Original Article

Hereditary Haemorrhagic Telangiectasia (HHT) Marked by *ACVRL1*^{C1120T} Variant Displays Hypopigmented Naevi and Frequent Bleeding Episodes if *CYP2C9* Co-Mutated: Clinical Notes & Rationale of Patient Registry

(hereditary haemorrhagic telangiectasia / personalized medicine / whole exome sequencing / NGS / hereditary diseases / haemorrhage)

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Abstract. Hereditary haemorrhagic telangiectasia (HHT) exhibits considerable phenotypic heterogeneity. Therefore, precise mutation screening and evaluation of patient risk must be determined in every HHT family. We present an HHT-2 case with an initial life-threatening bleeding episode that led to identification of a relatively large HHT family. Exome sequencing of the family members determined HHT-associated *ACVRL1*^{C1120T} variant resulting in Arg374Trp substitution at the Ser/Thr-kinase domain region. The affected members display typical epistaxis symptomatology from early childhood resulting in sideropoenia. In addition, the HHT patients also displayed dermatology findings such as facial teleangiectasias and trunk/limb white spots representing post-inflammatory hypopigmentation. Interestingly, co-segregating with modifying cytochrome P450 (*CYP2C)* variant in the HHT patients led to NSAID intolerance marked by increased frequency of bleeding episodes. No arterial-venous malformation of the visceral organs and brain or association with cancer were observed. The heterogeneity of clinical presentation and the role of other variants support the need of regular patient monitoring and development of a nation-wide patient registry.

Introduction

Hereditary haemorrhagic telangiectasia (HHT), also known as Osler–Weber–Rendu disease or syndrome, is inherited as autosomal dominant disorder with typical abnormalities of blood vessel formation called telangiectasias that are prone to recurrent bleeding resulting in iron deficient anaemia (IDA). HHT has an estimated prevalence of one case per 10,000 population. Most striking in HHT are the arteriovenous malformations (AVMs) that may affect larger organs and cause serious medical emergencies. As AVMs bypass the capillaries, the potential of forming an abscess is higher especially in the brain. Once the AVMs become haemodynamically important, high-output cardiac failure may develop. In addition, AVMs within the liver may also cause portal hypertension. Especially, AVM in the thoracic location may cause severe haemorrhage.

Initially, HHT has been linked to the 9q33-34 region (HHT type 1) and to the 12q13 region (HHT type 2). Later, mutations of endoglin (*ENG*) and activin-like receptor (*ACVRL1*) genes, respectively, were identified in HHT. Next, additional mutations in the signalling me-

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Abbreviations: ACD – anaemia of chronic disease, ACVRL1 – serine/threonine-protein kinase receptor R3, ALK-1 – activin-like receptor kinase 1, AVM – arteriovenous malformations, BMP – bone morphogenetic protein, CBC – circulating blood cells, CT – computational tomography, EDTA – ethylenediaminetetraacetic acid, ENG – endoglin, GP – general practitioner, Hb – haemoglobin, HHT – hereditary haemorrhagic telangiectasia, IDA – iron deficient anaemia, i.v. – intravenous, MCV – mean corpuscular volume, MR – magnetic resonance, NSAID – non-steroidal anti-inflammatory drugs, SMAD4 – SMAD family member 4, TGF-β – tumour growth factor β, VCF – variant call format, VAF – variant allelic frequency, VEGF – vascular endothelial growth factor, WES – whole exome sequencing.

diator SMAD4 were also associated with HHT. Thus, all HHT gene targets are involved in activin/BMP/TGF-β signalling (Van den Driesche et al., 2003). Mutations may result in changes such as missense, nonsense, splice site, insertion, indel, deletion or duplication. Instrumental were mouse models with *Eng* mutation displaying yolk sac vascular abnormalities (Li et al., 1999). Nevertheless, mice lacking either *Eng* or *Alk1* have defects in vascular development and cannot survive past midgestation. Together, the observed molecular defects in HHT support a superior role of TGF-β in maintaining vascular integrity and unravelling the potential contribution to pathogenesis of vascular disorders. Upon BMP/TGF signal disturbance, the vessels become more fragile and prone to dilation, leading to HHT clinical phenotype.

