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SHORT COMMUNICATION

The Stratified Population Screening of Hereditary Hemorrhagic Telangiectasia

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Abstract

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant multisystemic vascular disease with a wordwide prevalence of 1:5000–1:10000. We introduce our algorithm for the stratified population screening of HHT. Probands are selected from the consecutive hospital database review for HHT (I7800) and recurrent epistaxis (R0400) and the review of patient records referred by family practicioners. A proportion of probands might be de novo diagnosed with HHT in the 10-year study period. The checkup of probands consists of physical examination, arteriovenous malformation exploration and and genetic testing (*ACVRL1* and *ENG* sequence analysis). The family screening of HHT consists of physical examination and screening for the family-specific mutation of each at-risk individual, and furthermore, arteriovenous malformation exploration in individuals with suspected/definite HHT and/or carrying the mutation. Twenty-five definite HHT patients were explored: 7 of them by the I7800 review, 1 by the R0400 review, 3 were de novo diagnosed, and the remaining 14 were explored by the systematic family screening. Considering the 20 patients alive at the end of the study period and the unavailable 5 potential HHT patients and 12 at-risk family members, the HHT prevalence is estimated to be 1:6090–1:11267 in our study area, implying our algorithm's effectivity in the stratified population screening of HHT.

Keywords Hereditary hemorrhagic telangiectasia · Prevalence · Stratified screening · *ACVRL1* · *ENG* · Founder effect

Introduction

Hereditary hemorrhagic telangiectasia (HHT; OMIM # 187300) is an autosomal dominant vascular disease described by the four Curacao criteria: 1. spontaneous and recurrent nosebleeds; 2. multiple telangiectases at characteristic sites (lips, oral cavity, fingers, nose); 3. visceral lesions as gastrointestinal telangiectasia and arteriovenous malformations (AVM) predominantly in the lungs, liver and brain; and 4. family history with a first degree relative with HHT.

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Diagnosis is definite if 3 or 4 criteria are present, possible if 2 criteria are present and unlikely if fewer than 2 criteria are present [1].

The wordwide prevalence of HHT is estimated to be 1:5000–1:10000 [2]. All causative genes identified to date encode proteins of the transforming growth factor-beta (TGF-β) superfamily, controlling angiogenesis. Approximately 85% of HHT cases have heterozygous family-specific mutations either in the *ENG* (GenBank Accession No. NG_009551, encodes endoglin) or *ACVRL1* (GenBank Accession No. NG_009549, encodes activin receptor-like kinase 1) genes, causing HHT type 1 (OMIM #187300) and 2 (OMIM #600376), respectively [2]. All types of mutations were described throughout both genes, 506 *ENG* and 571 *ACVRL1* variants are known to date, the majority of them is pathogenic [3].

Due to this extreme allele heterogeneity, sequencing analysis covering exons and flanking intronic regions is the mainstay of genetic testing in HHT. Once the causative mutation is identified in the proband, at-risk kindreds, especially young individuals fulfilling 1 or 2 diagnostic criteria might be screened to confirm or exclude HHT [4]. Similarly, if a novel suspected pathogenic mutation is detected in a proband, genetic screening of at-risk individuals within the family is offered to evaluate the co-segregation of the mutation with disease status [5].

