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Two novel ENG variants in families with type 1 hereditary haemorrhagic telangiectasia

Dillon Mintoff[1,2]; Nikolai P Pace[3,4]; Nicola Carroll[5]; Jenna Jenkinson[5]; Isabella Borg[1,3,6]*

[1]Department of Pathology, Faculty of Medicine and Surgery, University of Malta, Malta.

[2]Department of Dermatology, Mater Dei Hospital, Malta.

[3]Centre for Molecular Medicine and Biobanking, University of Malta, Malta.

[4]Department of Anatomy, Faculty of Medicine and Surgery, University of Malta, Malta.

[5]Molecular Genetics Laboratory, South East Scotland Genetic Service, Western General Hospital, Edinburgh, Scotland.

[6]Medical Genetics Unit, Pathology Department, Mater Dei Hospital, Malta.

***Corresponding Author: Isabella Borg**

Department of Pathology, Faculty of Medicine and Surgery, University of Malta, Malta.

Email: Isabella.borg@um.edu.mt

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Abstract

Hereditary Haemorrhagic Telangiectasia (HHT) is a rare autosomal dominant genodermatosis characterised by cutaneous and visceral telangiectasia, recurrent epistaxis, and Arterio-Venous Malformations (AVMs). In this manuscript, we describe two novel ENG variants in Maltese-Caucasian patients presenting with a variable inter- and intrafamilial clinical phenotypes. This report highlights the importance of diagnostic suspicion of HHT in the presence of cutaneous and/or mucosal telangiectasia and/or AVMs.

Keywords: Hereditary Haemorrhagic Telangiectasia (HHT); Osler-weber-rendu; ENG.

Introduction

HHT, also known as Osler-Weber-Rendu syndrome, affects an estimated 1:5,000-1:10,000 persons [1,2]. A diagnosis of HHT is based on clinical criteria brought forward by Shovlin and colleagues (Curaçao criteria) 2 namely, multiple mucocutaneous or visceral telangiectasia, recurrent epistaxis (especially night-time bleeds), visceral Arterio-Venous Malformations (AVMs) and a first degree relative with a diagnosis of HHT. Herein, we report two novel *ENG* variants in multiple patients of Maltese Caucasian ethnicity suffering from HHT.

Case reports

Proband 1

A 27-year-old female was referred to the genetics clinic in view of a history of epistaxis, mucocutaneous telangiectasia and a pulmonary AVM which was diagnosed during investigation for postpartum hypoxia. Her past medical history was significant for rectal bleeding in the setting of endoscopically detected telangiectasia of the gastric antrum and the duodenum. Having satisfied three of Curaçao's four diagnostic criteria, the patient was clinically diagnosed with HHT (Table 1).

Proband 2

A 34-year-old male was referred to the genetics clinic after being found to have a dominant arteriovenous fistula in the lower lobe of the right lung, receiving pulmonary arterial supply from the right lower lobe pulmonary artery and draining via the pulmonary vein inserting directly into the left atrium. Smaller fistulae were noted in the apical segment of the upper and lower lobes of the right lung. The patient also had a right-sided aortic arch as well as an aberrant left subclavian artery. These complex radiological findings raised the suspicion for a diagno-

sis of HHT. Relevant clinical history was significant for epistaxis and examination revealed telangiectasia on the tongue. Having satisfied three of Curaçao's criteria 2, a clinical diagnosis of HHT was made (Table 1).

Cascade studies

In both probands, targeted capture and sequencing of an HHT gene panel (ACVRL1, ENG, EPHB4, GDF2, RASA1 and SMAD4) was performed using a custom-designed Twist Bioscience panel for library construction and enrichment, and an Illumina MiSeq® platform for paired-end DNA sequencing. Variants of interest were validated using Sanger sequencing. DNA was also analysed using the MLPA P093-C2 and P158-D1 kits, which measure the copy number of the coding exons of the ACVRL1, ENG and SMAD4 genes.