While most of the patients are mildly symptomatic, in cases of severe AVM appearance the therapeutic intervention becomes very important. Besides surgical or laser therapies, the systemic approach also exists. For example, bevacizumab, a recombinant humanized monoclonal antibody that blocks angiogenesis via VEGF inhibition, represents an i.v. approach that improves bleeding episodes in HHT and its other clinical parameters (Kritharis et al., 2018). Another approach that was developed quite recently for HHT is represented by somatostatin (octreotide), as evidenced by a steady increase in the haemoglobin level, decreased endoscopic interventions, and decreased blood transfusions (Houghton et al., 2019).

Here, we present the HHT-2 family with *ACVRL1* mutation and discuss several yet unknown features of the phenotype, and we also present genetic observations in order to support development of personalized genetic testing and to shed light on the prevention of clinical complications associated with HHT in the Czech Republic. In addition, based on larger genetic data utilized from whole exome sequencing (WES), we also noted additional co-segregating mutations that are disease severity modifiers in this HHT family.

Material and Methods

Ethics of obtaining DNA samples

Informed written consent was obtained from all patients in compliance with the Declaration of Helsinki and approved by the Ethics Committee of the General University Hospital in Prague. Display of clinical and laboratory data was permitted by all human subjects. Use of the photographs and details that prevent revealing identity of the particular person was permitted by each subject. Human peripheral blood samples were collected into EDTA-containing tubes. The mononuclear fraction was lysed and DNA isolated according to the manufacturer's recommendations (DNA easy, Qiagen, Germany).

Sequencing and analysis of the pathogenic DNA variants

Genomic DNA was used for construction of genomic libraries designed for whole exome sequencing as prepared by Roche Nimblegen SeqCap Kit (Indianapolis, IN) covering 47 Mb of all medically relevant genes; sequencing runs were performed in an Illumina HiSeq 2500 platform (San Diego, CA) with reaching the mean coverage depth of 50×. Further details on data analysis are shown in the Results section and legends. Bioinformatic tools were in part developed by project CZ.01.1.02/0.0/0.0/16_083/0010326. Briefly, NGS data were processed using an 'in house' approach, mostly utilizing standard tools such as analysis of data quality (FastQC, FastQ screen, multiQC), mapping to the genome (GRCh387) via alignment tools (BWA MEM) to form SAM/BAM files after stripping the unwanted artificial sequences. Later, the mapped data as VCF files were annotated using dbSNP or ClinVar databases. This resulted in data of sequencing depth higher than 10 readings of each base with variant allele frequency exceeding 10 %.

Results

Clinical and laboratory data of the HHT family

We present an 80-year old female patient who was initially admitted to our hospital via emergency room after presenting with deep microcytic anaemia of Hb = 45g/l, with signs of dyspnoea and prior anal bleeding after use of non-steroid anti-inflammatory drugs (NSAIDs, ibuprofen) and also previously accompanied by chronic epistaxis. Laboratory findings were very low ferritin levels, low saturation of transferrin, and low serum iron level, all pointing to chronic sideropenic anaemia of bleeding aetiology. The patient was stabilized with 6-transfusion units of erythrocytes. Colonoscopy excluded the presence of anal tumour; flow cytometry indicated absence of a clonal blood cell population. The patient was next referred to the haematology department to finalize her diagnosis and set the regular visit programme and provide iron supplementation.