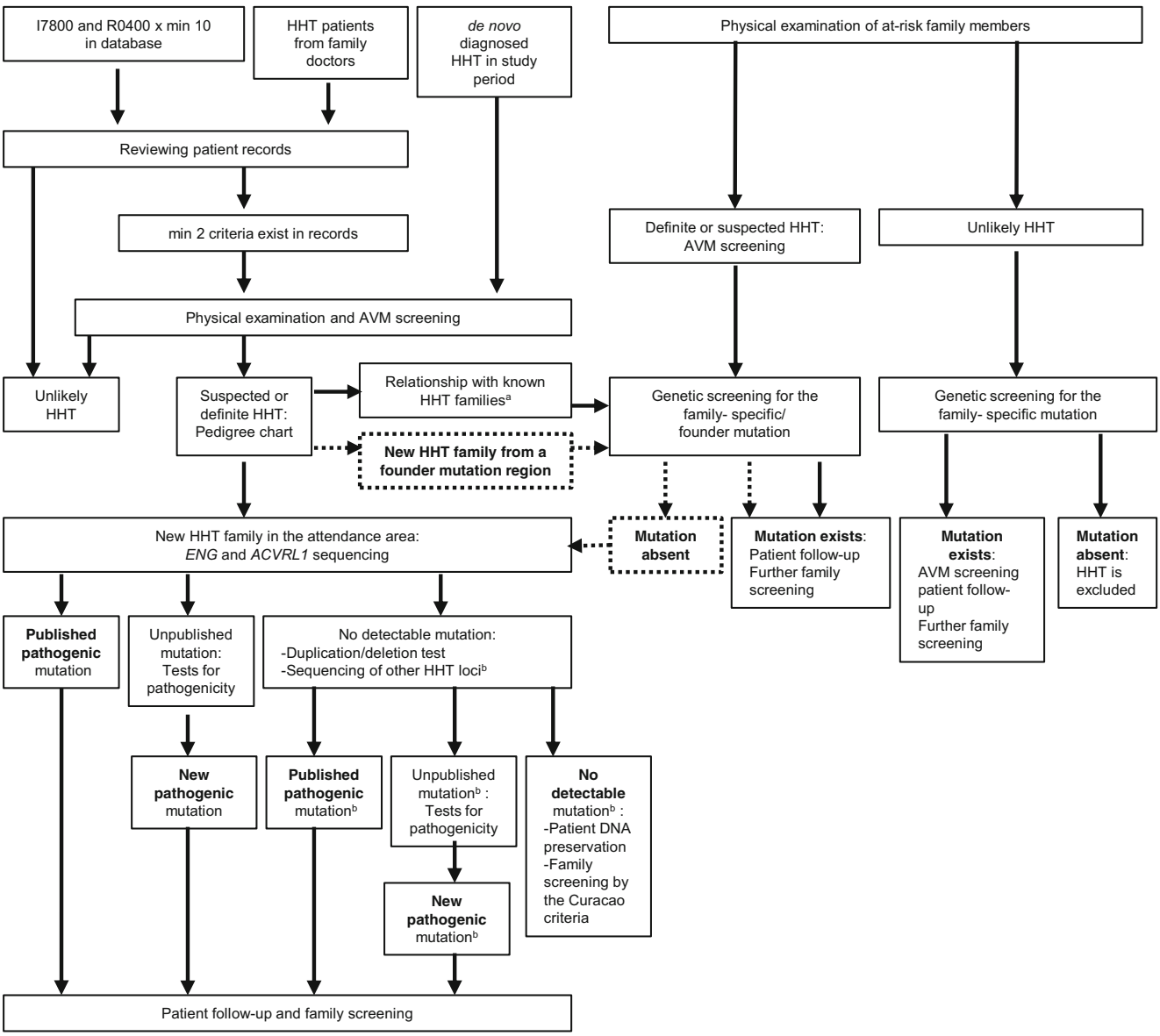

Fig. 1 The algorithm for the stratified screening of HHT. The algorithm is based on the International guidelines for the diagnosis and management of HHT [4]. In large diversified families relationship with known HHT probands might be explored by the pedigree charts.[a] The algorithm for new HHT families from a HHT founder mutation region is indicated with dotted lines. In our study *ENG* and *ACVRL1* sequencing revealed the causative mutation in each HHT family (see later), therefore extended genetic tests were not necessary[b]

Recurrent mutations among unrelated families, living at large distances from each other, are reported. If an identical mutation is detected in unrelated families living or originating within a geographic region, a founder effect is suspected [6–8].

