

## Brief Report

### Genetic Diagnosis of Hereditary Hemorrhagic Telangiectasia: Four Novel Pathogenic Variations in Turkish Patients

#### Baysal et al. Two Novel ENG Pathogenic Variations in HHT

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**Aims:** Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disorder characterized by telangiectasia, epistaxis and vascular malformations. Pathogenic mutations were found in *ENG*, *ACVRL1*, *SMAD4* and *GDF* genes. In this study we tried to present our database of HHT patients regarding the phenotype-genotype relations and discuss two novel *ENG* gene pathogenic variations in two unrelated families.

**Methods:** Next Generation Sequencing analysis performed in peripheral blood of nine HHT patients in four unrelated families. All patients were diagnosed with HHT according to Curaçao criteria. Data of treatment and screenings of visceral involvement were recorded from files.

**Results:** We have found pathogenic variation either in *ENG* or *ACVRL1* gene in each family. Two novel pathogenic variations in *ENG* gene including NM\_000118.3 (*ENG*): c.416delC (p.P139fs\*24) and NM\_000118.3(*ENG*): c.1139dupT (p.Leu380PhefsTer16) was found in same family. NM\_000020.2(*ACVRL1*): c.1298C>T (p.Pro433Leu) pathogenic variation in *ACVRL1* gene in our first family and a novel heterozygous likely pathogenic NM\_000020.2(*ACVRL1*): c.95T>C (p.Val32Ala) variation found in our second family. Seven of the nine patients treated with thalidomide for controlling bleeding episodes. All patients responded to thalidomide. In one patient; respond to thalidomide was lost and switched to bevacizumab.

**Conclusion:** In HHT certain type of mutations correlates with disease phenotypes and with next generation sequencing method, new pathogenic variations can be revealed which might help managing HHT patients.

**Keywords:** Hereditary hemorrhagic telangiectasia, *ENG* mutations, Genotype, Phenotype

Hereditary hemorrhagic telangiectasia, also known as Osler-Rendu-Weber syndrome is an autosomal dominant disorder with a prevalence of 1/10.000, characterized with angiodysplastic lesions. Expression of the disease is widely variable with an age related penetrance. (1). Diagnosis of HHT is made with Curaçao criteria which is consisted of epistaxis, telangiectasia, vascular malformations and autosomal dominant penetrance. Three criteria are required for definite diagnoses of HHT (2).

The genetic abnormalities of HHT are pertinent to TGF-beta/BMP signaling pathway which is the mainstay of the regulation of various physiological processes, particularly angiogenesis (3).

*ENG* (\* 131195), *ACVRL1* (\* 601284), *SMAD4* (\* 600993) and *GDF2* (\* 605120) genes have been identified as the underlying cause (4). HHT phenotypes are due to the mutations in the coding region of *ENG* and *ACVRL1* genes in the majority of patients (5) and *SMAD4* gene mutations in the 2-3% of patients (6). Mutations of *BMP9* gene is rarely reported in HHT patients. Still, mutation may not be found in more than 15% patients who are clinically diagnosed as HHT (7). Patients with *ENG* mutations classified as HHT1 and patients with *ACVRL1* mutations classified as HHT2. To date, more than 400 distinct mutations have been reported in the *ENG* including deletions, insertions, duplications, nonsense and missense mutations and 246 mutations in the *ACVRL1* gene (7-9).

Here we aimed to present the preliminary results of genetic analysis of HHT in our region and report two novel *ENG* variations observed in two unrelated HHT families.

## 2. Materials and Methods

### 2.1 Subjects

Four female and five male patients from 4 unrelated families diagnosed with HHT were included in this study. All patients were diagnosed with HHT according to Curaçao criteria. Data of bleeding episodes, requirement of transfusion and iron replacement, antiangiogenic treatments and screenings of visceral involvement were recorded from files. Ethical approval was obtained from the local ethical committee and written informed consent was obtained from all the patients.

### 2.2 Genetic Analysis

All individuals were subjected to genetic counseling and molecular testing. DNA was isolated from the peripheral blood sample of the patients by using EasyOne DNA isolation system (Qiagen, Hilden, Germany). Custom designed QIAseq Targeted DNA Panel (Qiagen, Hilden, Germany) was used for sequencing of the entire coding region of *ACVRL1*, *ADAM17*, *ENG*, *GDF2*, *PTPN14*, *RASAI*, and *SMAD4* genes. QCI analysis (Qiagen, Hilden, Germany) was used to control quality parameters and Clinical Insight (Qiagen, Hilden, Germany) was used to determine variations. Variants were classified according to ACMG 2015 criteria (10).

