brief report

Echocardiographic screening discloses increased values of pulmonary artery systolic pressure in 9 of 68 unselected patients affected with hereditary hemorrhagic telangiectasia

Carla Olivieri, PhD[1,], Lucav Lanzarini, MD[2,*], Fabio Pagella, MD[3], Lucia Semino, MD[3], Sabrina Corno, MD[3], Cristina Valacca, PhD[1], Henry Plauchu, MD[4], Gaetan Lesca, PhD[5], Martine Barthelet, MD[6], Elisabetta Buscarini, MD[7], Cesare Danesino, MD[1,8]*

Background: Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterized by the presence of telangiectases and arteriovenous malformations. In some families in whom a form of idiopathic pulmonary arterial hypertension cosegregated with HHT, mutations in the *ACVRL1* gene were present. **Purpose:** We noninvasively measured the pulmonary artery systolic pressure (PASP) in a group of patients with HHT. **Methods:** Doppler transthoracic echocardiography and mutation analysis by direct sequencing were used. **Results:** We studied 68 patients (age 19–84 years, mean 50.75 + 15.11; 32 females) and PASP measurement was possible in 44 (64. 7%); in addition, 9 of them (20.5%) showed elevated values. Molecular analysis identified mutations in the *ACVRL1* gene in 7 of these 9 subjects. Even on exclusion of relatives of the single case with known pulmonary hypertension, 5 of 37 patients (13.5%) still showed values higher than those of controls. **Conclusion:** The data indicate that elevated PASP values are a frequent and previously unrecognized complication of HHT. Because clinically significant pulmonary artery hypertension (a relevant cause of morbidity and mortality) may subsequently develop in these patients, we propose that the measurement of PASP should be included among the parameters recorded for all patients undergoing Doppler transthoracic echocardiography during routine clinical screening. ***Genet Med* 2006:8(3):183–190.**

Key Words: *hereditary hemorrhagic telangiectasia, pulmonary artery systolic pressure, ACVRL1 gene, Doppler transthoracic echocardiography, ACVRL1 mutations*

Hereditary hemorrhagic telangiectasia (HHT) (MIM 187300) is an autosomal dominant vascular dysplasia with the following characteristics: epistaxes and telangiectases are present in more than 95% of patients[1,2]; telangiectases involve the skin and mucosae (causing epistaxes and gastrointestinal bleeding that may be severe enough to require transfusions); visceral arteriovenous malformations (AVMs) are mainly observed in the liver (57% of patients), lungs (34%), and brain (9.7%)[2] and may cause severe life-threatening complications. Neurologic complications (strokes, cerebral abscesses, seizures) may be prevented with appropriate treatment of pulmonary AVMs.

The diagnosis of HHT can be confirmed, according to Curaçao's criteria,[3] when three of the four suggested diagnostic criteria (epistaxes, telangiectases, visceral lesions, first-degree affected relative) are present. The phenotype is highly variable, and penetrance is usually complete by the age of 40 years.[1]

Approximately 80% of patients with HHT carry mutations in either of two genes: Endoglin (*ENG*, OMIM 131195) (HHT1) or activin receptor-like kinase 1 (*ACVRL1*, OMIM 601284) (HHT2).[4,5] Evidence for a third locus has also been reported.[6,7] Association of the HHT phenotype with juvenile polyposis and mutations in the *MADH4* gene have recently been demonstrated[8] as well.

Pulmonary arterial hypertension (PAH) is a progressive disorder in which an increased pulmonary vascular resistance is caused by occlusion of the smallest pulmonary arteries; subsequently, right ventricular failure may occur.[9] A diagnosis of idiopathic pulmonary hypertension is proposed when PAH is observed in the absence of any known predisposing condition

From the [1]Biologia Generale e Genetica Medica, Università di Pavia, Pavia, Italy; [2]Divisione di Cardiologia, IRCCS Policlinico "S. Matteo," Pavia, Italy; [3]Clinica Otorinolaringoiatrica, Università di Pavia, IRCCS "S. Matteo," Pavia, Italy; [4]Génétique Clinique, Hôpital Hotel Dieu and Università "C. Bernard," Lyon, France, coordinator of HHT French-Italian network; [5]Laboratoire de Génétique, Hôpital Edouard Herriot and Università "C. Bernard," Lyon, France; [6]Service d'exploration fonctionnelle cardiovasculaire, Hôpital Louis Pradel, Lyon, France; [7]Unità Operativa di Gastroenterologia, Ospedale Maggiore, Crema, Italy; [8]Genetica Medica, IRCCS Policlinico "S. Matteo," Pavia, Italy.