A complete population screening programme for a rare disease like HHT would be unintelligibly expensive and time consuming. On the other hand, given a rare multisystemic disorder, HHT patients are checked up by several medical disciplines even for decades, without diagnosing the underlying disease. As epistaxis is the most common symptom and genetic testing of HHT is now available, an otorhinolaryngological and genetic based stratified screening of HHT seems to be practical.

Method

Informed consent was obtained from all individual participants included in the study.

The stratified screening algorithm for HHT developed in the primary attendance area of the Ferenc Markhot County Hospital, Eger, Hungary (population of 225.339) is shown in Fig. 1. The objectives of the stratified screening were 1. individuals with outpatient and inpatient records encoded with hereditary hemorrhagic telangiectasia (I7800 in the International Classification of Diseases – ICD 10) and recurrent epistaxis (R0400 with an arbitrary cut-off value of minimum 10 times) between 01.06.2007 and 31.05.2017; 2. in order to identify HHT patients in the area followed-up at other institutions, a HHT guidance was yearly sent to each family practitioner at our attendance area, prompting them to refer their known or possible HHT patients to our department between 01.01.2013 (the set-up date of our HHT study group) and 31.05.2017; 3. a number of patients might be de novo diagnosed with HHT (01.01.2013–31.05.2017) and 4. all available at-risk members of HHT families. According to our protocol, individuals with a minimum of 2 Curacao criteria in their medical records (mostly epistaxis and telangiectases) undergo an otorhinolaryngological physical examination completed with the inspection of characteristic telangiectases sites and a visceral AVM screening. The latter includes a contrast-enhanced magnetic resonance (MR) examination of the brain and a computed tomography (CT) of the lungs and

