epistaxis, mucocutaneous telangiectases, pulmonary AVMs, and family history of recurrent epistaxis. Birth history until childhood period was unremarkable. At nine years of age, he initially presented with cyanosis and polycythemia. The large pulmonary AVM at right upper lobe was identified and was treated by transarterial coiling (Figure 2G-I). Multiple small pulmonary AVMs at right upper lobe were also noted. A recurrent epistaxis started at 13 years old with mild severity (ESS 3.99). At 18 years old, the patient complained about an objective bruit at right mastoid area. The brain magnetic resonance imaging and angiography [MRI/MRA] demonstrated osteodural arteriovenous fistula [AVF] at right-sided clivus. Angiogram of the brain showed benign osteodural AVF at right-sided clivus (Figure 2C). Neither nidal type brain AVM nor pial AVF was observed, and only focal tiny capillary vascular malformations/micro-AVMs at right occipital (Figure 2E-F) and left posterior parietal lobes were noted. The osteodural AVF was treated due to irritating audible bruit. After three sessions of endovascular treatment, the fistula at right-sided clivus was completely obliterated (Figure 2D). Additional three sessions of transarterial coiling were performed and the last computed tomography angiography [CTA] of the chest at 20 years of age showed residual small pulmonary AVMs at right upper lobe.

His father (family 1, II-5) developed recurrent epistaxis at 30 years of age. Both his mother and sister had no history of recurrent epistaxis. His father and sister had no AVM or fistula on screening brain MRI.

On physical examination, tiny telangiectases were found at tongue and buccal mucosa of the proband and at lip and tongue of his father, as shown in Figure 2A and B. Neurological examinations of the proband and his father were unremarkable.

Family 2

The proband (family 2, III-3) was a 21-year-old man, who was clinically diagnosed with HHT due to recurrent epistaxis, mucocutaneous telangiectasia, pulmonary AVMs, spinal cord arteriovenous fistula [SCAVF], and extensive family history of recurrent epistaxis and/or visceral AVMs. He was born at 39 weeks' gestation by cesarean section because of fetal distress. At delivery, he had poor respiratory effort and was transferred to the neonatal intensive care unit.

He was intubated due to severe respiratory distress, grunting, and subcostal retraction. Examination, except for severe respiratory distress, was normal. His birth weight and head circumference were at 50th percentile with flat fontanel. A chest film was consistent with transient tachypnea of the newborn and he was quickly weaned from respiratory support. On the fifth day of life, he developed vomiting with bulging of anterior fontanel. Cranial ultrasound showed intraventricular bleeding. A brain CT revealed massive intracerebral hematoma at right frontal lobe with intraventricular hematoma and communicating hydrocephalus. No coagulopathy or bleeding disorder was noted. An emergent neurosurgery for clot removal was performed. Then, serial lumbar puncture every two days for releasing the intraventricular blood was done. His clinical status improved, and he was discharged from the hospital at two weeks of age. On follow-up, appropriated development was noted with acquired porencephalic cyst at right frontal region on brain MRI (Figure 3D). His history of recurrent epistaxis developed after the age of 12 with mild severity (ESS 3.28).

His grandmother (family 2, I-2) was incidentally found to have pulmonary AVM with history of recurrent epistaxis, and was diagnosed with HHT, at which time the proband was 13 years of age. The proband was screened for pulmonary AVM with chest CTA. The result showed multiple small pulmonary AVMs at right middle, right lower and left lower lobes (Figure 3J). The abnormal dilated vessels in the spinal canal at thoracic level were incidentally found (Figure 3F). Physical examination at that time revealed atrophy of both lower extremities, more pronounced on the left side. He also had mild distal weakness with left foot drop. Normal sensation of both lower extremities was noted. The MRI/MRA of the spinal cord revealed perimedullary SCAVF at T4 bony level with a large aneurysmal venous pouch. Focal high signal intensity of the spinal cord on T2-weighted images around the venous pouch was noted (Figure 3H). Spinal angiogram confirmed a high-flow perimedullary SCAVF at mid thoracic level with large venous pouch (Figure 3G). Transarterial embolization with liquid embolic material, n-butyl cyanoacrylate [NBCA] of the SCAVF was successfully performed without complication. The patient reported improvement of atrophy of both lower extremities and was able to regain his left lower extremity strength. Follow-up MRI/MRA of the spinal cord showed thrombosis of the venous pouch without evidence of residual SCAVF, and the signal of the spinal cord turned to normal (Figure 3I). Spinal angiogram at 20 years of age revealed no residual arteriovenous shunt. The brain MRI in this patient showed small capillary vascular malformation at left-sided medulla (Figure 3E).