Cesare Danesino, MD, Genetica Medica, Via Forlanini 14, 27100 Pavia, Italy.

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**C.O. (molecular investigations) and L.L. (clinical evaluation) equally contributed to this article.*

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such as pulmonary embolism, connective tissue disease, and lung or heart disease. Women are affected twice as commonly as men. The familial form of "primary pulmonary hypertension" (OMIM 178600) is observed in approximately 10% of overall cases.[10] It is rare, with an incidence of approximately 1 in 100,000 to 1 in 1,000,000, and inherited as an autosomal dominant trait with reduced penetrance; genetic anticipation was discussed by Deng et al. in 2000.[11] Finally, mutations in the *BMPR2* have also been identified.[12]

A form of PAH that is clinically and histologically indistinguishable from idiopathic pulmonary hypertension may occur in patients with HHT. In 2001, Trembath et al.[13] demonstrated mutations in the *ACVRL1* gene in patients who showed clinical features of both PAH and HHT; this observation was subsequently confirmed by other studies.[14,15] All of these studies were carried out in patients (or families) with a known history of PAH in whom symptoms of HHT could also be identified; the mutations that have been identified are summarized in Table 1.

We studied a group of 68 subjects affected by HHT who did not display any clinical evidence of PAH to assess pulmonary artery systolic pressure (PASP) values. We were interested in the possible increase of PASP values, as well as in the frequency of the increase, and in correlating, if possible, elevated PASP with genotype.

Table 1
Previously reported *ACVRL1*-PH–related mutations

Exon	Protein	Mut	Type	Reference
2	113fs(+1aa)	37 delC[b]	fs	13
5[a]	D179A	536 A>C[b]	mis	14
6	G211D	632G>A[b]	mis	14
6	254delD	[b]	del	13
7	C344Y	1031G>A	mis	14
8	R374W	1120C>T	mis	14, 15
8	R374Q	1121G>A	mis	14
8	W399S	1196G>C[b]	mis	14
8	R411W	1231C>T[b]	mis	13
8	R411Q	1232G>A	mis	14
10	S462X	1385C>G[b]	nons	15
10	R479X	1435C>T[b]	nons	15
10	R484WfsX493	1450C>T, 1450_1451insG[b]	fs	15
10	R484W	1450C>T[b]	mis	13
10	K487T	1460A>C[b]	mis	14
10	Q490X	1468C>T[b]	nons	13

[a]*ACVRL1* mutation found in a patient with PAH but no clinical signs of HHT.
[b]Mutations identified for the first time in patients with PAH and HHT.

MATERIALS AND METHODS

Patients

With Doppler transthoracic echocardiography (TTE), 68 consecutive patients (32 females) from 48 different families were screened, previously diagnosed with HHT by C.D., E.B., and F.P., according to Curaçao's criteria.[3]

None of the subjects studied had a personal clinical history suggesting PAH; cases 10 to 16 (Table 2) belong to a family with a known history of PAH in a single relative who was not included in the present study; the relatives tested were selected on the basis of HHT diagnostic criteria only.

In case 1 (Table 2), PAH was judged to be secondary to a mitral valve disease, whereas none of the other patients demonstrated any known risk factors (systemic disorders or exposure to chemicals). Six additional patients with HHT, known carriers of exon 10 mutations who were not included in the original group of 68, were studied in Lyon by B.M.

Doppler transthoracic echocardiography

Echocardiographic studies were performed on all patients using standard M-mode, two-dimensional, and Doppler echocardiographic evaluations. A commercially available GE-Vingmed ultrasound (System Five) (Horten, Norway) instrument and a 2.5 to 3.5 phased-array transducer were adopted for cardiac imaging, pulsed- and continuous-wave Doppler, and measurement of pulmonary pressure. A contrast echocardiographic study for verifying the presence of pulmonary AVMs was also performed in each patient. The Doppler method used for the evaluation of systolic pulmonary artery pressures is described in detail in a previous study.[16] Measurements represent an average of three normal sinus rhythm beats. Pulsed- and continuous-wave Doppler echocardiographic velocity tracings were recorded on paper strip charts at a speed of 100 mm/sec.