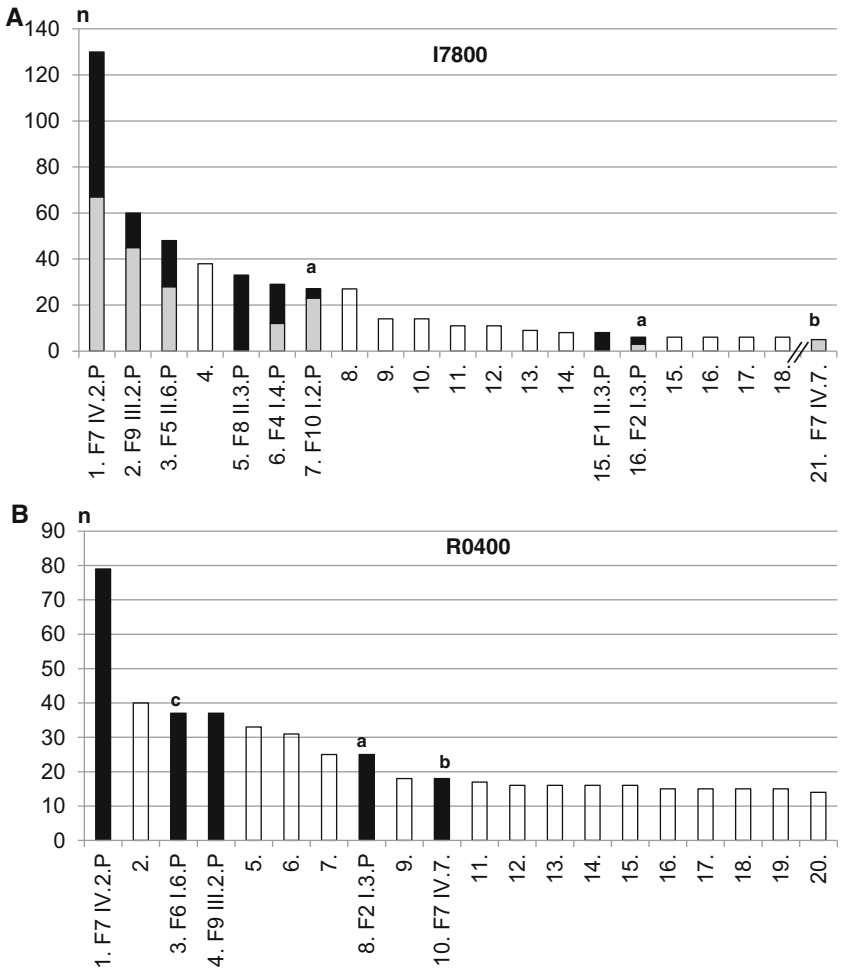

Fig. 2 Individuals encoded most often with HHT-I7800 (a) and epistaxis-R0400 (b) in the examination period. A: the number of medical records of definite HHT patients in the study period prior to the diagnosis of HHT by our study group is indicated with grey. Medical records of definite HHT patients after the diagnosis of HHT by our study group are indicated with black. For patient notations on pedigrees, see Online Resources 1 and 2. Patients F8 II.3. and F1 II.3. were de novo diagnosed with HHT. Individuals indicated with white are not HHT patients. **B**: HHT patients among individuals with epistaxis are indicated with black. I = individual ranking by the number of R0400 and I7800 medical records (n). For patient marking, see pedigree charts in Online Resources 2 and 3. F = family, P = proband. Three patients deceased in the study period [a]. Beyond the genetically confirmed HHT patients, one patient (F7 IV.7.) deceased prior to the availability of the genetic test [b]. One HHT proband was identified by the epistaxis review [c]

the liver (for adults). Pedigree charts are edited on the basis of information from probands. If the patient is the proband of a new HHT family in the attendance area, the sequencing analysis of exons and flanking intronic regions of *ACVRL1* and *ENG* is performed. Mutation pathogenicity is considered by checking the identified mutation in the HHT Mutation Database and in the literature [3]. If a proband is in relationship with known HHT families, screening for the family-specific mutation is performed. If a new HHT family is originating from a known HHT founder mutation region, the founder mutation screening is enabled as the first step (marked with dotted lines in Fig. 1). If the founder mutation is not detectable, *ACVRL1* and *ENG* sequencing analysis is necessary.

In the screening of known HHT families, a physical examination of each at-risk individual is performed at first. If the individual has definite or suspected HHT, AVM screening and genetic screening for the family-specific mutation are succeeded. The latter is done routinely even if HHT is unlikely, considering the age-related penetrance with occassionally late onset of symptoms. Family members who do not emerge or refuse the screening are defined as unavailable.

Table 1 The clinical and genetic data of probands and at-risk individuals with the family-specific mutation living in the study area