Extensive history of recurrent epistaxis and telangiectases in his family was noted. His grandmother had history of large pulmonary AVM embolization. His father (family 2, II-7) had pulmonary AVMs (Figure 3K) and small nidal type brain AVM. His aunt (family 2, II-5) had diffuse hepatic telangiectases.

On physical examination, tiny telangiectases were found at tongue of the proband, his father (family 2, II-7) and his aunt (family 2, II-6) (Figure 3A-C).

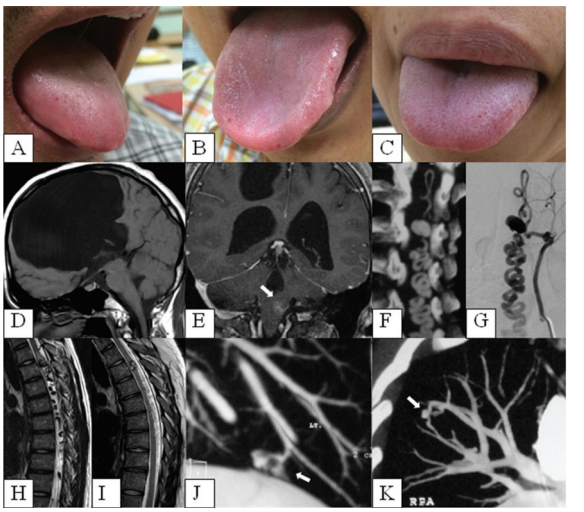

Figure 3. HHT phenotype in family 2: (A-C) Telangiectases on the tongues of the proband, his father and his aunt (II-6), respectively. (D) Brain MRI of the proband showed large porencephalic cyst involving right frontal region, a consequence from intraparenchymal hematoma in his neonatal period. (E) Contrasted brain MRI of the proband demonstrated focal enhancement (arrow) in left-sided medulla, suggestive of capillary vascular malformation/micro-AVM. (F) Chest CTA of the proband incidentally found a vascular enhancing lesion in the thoracic spinal canal. (G) Spinal angiogram of the proband revealed perimedullary AVF fed from dilated left fourth thoracic radiculopial artery with the fistulous connection at the site of the ectatic vein and caudally drained to perimedullary veins. (H) MRI of thoracic spine showed dilated serpenginous perimedullary veins around the spinal cord. Dilated venous pouch at T4 bony level with adjacent high T2 signal of spinal cord indicating myelopathy was also detected. (I) Follow-up MRI of thoracic spine after embolization showed thrombosed venous pouch and disappearance of abnormal dilated perimedullary veins and high T2 signal of spinal cord. (J, K) Chest CTA of the proband and his father showed small pulmonary AVM (arrow) at periphery of left lower lobe and right upper lobe, respectively.

Neurological examination of the proband showed mildly decreased strength of the left hip flexor and increased tone of the left leg. Normal neurological examination of the father and his aunt (family 2, II-6) was noted.

Family 3

The proband (family 3, III-2) was a 15-year-old man, who was diagnosed with HHT at 10 years of age. He had recurrent epistaxis, pulmonary AVMs, brain AVF, and family history of epistaxis and/or visceral AVMs. Birth history until childhood period was unremarkable. The proband and his younger twin brother (family 3, III-3) developed recurrent epistaxis at the age of 11 with mild severity (ESS 2.99 and 1.85, respectively).