The patient's history indicated that the proband-patient A (Fig. 1, pedigree) has suffered chronically from epistaxis starting at early childhood (first decade) and such bleeding symptoms also had her mother, siblings, as well as her son and grandson, all indicating a possible genetic trait of this phenomenon. The proband-patient A displayed variceal distortions at nasal mucosa and also similarly at perianal location. The bleeding of nasal origin was recorded to be as frequent as three times a month. The proband-patient A (and also her son, patient D, and grandson, patient F) also indicated that the bleeding episodes were much more frequent upon administration of NSAID (such as ibuprofen, aspirin). In addition, in her 5th decade the proband-patient A underwent extraction of varicosities on both lower extremities along

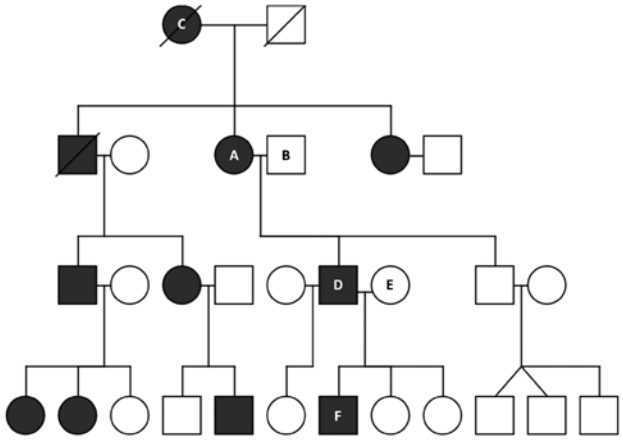


Fig. 1. Four-level pedigree: affected grandmother (C), her two daughters and son, the descendants of one of the daughters (A), and finally, the descendants of her progeny (D, F). Subjects indicated by letters (A-F) underwent exome-sequencing analysis. Rectangle = male, circle = female, horizontal lane indicates marriage, vertical lane indicates descendance. Dark = evidence of HHT, empty = no evidence of HHT according to the "Curaçao criteria".

venae saphenae magna & parva. Patient D also underwent variceal (small saphenous vein extraction) surgery in his 4th decade, while patient C and both siblings of the patient-proband A had varicosities that were not surgically removed. Additional findings in the patient-proband A were: diabetes mellitus type 2 requiring oral antidiabetic therapy, hypercholesterolemia with cholecystolithiasis and xanthelasma (near eyeshadow), which both required surgical removal in the 6th decade.

From this initial clinical inspection, the diagnosis of hereditary haemorrhagic telangiectasia (HHT) became definite according to the "Curaçao criteria" (spontaneous recurrent epistaxis, multiple telangiectasias in typical locations, first-degree family member with HHT) with the following exception: no proven visceral arterial-venous malformations (= AVM of the lung, liver, brain, or spine). We therefore searched for AVMs at the predicted locations using gastroscopy, colonoscopy, CT scan of upper chest, neck and head, chest X rays, and ultrasound of abdominal cavity. Evaluation of the colon of two affected members (A, D) by colonoscopy confirmed perianal varices; however, no benign polyps or colorectal cancer were detected. Gastroscopy (proband-patient A) revealed hiatus hernia with typical symptomatology of occasional diet-dependent gastroesophageal reflux that required intermittent use of proton pump blockers and special diet regimen avoiding gastroesophageal reflux irritants. No signs of liver disease (proband-patient A) except mild steatosis with no significant alteration of liver enzymes in the biochemistry panel or ascites or oesophageal varices were recorded. No signs of lung-derived bleeding such as haemoptysis or haemothorax were recorded. No cardiac disease or brain disease such as loss of consciousness, subarachnoid haemorrhage, stroke, or chronic headache were detected in search of AVMs. Moreover, the proband-patient A underwent magnetic resonance of the brain with the following findings: signs of brain atrophy (relevant to the age of 79) of intermediate grade and some very tiny spots of supratentorial gliosis, potentially of ischaemic vascular origin, albeit without any clinical correlates.

After very careful examination of the HHT family, both affected and non-affected members, we noted the following HHT-associated dermatologic findings, which included 2-4 mm hypopigmentation spots at the arms, forearms and trunk (Fig. 2, photographs). Some of these spots were thus subjected to biopsy. The histology (performed by professor Jiri Stork) revealed signs of post-inflammatory hypopigmentation, indicating that these spots could be a result of the previous vessel collapse and necrosis. In addition, during the haematological follow-up the patient-proband A also underwent acute dermatitis and eosinophilia (over 20,000 eosinophils/μl) with extensive pruritus. Extensive measures were applied such as differential diagnosis of eosinophilia; however, no signs of parasitic or clonal haematological disease were detected. Systemic administration of prednisolone 60 mg daily for five days was applied followed by dose de-escalation and concomitantly with dexamethasone-based ointment therapy, all resulting in complete healing of the acute dermatitis.