In proband 1, an ENG missense variant c.1280T>G (p.Val427Gly) in exon 10 was identified. A Sanger trace demonstrating the variant is shown in (Figure 1a). Cascade testing of relatives at risk was carried out. The proband's mother, maternal aunt and cousin were found to harbour the variant. Their clinical, genetic and investigational results are presented in Table 1.

In proband 2, the ENG sequence variant c.1583del (p.Pro528fs) in exon 12 was identified. A Sanger trace demonstrating the variant is shown in (Figure 1b). Cascade testing of relatives at risk was carried out. The proband's brother, son and daughter were all found to harbour the variant. Their clinical, genetic and investigational results are presented in (Table 1).

Apart from epistaxis in the proband's father, no other of Curaçao criteria for a diagnosis of HHT were met by the proband's parents. Nonetheless, bearing in mind that HHT mode of inheritance is Autosomal Dominant (AD), the proband's father (who experienced epistaxis) was screened for the familial variant; this was not identified on two separate occasions. His asymptomatic mother turned down targeted genetic testing. The late maternal and paternal grandparents did not fulfil any of Curaçao diagnostic criteria.

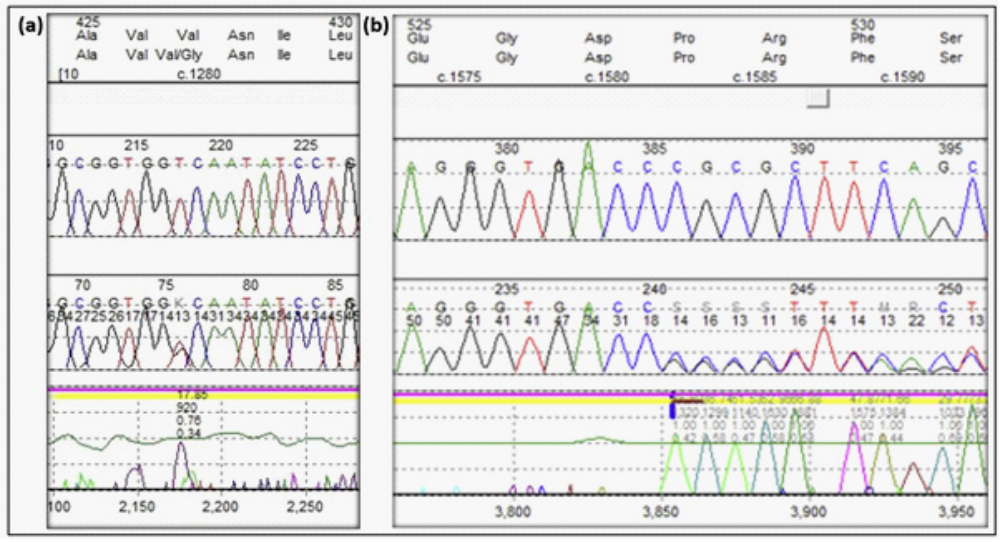

Figure 1: Sanger traces demonstrating the **(a)** ENG:c.1280T>G (left panel) and the **(b)** ENG:c.1583del (right panel) variants.