At 10 years of age, he presented with severe headache and loss of consciousness. The brain CT/CTA showed ruptured venous pouch of pial AVF at right frontal lobe (Figure 4D). Cerebral angiogram confirmed AVF with large venous pouch at right frontal lobe (Figure 4E). Slightly dilated feeders from right anterior cerebral artery and middle cerebral artery converge toward a single venous segment before emptying into large venous pouch and subsequent inferior frontal vein. Nidal type brain AVMs were also detected at right parietal, left frontal, left parieto-occipital and left occipital lobes (Figure 4B-C). Endovascular embolization of the pial AVF was successfully done with liquid embolic material, NBCA. No residual AVF on follow-up angiogram was noted (Figure 4F).

On screening, chest CTA of the patient and his brother revealed multiple small pulmonary AVMs at periphery of both lungs (Figure 4H-I). Screening brain MRI of his brother showed no abnormal intracranial vasculature but cortical maldevelopment at right inferior frontal lobe was incidentally detected (Figure 4G).

On physical examination, tiny telangiectasia at tongue was found in his brother (Figure 4A). Normal neurological examination of the proband and his brother was noted.

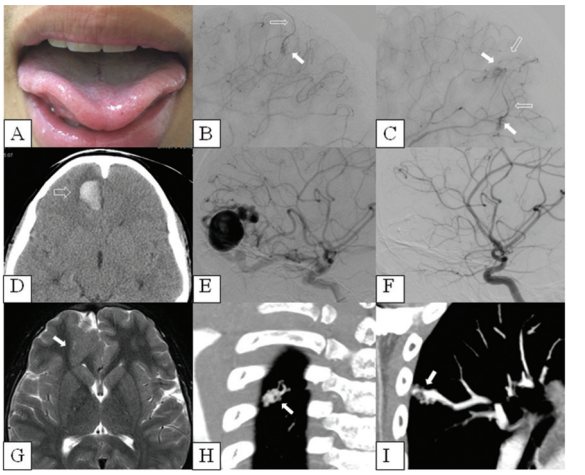

Figure 4. HHT phenotype in family 3: (A) Telangiectases on the tongue of younger twin brother of proband. (B) Right and (C) left internal carotid angiogram of the proband demonstrated supratentorial AVMs with small nidus (arrow) and superficial venous drainage (opened arrow) in bilateral parietal lobes. (D) Brain CT of the proband showed intraparenchymal hematoma (opened arrow) at right frontal lobe with mild perilesional brain edema. (E) Right internal carotid angiogram of the proband before and (F) after treatment showed pial AVF at right frontal lobe fed from right anterior and middle cerebral arteries and emptied to a large venous pouch and complete obliteration of pial AVF at right frontal lobe. (G) Brain MRI of twin brother of the proband showed thickening of the cortical grey matter at medial aspect of right inferior frontal cortex, compatible with cortical dysplasia. Also noted is a strip of ectopic gray matter (arrow) extending from the head of the right caudate nuclei to the orbitofrontal cortex, compatible with gray matter heterotopia. (H, I), Chest CTA of the proband and his younger twin brother respectively showed small pulmonary AVMs (arrow) at periphery of right upper lobes.

Discussion

HHT is an autosomal dominantly inherited vascular disorder characterized by recurrent epistaxis, mucocutaneous and gastrointestinal telangiectasia, and visceral (lung, liver, brain, or spinal cord) AVMs. Clinical manifestation of HHT has intra- and inter-familial variations, which we also observed in this report.

Spontaneous recurrent epistaxis is the most common manifestation of HHT. Average age of onset is approximately 12 years but may range from infancy to adulthood[11]. Most HHT patients have only mild epistaxis that does not require medical attention. In these reported patients, all have history of spontaneous recurrent epistaxis with an average age of onset of 13 years (median, interquartile range 11 to 20). Most of them have only mild symptoms, and only one case (family 2, II-7) has moderate symptom with a history of cauterization.

Mucocutaneous telangiectases occur in about 80% of affected individuals[7]. These lesions appear as small red to purplish spots, typically pinpoint to pinhead size and mostly occur on the face, oral cavity, or hands. The lesions generally manifest later in life than epistaxis,

but typically occur during youth. In presented study, telangiectases were found in all three families, except one affected individual (family 3, III-2), and only one or few telangiectases were found in characteristic sites of each patient.