Contrast echocardiography was performed in all patients according to Nanthakumar et al.[17] After having excluded the presence of intracardiac shunt, 10 to 30 mL of agitated saline were injected into a peripheral vein. Appearance of a cloud of bubbles in the left atrium occurring at least three cardiac cycles after first appearance in the right atrium was considered confirmation of right-to-left pulmonary shunting because gas bubbles do not survive a normal capillary bed. The cardiologist performing the echocardiographic studies was blinded to the clinical history of patients and to the results of other diagnostic and laboratory tests.

Sex and age-related reference values for PASP, obtained by the same method, were reported by McQuillan et al.[18]

Catheter-derived pulmonary artery pressure estimates were then proposed to the patients who showed clear clinical indications and was subsequently performed in case 1; the procedure was refused by cases 2 and 4.

Molecular analysis

DNA was obtained from peripheral blood after informed consent. Mutation analysis for *ACVRL1* and *ENG* genes was

Table 2
Italian patients: Clinical findings

Case	ID	Sex/age	ePASP (mm Hg)	Case	ID	Sex/age	ePASP (mm Hg)
1	**HHT01:A101**	**f/63**	**58**	36	HHT23:101	m/67	28
2	**HHT02:A101**	**m/72**	**50**	37	HHT23:201	m/40	nv
3	HHT02:A201	f/42	27	38	HHT24:101	f/55	27
4	**HHT03:A101**	**f/60**	**50**	39	HHT25:101	m/53	27
5	HHT03:A201	f/33	25	40	HHT26:E203	f/44	25
6	**HHT04:A101**	**m/71**	**47**	41	HHT26:E201	m/52	nv
7	HHT05:101	m/71	43	42	HHT27:A303	f/52	25
8	**HHT06:101**	**f/59**	**42**	43	HHT27:A311	m/53	25
9	**HHT07:A101**	**f/26**	**40**	44	HHT28:101	m/48	25
10	HHT08:A314	m/67	40	45	HHT29:A101	m/54	25
11	HHT08:A406	f/55	32	46	HHT29:A201	m/28	nv
12	HHT08:A506	f/29	30	47	HHT30:101	m/60	25
13	HHT08:A507	f/25	30	48	HHT31:S201	m/19	22
14	HHT08:A418	f/37	23	49	HHT31:S301	m/46	nv
15	HHT08:A407	f/52	28	50	HHT32:101	f/28	20
16	HHT08:A335	f/56	nv	51	HHT33:101	f/41	20
17	HHT09:101	m/66	40	52	HHT34:E201	f/29	nv
18	HHT10:101	f/59	35	53	HHT34:E203	m/26	nv
19	HHT10:201	f/56	nv	54	HHT35:101	f/65	nv
20	HHT11:A101	f/65	35	55	HHT35:201	f/40	nv
21	HHT11:A103	f/56	30	56	HHT36:101	f/50	nv
22	HHT12:101	f/84	35	57	HHT37:101	f/50	nv
23	HHT13:101	m/73	33	58	HHT38:101	f/59	nv
24	HHT14:101	f/47	32	59	HHT39:101	f/76	nv
25	HHT15:101	f/74	32	60	HHT40:101	m/28	nv
26	HHT16:101	m/67	32	61	HHT41:101	m/30	nv
27	HHT16:201	f/38	23	62	HHT42:101	m/38	nv
28	HHT17:101	m/25	30	63	HHT43:101	m/41	nv
29	HHT18:101	m/47	30	64	HHT44:101	m/42	nv
30	HHT19:101	m/56	30	65	HHT45:E101	m/51	nv
31	HHT20:101	m/64	30	66	HHT46:101	m/52	nv
32	HHT21:101	m/65	30	67	HHT47:101	m/70	nv
33	HHT22:101	m/44	29	68	HHT48:101	m/72	nv
34	HHT22:103	m/47	25				
35	HHT22:105	m/41	nv				

Cases belonging to the same families have the same family number in the ID. In bold, patients with ePASP values out of the 95% confidence interval (CI). ePASP, estimated pulmonary artery systolic pressure.

performed according to Olivieri et al.[19]; primers for exon amplification were obtained through the Genome Data Base[20] or designed by O.C. using Primer 3 Input Software.[21] Molecular analysis of the French patients was performed by L.G. as described in Lesca et al.[22]

RESULTS

A synopsis of TTE measurement of PASP and the patients' molecular results is included in Tables 2 and 3; the results from the French group are shown in Table 4.