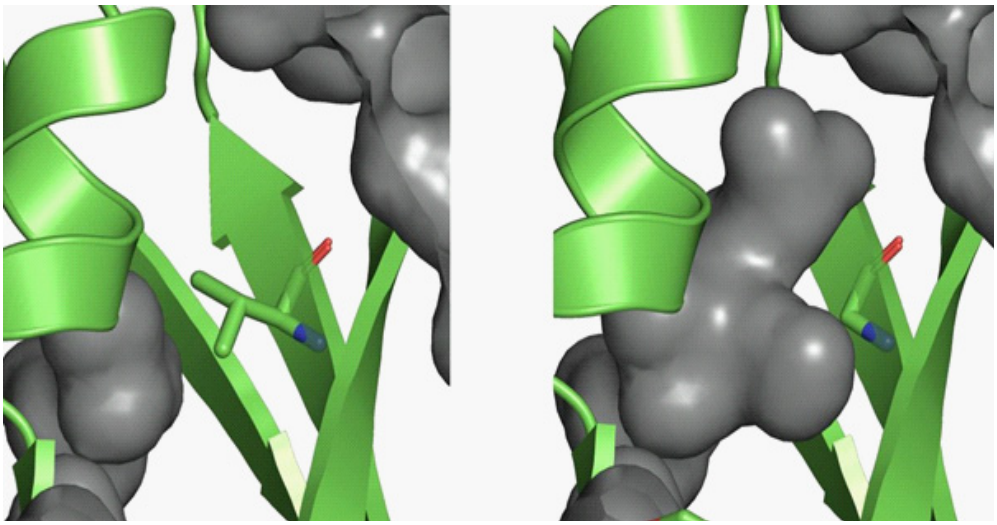

Figure 2: ENG wildtype Val427 (left) and variant Gly (right). Wild type and variant residues shown in ball-and-stick image. The substitution results in an expansion of protein cavity volume (grey shading) and a switch between the buried and exposed states of the wild type and variant residues. Image generated using PyMOL.

Table 1: Available genetic, clinical and investigational findings of probands and relatives.

Patient/Relation to proband	Variant Identified	Age at Diagnosis	Epistaxis	Telangiectasia			Arteriovenous Malformations		
				Cutaneous	Mucosal	Visceral	Pulmonary	Cerebral	Hepatic
Family 1			Variant: *ENG*:c.1280T>G (p.Val427Gly)						
Proband (Female)	Yes	28	Yes	Yes	Yes	Yes	Yes	Yes	No
Mother	Yes	54	Yes	No	No	No	Yes	NI	No
Maternal Aunt	Yes	42	Yes	Yes	No	No	No	No	No
Maternal Cousin 1	Yes	18	Yes	Yes	No	No	NI	NI	NI
Family 2			Variant: *ENG*:c.1583del (p.Pro528fs)						
Proband (Male)	Yes	36	Yes	No	Yes	No	Yes	No	No
Mother	NI	NA	No	No	No	NI	NI	NI	NI
Father	No	NA	Yes	No	No	No	NI	No	NI
Brother	Yes	35	Yes	Yes	Yes	No	NI	NI	NI
Son	Yes	6	Yes	Yes	Yes	NI	NI	NI	NI
Daughter	Yes	9	Yes	Yes	Yes	NI	NI	NI	NI

NA: Not applicable; NI: Not investigated.

Discussion

HHT is a rare autosomal dominant genodermatosis which exhibits locus heterogeneity, with variants in *endoglin (ENG)* (9q33-q34.1, HHT1: OMIM #187300), Activin-Like Receptor Kinase-1 (*ALK-1*) (12q.11-q14, HHT2: OMIM #600376) and *Mothers Against Decapentaplegic Homolog 4 (MADH4)*, encoding for SMAD4 (18q21.2, Juvenile polyposis/HHT syndrome; OMIM:17050) accounting for 80%-96% of patients with HHT [3-5]. Other less common variants such as those in an unidentified gene on chromosome 5q31.3-q32 in HHT3 [6] (OMIM #601101), unidentified gene on chromosome 7p14 in HHT4 [7] (OMIM #610655), and *GDF2* gene on chromosome 10q11.22 encoding Bone Morphogenic Protein 9 (BMP9) in HHT5 [8] (OMIM #611506), account for the remainder of documented HHT patients [5].

Patients with HHT exhibit a variable clinical phenotype. In the absence of a family history of HHT, epistaxis may be the presenting sign in up to 90% of patients with the eruptions of mucocutaneous telangiectasia lagging 5 to 20 years after the initial nose bleed [9]. Some patients may be diagnosed with HHT whilst being investigated for chronic anaemia (which is present in up to 50% of patients) [10] or after sustaining potentially fatal complications from previously undocumented visceral AVMs.

In this report, we describe two novel ENG variants. Both variants are absent from the genome aggregation database gnomAD as well as from an ethnically matched reference genome collection.