In the circulating blood cell (CBC) analyses, the diagnosis of iron deficiency anaemia (IDA) was confirmed to be coincident with the chronic blood loss via epistaxis and variceal bleeding (also requiring iron supplementation). Iron deficiency was also complicated by a concomitant iron absorption defect, as the patient was diagnosed previously with symptomatic hiatus hernia. Transient systemic inflammatory response and dermatitis with eosinophilia led to temporal elevation of the ferritin level and normocytic anaemia, which all imposed the possibility of coincident anaemia of chronic disease (ACD). This patient mostly presented in her routine laboratory check-ups, while on iron supplementation, as grade 1 anaemia with Hb range of 100–110 g/l with MCV ~80 fl without any significant alterations of other lineages. Usually, upon the patient's visits, the haemoglobin level coincided with a below-normal ferritin level, and often the patient reported hiatus hernia-based iron supplementation intolerance (nausea, vomitus) and several preceding bleeding episodes. In addition, lowering the haemoglobin level sometimes coincided with aggravation of dermatitis, while topic dexamethasone administration led to a better CBC outcome, again pointing to the cooperating effect of chronic inflammation on the anaemia phenotype.

Genetic WES analysis identifies HHT-2-associated ACVRL1 and additional variants

To identify the genetic association with clinical symptomatology, we used whole exome sequencing (WES)

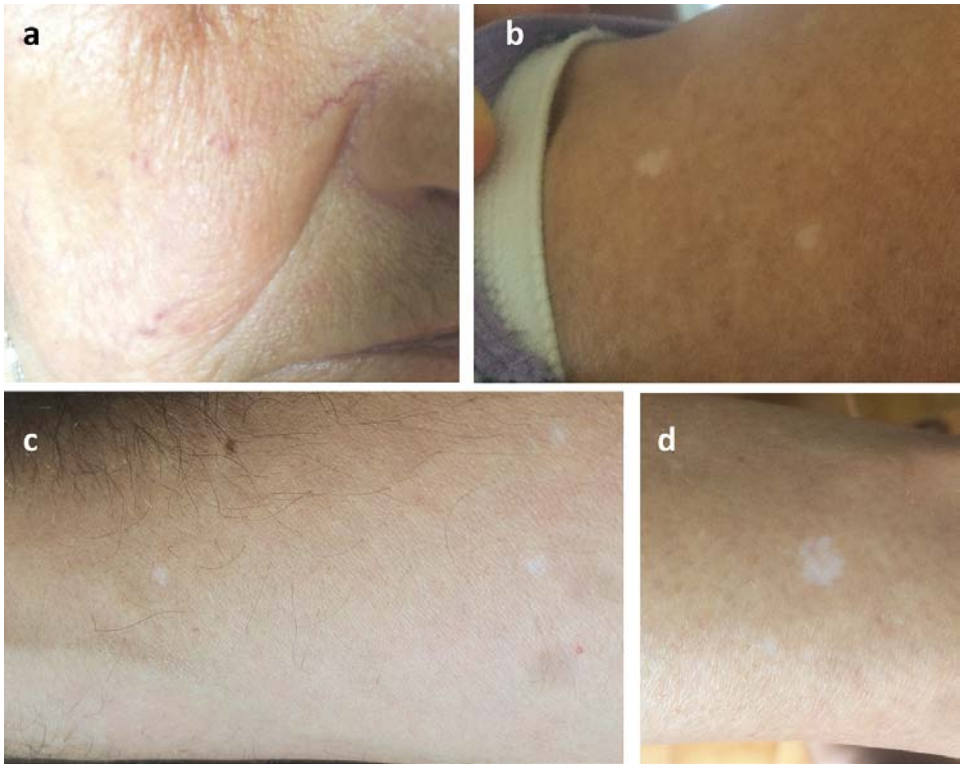


Fig. 2. Dermatologic findings: (a) telangiectasias on the face (proband-patient A), (b) white spots of patient A on the upper back, (c) white spots of patient D on the forearm, and (d) white spots on the forearm of the sister of the patient-proband A.