## 3. Results

Mean age of the patients was 59, 77 (29-76). All patients presented and diagnosed with intractable epistaxis. Mutation screening results and patient demographics are summarized in Table 1. Gastrointestinal bleeding was observed in one patient (patient No: 9) and heavy menstrual bleeding was observed in one (patient No: 7) patient.

Our first family has four affected individuals with HHT and all of them has a heterozygous novel likely pathogenic NM\_000020.2(*ACVRL1*): c.1298C>T (p.Pro433Leu) variation. Two different missense variations, NM\_000020.2:c.1298C>G (p.Pro433Arg) (10) and HGVS

NM\_000020.2:c.1297C>T(p.Pro433Ser) have previously been reported in the literature for the same codon of *ACVRL1* gene (11). Three of the four affected members of this family were treated with thalidomide.

The second family has two affected individuals who have a novel heterozygous likely pathogenic NM\_000020.2(*ACVRL1*): c.95T>C (p.Val32Ala) variation. Another missense variation; NM\_000020.2:c.95T>G (p.Val32Gly) has been reported in the literature for this position of *ACVRL1* gene (12). Both patients have clinical findings, family history, telangiectasias and epistaxis episodes. Combining these findings patients were treated with thalidomide.

In our third family we have 2 affected individuals. Two of the affected family members; A 49 year old and a 76 old mother had a novel NM\_000118.3(*ENG*):c.416delC (p.P139fs\*24) variation in the *ENG* gene This variation was not reported in dbSNP or ClinVar and classified "pathogenic" by our laboratory according to ACMG 2015 criteria (10) (PVS1, PM2, PP3). The bleeding history of the female patient (patient No:7) consisted of heavy menstrual bleeding alone in her early years treated with iron replacement and recurrent severe epistaxis after the age of 60 which required multiple attempts of surgical and local control. Diagnosis of HHT was based on the presence of 3 of the Curaçao criteria and her HHT Epistaxis severity score (ESS) were observed to be 7,31 (13). To control her epistaxis episodes, oral thalidomide 100 mg/day was initially commenced. She responded well to thalidomide for a year and reported only trivial episodes of epistaxis without a need of medical care. ESS at the first year of treatment were decreased to 4, 94. Hemoglobin levels were maintained above 10 g/dl. No adverse effects were observed. However, during the second year of thalidomide, epistaxis episodes became more frequently and her ESS level raised to 7, 19. Treatment was switched to bevacizumab. The second member of the same family, the son did report only trivial epistaxis episodes which have been managed with local measures. The proband from the 4<sup>th</sup> family has a novel NM\_000118.3(*ENG*):c.1139dupT (p.Leu380PhefsTer16) variation in *ENG* gene. This variation has not been reported in the literature or databases before and classified "pathogenic" by our laboratory according to ACMG 2015 criteria (14) (PVS1, PM2, PP3). This patient was responded to thalidomide treatment.

Initial treatment of patients were local compression and cauterization and iron supplements, all without a sustainable response. Seven of the nine patients were further treated with thalidomide 100 mg/day orally. In two patients no systemic treatment was necessary. The treatment was well tolerated with trivial side effects comprising grade one dizziness and nausea, which did not require additional medication (15). Epistaxis episodes decreased, in all patients. In one patient, thalidomide was switched to bevacizumab because of clinical effectiveness. *ACVRL1* mutation was observed in 6 patients. As categorized according to mutation status, six patients were observed as HHT2, while three patients were observed as HHT1. The detailed information HHT type and the phenotype correlations were given in Table 2.

## Discussion

In a hypothetical model of HHT called as “two-hit model”, it has been suggested that a germline heterozygous mutation in HHT gene may be regarded as the first hit while inflammation, vascular injury, hypoxia and angiogenesis may be regarded as the second hit to develop HHT. Similar to the multi-hit hypothesis of venous thromboembolism, haploinsufficiency alone caused by mutations may not be enough for the clinical picture; addition of other processes may explain the “intrafamilial phenotypic variability” of the disease (8, 9). As the processes of the second hit may be observed with aging, we may extrapolate that age may also be an additional hit in the variability of phenotype. Supporting this notion, in a retrospective study, epistaxis has been reported as the first symptom and other manifestations related to visceral organs tended to occur in more mature ages explained with the role of inflammation, vascular injury and ageing (16). In the second family of our cohort, mother and son had the same novel pathogenic variation with different phenotypes. Younger patient had a much milder phenotype while older patient had severe and challenging bleeding episodes and a non-sustained response to antiangiogenic treatments.