The *ENG*: c.1280T>G (p.Val427Gly) substitution is classified as a likely pathogenic variant based on PS4_Sup, PM2, PP1, PP3, PP4 (ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020). Several *in-silico* predictors support a deleterious effect of this substitution. To gain insight into the possible effect of the p.Val427Gly substitution on the stability, structure and function of the ENG protein, a computational approach was applied. Molecular modelling was conducted based on the crystal structure of *ENG* (PDB entry 5HZV) using different structural bioinformatics tools. The *ENG* p.Val427Gly substitution is predicted to be structurally damaging by Missense3D [11], as it results in an expansion of protein cavity volume and a switch between the buried and exposed states of the wild type and variant residues. The substitution has destabilising thermodynamic predictions from Dynamut; Δ Δ G= -2.691kcal/mol (using protein structure as predicted by AlphaFold [accession code AF-P17813-F1]) [12].

On the other hand, the ENG: c.1583del (p.Pro528fs) has not been previously published, but has one ClinVar entry. This frameshift indel creates a premature stop codon and is classified as pathogenic based on PVS1, PM2, PP1 (ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020).

Given that the proband's symptomatic brother was found to be carrying the familial pathogenic indel variant, and in view of the mode of inheritance being AD, it would be expected for either of the parents to harbour the variant. Since the father (whose history of epistaxis could be explained by other medical reasons including a history of hypertension, anticoagulation for a previous transient ischaemic attack and by occupational exposure to industrial spray and dust) did not carry the variant and the possibility of non-paternity was denied, a likely explanation for the origin of the variant is gonadal mosaicism in either of the proband's parents. The possibility of asymptomatic carriage [13] of the variant in the proband's asymptomatic mother is unlikely, given that the affected individuals in this family presented with epistaxis and telangiectasia in childhood.

ENG encodes for endoglin (CD105), a 180 kDa transmembrane glycoprotein that binds ligands of the transforming Growth Factor β (TGF β) [14] and Bone Morphogenic Protein (BMP) [15] families. Exons 1-12 of the ENG encode for the large extracellular domain of the protein whilst exons 13 and 14 encode for the transmembrane and intracellular domains of endoglin, with most variants affecting exons 2 to 11 and 13 [16]. Membrane-bound isoforms (the Long [L], the Short [S]) [17] and a soluble (cleaved) forms [18,19] of the protein have been described. Over 400 ENG variants have been identified, of which the commonest are deletion variants [20]. More than 75% of the described variants are classified as pathogenic [20]. It is likely that *ENG* variants cause HHT by haploinsufficiency [21], as a result of impaired endoglin folding rather than hindered proteomic interactions [15]. Endoglin is involved in the normal functioning of the vascular endothelium as well as other processes such as inflammation [22]. A diagnosis of HHT based on

Curaçao diagnostic criteria is highly predictive of patients harbouring pathogenic ENG or ACVRL1 variants [5]. ENG variants have been associated with an HHT phenotype associated with an increased incidence of pulmonary and cerebral vascular malformations in both paediatric [23] and adult [24] patients. On the other hand, hepatic vascular malformations are less likely in patients with ENG variants when compared with patients harbouring ACVRL1 variants [25]. Patients having ENG variants are likely to experience epistaxis earlier and more severely than patients with other types of HHT-associated variants [25] and are more likely to be diagnosed earlier than patients with ACVRL1 variants [24]. Patients who are actively screened and treated for potential HHT-related visceral complications have been found to have a lifespan comparable to non-HHT patients, justifying this approach [26].

Conclusion

In this report we describe two novel ENG variants thereby expanding the mutational spectrum of this rare genodermatosis. No definitive genotype-phenotype associations have been established, as affected patients exhibit a variable inter- and intrafamilial clinical phenotypes. HHT can be suspected by the clinical identification of any of Curaçao clinical criteria thus making an early diagnosis possible.

Declarations

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Conflict of interest: Nil to disclose.

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