to identify all variants co-segregating with the disease, and for that purpose we utilized samples from affected HHT-2 individuals A, D, and F and from HHT-2 unaffected relatives B and E as controls. All variants were listed by ClinVar and are shown in Table 1. Peripheral blood mononuclear cells were used for the sequencing, a library was constructed, and sequencing was performed using a Hi-Seq platform (Illumina).

The proband A carried a variant of *ACVRL1* gene (also known as *ALK1*, activin-like receptor kinase 1), namely the C1120T variant resulting in Arg374Trp substitution at the Ser/Thr-kinase domain region that is known to affect downstream signalling via SMAD1/5 (Ricards et al., 2010). C1120T was associated with HHT-2, associating with AVM-associated pulmonary arterial hypertension. HHT-2 was clinically recorded in proband A, as well as along the C1120T variant segregation in family members D and F. No *ACVRL1* mutation was detected in the controls, B and E. The bleeding episodes with frequency >1× monthly were observed in all affected family members carrying the *ACVRL1*^{C1120T} pathogenic variant from early childhood (usually after age of 10).

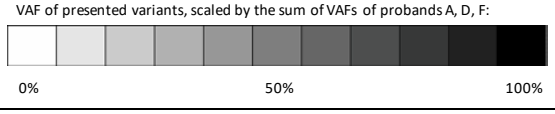
There were variants that were detected in the HHT-2 patients but not in controls and segregating with the disease with a potential effect on the HHT-2 phenotype. Most strikingly, the *ACVRL1*^{C1120T} variant was linked with *CYP2C* variant 9 (rs1057911) that causes amino acid substitution of lle359Leu in cytochrome p450. This variant has previously been observed to act behind the defective degradation and lower tolerance of warfarin and anti-inflammatory NSAID agents. This *CYP2C9* variant affects the metabolism of many additional xenobiotics (besides ibuprofen and warfarin), including phenytoin or tolbutamide. Indeed, anamnestic data indicated that HHT-2-affected family members reported both much higher occurrence and longer duration of bleeding upon the NSAID exposure. Additional variants modifying the clinical phenotype of this family are listed in Table 1 and include: *SLC30A8* variant representing diabetes mellitus type 2 susceptibility, *PON1* variant representing coronary artery disease susceptibility, *FCN3* variant of immunodeficiency due to ficolin 3 deficiency, *FRAS1* variant for cryptophthalmos syndrome, and *SIAE* variant for autoimmune disease 6.

Discussion

Here we present a family carrying the *ACVRL1* gene variant C1120T that stayed behind the HHT-2 phenotype. However, mutations within the *ACVRL1* gene may have very different phenotypes, and therefore their clinical monitoring and analyses of the phenotypes appear to be very important for both clinical and predictive reasons. For example, in two families with *ACVRL1* exon 8 mutations, one with the T1193A mutation had high prevalence of AVM, while in another family with the C1120T mutation, no AVMs were noted (Kjeldsen et al., 2001). Our presented family with C1120T mutation-mediated HHT-2 had no AVMs despite several clinical