Pulmonary AVMs are presented in 50% of HHT patients and have been associated with life-threatening complications, such as stroke, transient ischemic attack, cerebral abscess, massive hemoptysis, and spontaneous pneumothorax[12]. Current international guidelines recommend all patients with HHT should be screened for pulmonary AVMs using transthoracic contrast echocardiography with unenhanced thoracic CT with thin-cut reconstructions for confirmation[13]. Pulmonary AVMs can be either single, multiple, or diffuse. Pulmonary AVMs may be asymptomatic (if small in size and shunting volume) or may result in infectious and ischemic complications. Diffuse or large pulmonary AVMs with large shunting volume can cause dyspnea, fatigue, hemoptysis, cyanosis, or polycythemia. In these reported patients, five cases had pulmonary AVMs. Only one case (family 1, III-2) with large pulmonary AVM had polycythemia, which is an unusual presentation. Polycythemia was improved after transarterial coil embolization. The other four cases with multiple small pulmonary AVMs were asymptomatic.

Brain AVMs occur in approximately 10% to 20% of affected individuals[14]. Brain AVMs in HHT can be classified into three types, 1) large single-hole pial AVF, 2) AVMs with nidus, and 3) micro-AVMs or capillary vascular malformations[14,15]. These lesions tend to be in supratentorial and superficial compartments. The large AVMs/AVFs typically but not exclusively become clinically manifest in young children, whereas small AVMs are typically found in older age groups. Micro-AVMs are typically discovered in older children and young adolescents. Approximately 60%, 40%, and 10% of HHT patients with brain AVMs have micro-AVM/capillary vascular malformations, nidal type AVMs, and pial AVFs, respectively[15]. On the basis of different angioarchitecture of these lesions, the natural history and presentation also differ substantially. Capillary vascular malformations are often discovered incidentally and are not associated with symptoms, such as seizures, headache, and hemorrhage. Nidal type AVMs are typically asymptomatic with either detected incidentally or during screening. Pial AVFs generally have a high shunt volume and angiographic features that predict a poor natural history, including marked feeding artery enlargement, arterial stenoses, feeding artery aneurysms, multiple draining veins, venous ectasia, and a pseudophlenitic pattern[14,15].

In a recent study of natural history of brain AVMs in patients with HHT, the authors found an overall bleeding rate of 1% per year with a rupture rate of 0.4% per year for unruptured brain AVMs and 10% per year for ruptured brain AVMs[16]. Although the study did not stratify AVM types, it was the first to demonstrate that the brain AVM rupture rate is similar to that of sporadic AVMs[16]. The prevalence of brain AVMs and risk of rupture in children with HHT are unknown. Ruptured brain AVM in the neonatal period is rare. However, it is usually catastrophic and uniformly fatal[17]. Only one survival of a neonate with HHT and intracerebral hemorrhage was reported[18]. According to hemorrhagic risk, brain AVM obliteration is required to effectively eliminate the risk of future hemorrhage. Treatment of brain AVMs should be considered on an individualized manner with careful consideration of the angioarchitecture of the lesion, natural history, and patient comorbidities[13]. Effective treatment strategies include endovascular embolization, stereotactic radiation, and microsurgery as well as combined treatment approaches.

In the present study, all types of brain AVMs in HHT were observed. Capillary vascular malformations/Micro-AVMs were identified in two cases and nidal type AVMs were found in two cases. Pial AVF was observed in one case, however it had unusual angiographic features. Pial AVFs typically have high shunt volume that results in enlargement of feeding artery, significant venous ectasia, and secondary change from local hypoxemia such as perinidal angiogenesis. However, the angiographic feature of the pial AVF in our case is unique, disproportionate minimally dilated feeding arteries and huge venous ectasia.