	Pedigree symbol	Sex/Age	HHT symptoms	Mutation	Screening method
Family 1 proband	II.3.	M/54	E, T	*ACVRL1 c.625 + 1 G > C*	de novo
Definite HHT	I.4.	F/79[a]	E, T	+	family screening
	III.2.	M/37	E, T	+	family screening
Probable HHT	III.1.	M/40	E	+	
Family 2 proband	I.3.	F/82[a]	E, T, H	*ACVRL1 c.625 + 1 G > C*	I7800 database
Definite HHT	II.2.	M/61	E, T	+	family screening
Probable HHT	III.2.	M/35	E	+	
Unlikely HHT	IV.1.	F/6		+	
	IV.2.	M/1		+	
Family 3 proband	I.3.	F/39	E, T, H	*ACVRL1 c.625 + 1 G > C*	de novo[d]
Definite HHT	II.2.	F/10	E, T	+	family screening
Probable HHT	II.1.	M/12	T	+	
Family 4 proband	I.4.	M/57	E, T, H	*ACVRL1 c.625 + 1 G > C*	I7800 database
Definite HHT	II.3.	M/34	E, T	+	family screening
Probable HHT	II.2.	M/37[b]	E	+	
Family 5 proband	II.6.	F/57	E, T, H, G	*ACVRL1 c.625 + 1 G > C*	I7800 database
Definite HHT	II.2.	F/67[a]	E, T, H, G	+	family screening
	III.4.	F/34	E, T	+	family screening
Family 6 proband	I.6.	F/74	E, T, H	*ACVRL1 c.613 delG*	R0400 database
Family 7 proband	IV.2.	F/75	E, T	*ENG c.817–2 A > C*	I7800 database
Definite HHT	IV.7.	M/73[a]	E, T	not tested[c]	I7800 database
	IV.14	F/69	E, T	+	family screening
	IV.16.	M/69	E, T	+	family screening
	V.10.	F/52	E, T, P	+	family screening
	VI.11.	M/25	E, P	+	family screening
Family 8 proband	II.3.	M/64	E, T, P	*ENG c.817–2 A > C*	de novo[d]
Definite HHT	II.5.	M/54	E, T, P	+	family screening
	III.1.	M/42[b]	E, T	+	family screening
Family 9 proband	III.2.	M/52	E, T, P	*ENG c.360 + 1 G > A*	I7800 database
Definite HHT	IV.1.	M/28	E, T, P	+	family screening
Family 10 proband	I.2.	F/83[a]	E, T, P, H, G	*ENG c.816 + 5 G > A*	I7800 database

For individual's position on pedigrees see Online Resources 1 and 2.

F, female; *M*, male; *E*, epistaxis; *T*, telangiectases; *G*, gastric lesion; *H*, hepatic AVM; *P*, pulmonary AVM. + refers to individuals heterozygous for the family-specific mutation

Five HHT patients died in the investigation period. [a] AVM screening was incomplete in two patients.[b] One patient died prior to the availability of genetic testing.[c] The screening method is interpreted in definite HHT patients. Two probands were successfully screened for a local founder mutation.[d] The common ancestry of families 1–5 sharing the *ACVRL1 c.625 + 1 G > C* mutation is unequivocal [9]

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Results

In the 10-year study period, 829 medical records of 141 individuals were encoded with I7800, 416 records out of them belonged to 22 HHT patients. Seven of them (with 6 probands) were diagnosed with definite HHT prior to the set-up of our HHT study group (Fig. 2). The remaining 15 patients were diagnosed with HHT by our study group in other manners and I7800 was encoded afterwards. For recurrent epistaxis, 776 records of 45 patients were reviewed, and beyond the 4 HHT patients with 159 records already diagnosed by the prior I7800 review, an additional proband with 37 records of R0400 was found to have definite HHT (Fig. 2). Three probands were de novo diagnosed with HHT, two of them (F3 I.3.P and F8 II.3.P) were successfully screened for a founder mutation. Altogether, 10 HHT families with 5 different family specific mutations were identified (Table 1). Four out of these mutations are splice-site variants (*ACVRL1 c.625 + 1 G > C, ENG c.817–2 A > C* and *ENG c.360 + 1 G > A* variants are pathogenic, *ENG c.816 + 5 G > A* is suspected pathogenic), and the remaining one (*ACVRL1 c.613delG* in family 6) is a pathogenic nonsense variant resulting in an early stop codon. The pedigree charts of families with *ACVRL1* and *ENG* mutations are found in Online Resources 1 and 2, respectively. Both haplotype analysis and genealogical examination confirmed the common ancestry of families 1–5 sharing the *ACVRL1 c.625 + 1 G > C* mutation [9]. Upon family screening, 14 additional definite HHT patients were identified. Four individuals with the family-specific mutation were diagnosed with probable HHT, while two children with the family-specific mutation are asymptomatic for the present. Twenty-nine at-risk family members were found to carry the wild-type *ENG* and *ACVRL1* alleles, none of them showed any HHT symptoms.

Family practicioners did not refer any additional HHT patients to our department.