Table 1. Exome sequencing data

Gene	Gene name	Amino acid substitution	Related condition	VAF				
				A	D	F	E	B
IL4R	Interleukin-4 receptor subunit α	p.Ile75Val	Resistance to atopy					
SLC30A8	Solute carrier family 30 member 8	p.Arg276Trp	Susceptibility to diabetes mellitus type 2					
PON1	Paraoxonase 1	p.Leu55Met	Susceptibility to coronary artery disease, Microvascular complications of diabetes					
FCN3	Ficolin 3	deletion/ frameshift	Immunodeficiency due to ficolin 3 deficiency					
SIAE	Sialic acid acetylesterase	p.Met89Val	Autoimmune disease 6 (AIS6)					
FRAS1	Fraser extracellular matrix C1	insertion/ frameshift	Cryptophthalmos syndrome					
CYP2C9	Cytochrome P450 2C	p.Ile359Leu	Warfarin and NSAID response					
ACVRL1	Activin A receptor like type 1	p.Arg374Trp	Hereditary hemorrhagic telangiectasia type 2					
ADD1	Adducin 1	p.Gly460Trp	Efficacy of response - furosemide and spironolactone					
PRODH	Proline dehydrogenase 1	p.Arg431His	Schizophrenia 4 (SCZD4)					
PKD1	Polycystin 1	p.Arg324Leu	Polycystic kidney disease, adult type (PKD1)					
FRZB	Frizzled related protein	p.Arg200Trp	Osteoarthritis (OS1)					
FCGR2A	Fc fragment of IgG receptor IIa	p.His166Arg	Susceptibility to lupus nephritis, severe Malaria, trastuzumab response					
AGRP	Agouti related neuropeptide	p.Ala67Thr	Late-onset obesity					
FCGR3A	Fc fragment of IgG receptor IIIa	p.Phe176Val	Not-specified efficacy of rituximab, trastuzumab response					
ADRB2	Adrenoceptor β 2	p.Thr164Ile	Reduced response to β-2-adrenoreceptor agonist					
ABCB1	ATP binding cassette B1	p.Ser893Ala	Susceptibility to inflammatory bowel disease 13					
MPO	Myeloperoxidase	splicing	Myeloperoxidase deficiency (MPOD)					
AURKA	Aurora Kinase A	p.Phe31Ile	Susceptibility to colon cancer					
CCR5	C-C motif chemokine receptor 5	deletion/ frameshift	Susceptibility to West nile virus, HIV type 1, Multiple sclerosis modifier					
AMPD1	Adenosine monophosphate deaminase 1	p.Lys320Ile	Muscle AMP deaminase deficiency (MMDD)					
IRS2	Insulin receptor substrate 2	p.Gly1057Asp	Susceptibility to diabetes mellitus type 2					
ATIC	IMP cyclohydrolase	intron variant	Efficacy of methotrexate response					
LIPC	Lipase C, hepatic type	p.Thr405Met	Hepatic lipase deficiency					
UMPS	Uridine monophosphate synthetase	p.Gly213Ala	Leucovorin response - Toxicity/ADR					
MRC1	Mannose receptor C-type 1	p.Gly396Ser	Multibacillary leprosy					
KIAA0586	KIAA0586	deletion/ frameshift	Joubert syndrome, Short-rib thoracic dysplasia 14 with polydactyly					
INSL3	Insulin like 3	p.Arg102His	Cryptorchidism					
ADH1C	Alcohol dehydrogenase 1C	p.Ile350Val	Alcohol dependence					
TLR5	Toll like receptor 5	p.Asn592Ser	Legionellosis					
CYP2C9	Cytochrome P450 2C9	p.Arg144Cys	Warfarin response					
CYP2C8	Cytochrome P450 2C8	p.Lys399Arg	Rosiglitazone response - Metabolism/PK					
SAA1	Serum amyloid A1	p.Ala70Val	Serum amyloid A variant					
PRRT2	Proline rich transmembrane protein 2	deletion/ frameshift	Benign familial infantile seizures, Infantile familial convulsions and paroxysmal choreoathetosis					
VDR	Vitamin D receptor	p.Met1Thr	Vitamin D-dependent rickets					
CYP2B6	Cytochrome P450 2B6	p.Gln172His	Nevirapine response - Other					
AQP7	Aquaporin 7	p.Gly264Val	Glycerol quantitative trait locus (Defective glycerol release during exercise)					
SDC3	Syndecan 3	p.Val208Ile	Association with obesity					
ABO	α 1-3-Galactosyltransferase	deletion/ frameshift	ABO blood group system					
TPCN2	Two pore segment channel 2	p.Met484Leu	Skin/hair/eye pigmentation, variation in, 10 (SHEP10)					
CYP2B6	Cytochrome P450 2B	p.Lys262Arg	Efavirenz response					
MYH7	Myosin heavy chain beta (MHC-β)	p.Arg1845Trp	Autosomal dominant myosin storage myopathy, Hypertrophic cardiomyopathy					