History of intracerebral and intraventricular hemorrhage during neonatal period was noted in the proband of family 2. Although the imaging at neonatal period was not available, the follow-up brain MRI showed subsequent porencephalic cyst at right frontal region at 13 years of age. However, neither pial AVF nor nidal type brain AVM was detected on brain MRA. According to the diagnosis of HHT and no underlying coagulopathy, the cause of hemorrhage at that time was most likely from ruptured small brain AVM, which either spontaneously thromboses due to the pressure of the clot or was unintentionally removed during clot removal. Current guidelines recommend that children who are considered to be high risk for HHT should be screened for brain AVMs in the first

six months of life and referred positive individual to a neurovascular expert center for further management. Early screening will detect a treatable brain AVM before the development of a life-threatening or debilitating complication[13].

Another type of neurovascular malformations found in the present study was dural arteriovenous fistula [DAVF], which was found in the proband of family 1. Previous studies of neurovascular manifestations in HHT also found this vascular malformation but with only one case for each study[6,15,19]. Only the study of Komiyama et al[6] stated that in their DAVF case, the shunt was located at clivus. Cortical maldevelopment was observed in one patient (family 2, III-3). Bergerot et al[20] reported cortical maldevelopments in 13 of 162 HHT patients (8%). Twelve of them were polymicrogyria, predominantly located in perisylvian areas. The remaining patient had focal cortical dysplasia. It remains unclear that either DAVF or cortical maldevelopment is related to HHT.

Spinal cord AVMs are rarely found in HHT patients with an estimated prevalence of less than 1%[9]. Systematic review of 26 HHT patients with spinal AVMs by Brinjikji et al[21] showed that most of the cases are presented before 18 years old. Striking male predilection in 72% of patients was noted. Nearly half of the patients had intramedullary or subarachnoid hemorrhage on presentation. Paraplegia/paresis or quadriplegia/paresis was the most presenting symptom (58%) and headache secondary to subarachnoid hemorrhage was the second most (19%). The fistula mostly occurred in the thoracic spinal cord (68%) following with cervical spinal cord (20%). All patients in the study of Brinjikki et al. had perimedullary fistulae. Venous varices were nearly specific feature of spinal cord AVMs in HHT patients[21]. Endovascular embolization is the preferred treatment modality for spinal vascular malformations in HHT patients. The rate of angiographic or clinical improvement is over 70% and complications are rare[21,22]. Our patient's demographic data, clinical symptoms, and angiographic features are similar to the previous studies. Good treatment outcome after transarterial NBCA embolization in our patient was noted. Current recommendations do not support routine screening for spinal cord AVMs[13], as these lesions are uncommon in HHT patients. However, given the above evidence of poor natural history but good treatment outcome in these lesions, it is our opinion that MRI screening may be appropriate in HHT patients.

Hepatic vascular malformations including telangiectases and intrahepatic shunts affect 30 to 70% of HHT patients, but only about 8% are symptomatic[13]. The clinical manifestations include high output cardiac failure, portal hypertension, biliary ischemia, and rarely, acute liver failure. The presence of telangiectases and dilatation of the common hepatic artery are typical for HHT liver involvement[23,24]. Arterial embolization is not recommended because of high rate of complications. Liver transplantation is the definitive treatment in cases of intractable liver failure, high output cardiac failure, or biliary complications[13]. In the present study, one family member (II-5) in family 2 had diffuse hepatic telangiectases; however, no abnormal symptoms or blood chemistry was noted.

Four genes encoding TGF-β signaling pathway including *ENG*, *ACVRL1*, *SMAD4*, and *GDF2* were identified to be the causing genes of HHT. In this report, all three families were found to have mutations only in *ENG* gene. Previous reports demonstrated variable ratio of *ENG*/*ACVRL1* gene mutations in different ethnics (Table 2; 0.51 in French, 0.72 in Canada, 1.17 in Utah, USA, and 1.48 in Japan)[6,25-27]. Further research for genetic analyses in Thai HHT patients is recommended to demonstrate gene mutation prevalence.

A large HHT case series from Japan described types of mutations in *ENG* gene including missense (25.9%), splicing (25.9%), frameshift (18.5%), nonsense (18.5%), and deletion (11.1%)[28]. Common mutations in *ENG* gene have not been demonstrated.