Endoglin is a 180 kDa transmembrane glycoprotein, functions as co-receptor for TGF-beta receptor complex which regulates *ALK1* and *SMAD* signaling (4, 17). Both truncating and nontruncating mutations in *ENG* are demonstrated to be associated with HHT1 causing the genes behave as null alleles, defined as haploinsufficiency. As endothelial cells are depleted with endoglin due to this haploinsufficiency, all physiological processes mediated with TGF-beta becomes abnormal which leads to angiodysplasia (17-19). The severity of phenotype is reported to be various between patients who harbor *ENG* mutation though no specific variant is demonstrated to be related with a more severe phenotype while in patients with missense mutations, a milder phenotype is reported (10, 20-22). There were 507 reported variations in the *ENG* gene in the Hereditary Hemorrhagic Telangiectasia Mutation Database of Utah University (23). Our patients' variation was not reported in this database.

As HHT is classified according to mutations of either *ENG* or *ACVRL1* genes phenotype has also been reported to vary in HHT1 and HHT2 such as pulmonary AVMs more common in HHT1 while hepatic AVMs in HHT2 (20, 22). In our study, we observed similar penetrance as pulmonary penetrance in HHT patients and hepatic AVMs in HHT2 patients. None of our patients had cranial AVMs. In our limited analysis; we found more *ACVRL1* mutated patients than *ENG* mutated patients. Diagnosis of HHT made with clinical findings; however different type of mutations could cause different type of disease. Therefore mutational analysis provide disease classification (HHT1, HHT2, HHT4) and genotype phenotype correlations. Thalidomide and bevacizumab are the most used agents in HHT. Thalidomide is mainly used in multiple myeloma and bevacizumab in several cancer types (24-26). The use of both drugs relies on a rational logic, but they are not curative treatment options, and there are still large gaps and unmet needs in the treatment of HHT patients. In our limited number of patients with *ACVRL1* mutation, we observed a positive effect of thalidomide which should be evaluated with further studies.

### Conclusion

This study has two distinct yet adjunctive messages. First is the observation of 4 novel pathogenic *ENG* variations, which support the genetical diagnosis of HHT within families, and the second message is the observation which needs further supportive data regarding the contribution of other factors to the phenotype of HHT including age and inflammation.

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**Table 1. Summary of Patients Demographic information and Mutational Analysis**

Patient No.		Gender	Age	Variation	Visceral AVM	Treatment
1.	Family-1	F	66	NM_000020.2(ACVRL1): c.1298C>T (p.Pro433Leu)	Hepatic	Thalidomide
2.	Family-1	M	46	NM_000020.2(ACVRL1): c.1298C>T (p.Pro433Leu)	None	Thalidomide
3.	Family-1	M	57	NM_000020.2(ACVRL1): c.1298C>T (p.Pro433Leu)	None	Thalidomide
4.	Family-1	F	27	NM_000020.2(ACVRL1): c.1298C>T (p.Pro433Leu)	Hepatic	None
5.	Family-2	F	65	NM_000020.2(ACVRL1): c.95T>C(p.Val32Ala)	Hepatic	Thalidomide
6.	Family-2	M	64	NM_000020.2(ACVRL1): c.95T>C(p.Val32Ala)	None	Thalidomide
7.	Family-3	F	76	NM_000118.3(ENG): c.416delC (p.P139fs*24)	None	Bevacizumab
8.	Family-3	M	49	NM_000118.3(ENG): c.416delC (p.P139fs*24)	None	None
9.	Family-4	M	58	NM_000118.3(ENG): c.1139dupT (p.Leu380PhefsTer16)	GIS, Pulmonary AVM	Thalidomide

AVM: Arteriovenous malformation GIS: Gastrointestinal System

**Table 2. Clinical Features and Genetic Classification**

	HHT1	HHT2
Number of patients	3	6
Age, mean (years)	61,00	55,42
Gender	2M,1F	3M,4F
Epistaxis	All patients	All patients
Gastrointestinal bleeding	1/3	None
Visceral AVM	Pulmonary AVM	1/3
	Hepatic AVM	None
	Brain AVM	None