The first column represents the gene abbreviation, followed by the gene name and the effect of each variant on the protein structure – shown are missense (amino acid substitution), frameshift (insertion/deletion) and intron variants. Related clinical condition annotated by the ClinVar database for the specific variant is presented in the fifth row. Heatmap on the right represents variant allelic frequency (VAF) in each of the sequenced probands. Variants are scaled by the sum of VAFs of probands A, D, and F.

visualization techniques used. The mutations of *ACVRL1* often lead to frequent bleeding episodes; however, the C1120T variant is relatively milder than T1193A (Kjeldsen et al., 2001). Despite that, patient-proband A presented with a life-threatening bleeding episode requiring emergent blood transfusions at her Hb = 45 g/l level. Therefore, relying only on the prediction of milder phenotype in the case of C1120T variant is not accurate, and

Table 2. Proposed clinical examinations and rationale for patients with HHT

Rationale	Clinical examinations
Prevention of disease-related complications	Blood cell analysis, flow cytometry, haemostasis
Prevention of organ failure	Iron metablism and blood biochemistry analysis
Cancer prevention	Exome sequencing, genetic counselling
Prevention of AVM rupture	CT, MR, X-rays, or ultrasound

such patients require more careful monitoring. It should also be noted that unlike other HHT variants, the tested HHT *ACVRL1*^{C1120T} family members reported the bleeding episodes relatively early. Indeed, the HHT clinical and phenotypic diversity in dependence on the type of mutation is supported by molecular testing of ~20 different *ACVRL1* variants *in vitro* (Ricard et al., 2010), as evidenced by clinical data accumulated for at least 50 variants previously (Van den Driesche et al., 2003). Clinical evidence supports the effect on thrombocyte aggregation in the HHT *ACVRL1*^{C1120T} case to be augmented by NSAID upon *CYP2C9* co-mutation.

For monitoring of HHT variants in the Czech Republic, there neither exists a patient registry nor are provided counselling recommendations for HHT patients despite the fact that this disease must involve at least one thousand individuals. Such genetic data regarding HHT would be an important future addition to the GeneReviews® (McDonald et al., 2000) containing comprehensive information focused on HHT diagnosis, treatment and prevention. HHT variants obtained by NGS in the future will be monitored via ClinVar. Thus, a general need for the Czech HHT registry appears to be clinically important, as summarized in Table 2: first, timely diagnosis is essential in order to prevent disease-related complications such as bleeding, stroke, abscess, or other emergent health complications. To accurately and comprehensively identify the HHT variants (in one of the three genes involved) with co-segregating mutations and to exclude other genetic causes, we recommend the use of WES, which nowadays appears to be a very efficient strategy to identify pathogenic variants that stay behind HHT or additional diseases including cancer. We believe that WES will soon become one of the key and relatively inexpensive strategies for most diseases with expected genetic background, which also include predisposition syndromes of low penetrance. The ethical point of obtaining DNA sequence analysis of such extent, however, cannot be easily underestimated. The informed consent must therefore contain specific parts that secure public spreading of the genetic information and that will make the patient/client fully responsible for its clinical use.

Genetic counselling to families might be beneficial for excluding HHT complications from other causes during the visits at the general practitioner. For those who carry *SMAD4* variants, the association with tumorigenesis has been previously indicated, and therefore *SMAD4* variants should be counselled not as mild as the other HHT variants and tumour screenings should be more intensive and frequent. CBC analyses and visits at the haematology practice should be done at least once a year in those HHT patients requiring iron supplementation, also for assessment of sideropenic anaemia. Visualization techniques such as CT, MR, X-rays, or ultrasound for search of variant-carriers associating with AVM appears to be mandatory to exclude or confirm AVM and provide appropriate prevention. Finally, we believe that directing resources to prevention of HHT complications may prevent higher costs of emergency medicine.

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