Table 2. Previous reports about genetic mutations in HHT in different ethnics

Sources	Number of patients*/families#	Genetic mutations				
		ENG	*ACVRL1*	*SMAD4*	*GDF2*	*ENG/ACVRL1 ratio*
Abdalla et al.[26], 2005	31#	13	18	NA	NA	0.72
Lesca et al.[25], 2006	136*	40	79	1	NA	0.51
Bayrak-Toydemir et al.[27], 2006	34#	14	12	NA	NA	1.17
Komiyama et al.[6], 2015	66#	34	23	-	NA	1.48
The present study	3#	3	-	-	-	NA

HHT = hereditary hemorrhagic telangiectasia; NA = Not available

Negative (-) signs indicate absence for specific mutation

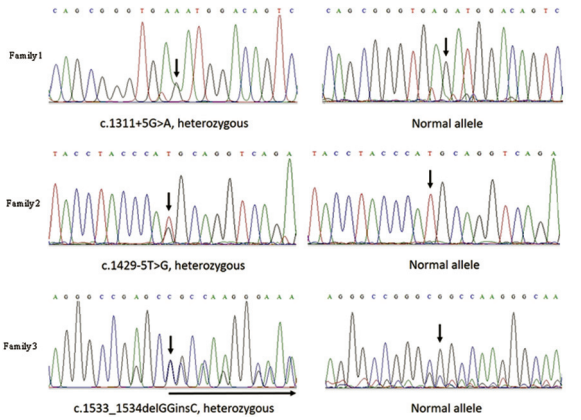

Figure 5. Sequencing results of three HHT family members. Family 1, *ENG* c.1311+5G>A (splicing defect); Family 2, *ENG* c.1429-5T>G (splicing defect); Family 3, *ENG* c.1533_1534delGGinsC (p.A511Afsx7). Arrows indicate mutation sites. Normal allelic controls are from unaffected member of each family.

In this report, two families were found to have splicing mutations and one family with frameshift mutation (Figure 5). One splicing mutation (c.1311+5G>A) is a known HHT-associated mutation in one parent patient and two affected children in HHT mutation database (http://www.hhtmutation.org). Another splicing mutation (c.1429-5T>G) and frameshift mutation (c.1533_1534 delGGinsC) are novel mutations. This frameshift mutation causes premature stop codon and shortening of polypeptide chain from 626 amino acids to 517 amino acids. These three mutations possibly lead to non-functional protein. Further functional test of these mutations is in process.

Patients with *ENG* mutations are more frequent to have pulmonary and brain AVMs (65.2% and 31.8%, respectively) than patients with *ACVRL1* mutations (16.7% and 2.8%, respectively)[6]. However, patients with *ACVRL1* mutations are more common to have hepatic AVMs more than *ENG* mutations[5,6]. Furthermore, symptomatic pulmonary AVMs are more frequent in patients with *ENG* mutations, whereas severe liver involvement is only detected in patients with *ACVRL1* mutations[29]. There is preference of brain AVMs and symptomatic pulmonary AVMs in our studied families. This is a possible reason why we found only *ENG* mutations in the present study.

Locations of AVMs in these three families are variable including pulmonary, brain, spinal cord, and hepatic AVMs, which confirmed intra-familial variation. The present study supports that patients with clinical definite HHT by the Curaçao criteria have high chance to demonstrate pathologic variants from one of the known disease causing genes.

This case series is a preliminary study of Thai HHT patients only in Ramathibodi Hospital. Further national research to explore the incidence, clinical characteristics, and genetic background of HHT in Thai population is warranted.

Conclusion

The present study is the first HHT series in Thailand with comprehensive clinicoradiological and genetic analyses. Various clinical manifestations among family members were observed. Pulmonary AVMs and CNS AVMs are the major causes of morbidity in these patients. Only *ENG* gene was found to be a cause of HHT in the presented study.

What is already known on this topic?

Information about clinical manifestations and genetic mutations of HHT is well-establish in many countries. However, there is limited data about genetic mutation in Thai population.

What this study adds?

The presented study revealed genetic mutations of Thai families with HHT and support that patients with clinical definite HHT by Curaçao criteria have high chance to demonstrate pathologic mutations.

Potential conflicts of interest

The authors declare no conflict of interest.

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