In summary, 25 definite HHT patients were found in the study area. Considering the 20 alive definite HHT patients, the unavailable 5 potential HHT patients and 12 at-risk individuals, the estimated prevalence of HHT is 1: 11267–1: 6090 (Table 2).

Discussion

Our study population seems to be limited to screen for a rare disease, but 1. HHT is not extremely rare and 2. this study area was covered completely by the hospital database and each family practicioner was accessible.

Nearly half of the I7800 medical records belonged to patients with diseases other than HHT. As I7800 is one of the few ICD 10 codes including „telangiectasia", patients with any other causes of telangiectasia are often encoded as having HHT. Despite this unclear encoding practice, 6 out of the top 7 patients encoded with I7800 were definite HHT patients (Fig. 2).

As epistaxis is the most common symptom of HHT, the review of recurrent epistaxis patients' medical records is self-explanatory. Indeed, 5 out of the top 10 patients had HHT, although 4 of these 5 patients were already identified by the prior I7800 review. Beyond the 4 patients identified by the prior I7800 review, one additional HHT patient was

Table 2 The summary of the results by the different HHT patient exploration methods and the estimated maximal point-prevalence of HHT in the study population at the end of the investigation period

	Patient number	Patient notation on pedigree charts[a]	Estimated maximal prevalence
Database, I7800	7	see in Table 1	
Database, R0400	1		
de novo diagnosed	3		
Family screening	14		
Overall	25		
Patients deceased in the study period	-5		
Patients alive at the end of the study period	20		1: 11267
Further potential HHT patients with incomplete HHT diagnosis or being unavailable[b]	+5	F3 I.1., F4 II.1.and II.2., F7 V.12. and V.22.	1: 9014
Further unavailable potential at-risk individuals[c]	+12	F5 II.1., F7 IV.9., IV.10., IV.18., V.13., V.21., V.24. and VI.10, F8 II.2., F9 II.5. and III.6., F10 II.1.	1: 6090

For individuals' positions on pedigrees see Online Resources 1 and 2[a]

Unavailable potential HHT patients fulfil one or more Curacao criteria (mainly epistaxis) by hearsay [b], while in potential at-risk individuals the disease status is unknown [c]

explored by the epistaxis review (Fig. 2). Although the ratio of patients identified by the I7800 and R0400 reviews might vary according to the local encoding practice, the successive I7800 and R0400 reviews are suggested.

Two of the 3 HHT probands diagnosed de novo were screened successfully for a local HHT founder mutation (Table 1). This option is substantially faster and cheaper than the *ACVRL1/ENG* sequence analysis [8].

The majority of HHT patients was identified by the family screening, emphasizing its significance in the exploration of HHT. Furthermore, co-segregation analysis by family screening might confirm the pathogenicity of a suspected pathogenic mutation (*ENG* c.*816 + 5 G > A* in our study) [5, 10]. Children (F2 IV.1. and IV.2.) and young adults with the mutation need AVM screening, as AVMs might precede the onset of epistaxis and telangiectases [4]. In contrast, in children and young adults with the wild-type *ACVRL1* and *ENG* alleles (F1 III.6., F5 IV.1., F9 IV.2.) HHT can be excluded.

Founder effects (*ACVRL1 c.625 + 1 G > C* in families 1–5 and *ENG c.817–2 A > C* in families 7 and 8) significantly influenced the HHT status in our limited study population. The bigger the cluster of HHT patients with a founder mutation, the more remarkable is its influence to the *ACVRL1/ENG* ratio, affecting the population's HHT phenotype [6].

Either the age-related penetrance with an occassionally long asymptomatic period or the fear of the forthcoming disease might account for the high ratio of unavailable individuals (including patients refusing the family screening). Considering the unavailable potential HHT patients and at-risk individuals, the estimated HHT prevalence in the study population corresponds with the literature data, implying our algorithm's effectivity in the stratified population screening of HHT. On the other hand, we must emphasize that literature data for the HHT prevalence are estimated values.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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