Table 3
Italian patients: Molecular findings

Case	Mutation				References
	Gene	Exon	Genome	Protein	
29	*ACVRL1*	E3	c. G152A	C51Y	34
68	*ACVRL1*	E3	c. $\Delta T_{164}GGTGC_{169}$	ΔL55V56 *in frame*	Unpublished
42, 43	*ACVRL1*	E3	c. G172T	E58X	19
23	*ACVRL1*	E3	c. 203 ins G	G68 fs X166	Unpublished
24	*ACVRL1*	E3	c. G235A	G79R	Unpublished
20, 21, 45, 46	*ACVRL1*	E3	c. $\Delta C_{289}ACAAC_{294}$	ΔH97N98 *in frame*	19
17	*ACVRL1*	E4	c. 321 del A	Q107 fs X121	Unpublished
59	*ACVRL1*	E4	c. C430T	R144X	35
67	*ACVRL1*	E7	c. 824duplGGCT	L273 fs X392	Unpublished
9	*ACVRL1*	E8	c. T1127A	M376K	Unpublished
1	*ACVRL1*	E8	c. T1139G	V380G	36
6	*ACVRL1*	E8	c. C1199A	A400D	19
2, 3	*ACVRL1*	E8	c. G1232A	R411Q	37
10–16	*ACVRL1*	E10	c. C1435T	R479X	22
4, 5	*ACVRL1*	E10	c. C1450T	R484W	13
65	*ENG*	E3	c. C277T	R93X	38
58	*ENG*	E6	c. C_{780}Ins (Dupl C_{768}-G_{775})	S260 fs X358	Unpublished
52, 53	*ENG*	E6	g. IVS 6 + 5 g->c	Splicing	Unpublished
26, 27	*ENG*	E11	c. ΔG1478	S492 fs X516	Unpublished
40, 41	*ENG*	Linkage			

The TTE method permitted PASP measurement in 44 of 68 patients (64.7%) whose mean age was 50.75 ± 15.11 years (range 19–84 years); 23 were females.

The absence of tricuspid valve regurgitation prevented PASP measurement in 24 patients; this finding, associated with normal right ventricular morphology and normal 12-lead electrocardiogram (ECG), suggests normal right ventricular function, rendering PAH extremely unlikely in this subset of patients.

Table 4
French patients: Clinical and molecular findings

ID	Sex	Age	Mutation				ePASP
			Gene	Exon	Type	Protein	
26764	F	51	*ACVRL1*	E10	Nonsense	R479X	Normal
27769	F	70	*ACVRL1*	E10	Missense	R479Q	Normal
23974	M	63	*ACVRL1*	E10	Nonsense	R479X	Normal
25398	M	74	*ACVRL1*	E10	Nonsense	R479X	Normal
26600	F	53	*ACVRL1*	E10	Duplication	Dupl R484	Normal
22480	F	59	*ACVRL1*	E10	Missense	R479L	25 mm Hg
26480	F	57	*ACVRL1*	E10	Missense	R484Q	35 mm Hg

ePASP, estimated pulmonary artery systolic pressure.

The PASP values observed in our group of patients with HHT (44 cases, mean: 30.83 ± 7.87 mm Hg) were compared with the reference values for the different age groups from the large study by McQuillan et al.[18] (3790 controls: mean 28.3 ± 4.9 mm Hg) and entered in Figure 1A (males) and B (females). No statistical tests were applied to the two groups because of the large difference in their size.

Nine unrelated patients with HHT (four females) showed PASP values higher than 1 standard deviation (SD) for their age group, and six of them (four females) had PASP values outside the 95% confidence interval (CI); seven of nine subjects (cases 1, 4, 7, 8, 9, 10, and 17) with elevated PASP values showed contrast echocardiographic evidence of right-to-left pulmonary shunting.

In case 1, a previously undiagnosed significant mitral stenosis plus regurgitation was first identified by echocardiography performed for the purposes of the present study; the increased value of PASP in this case was judged to be mostly secondary to the valvular abnormality, and this case was not considered in the analysis of results or in the discussion.

The involvement of *BMPR2* was excluded in the only family whose size made it suitable for haplotype analysis (cases 10–16, data not shown).

After the identification of mutations in exon 10 of *ACVRL1* (Table 3) in two of nine subjects with increased PASP values,

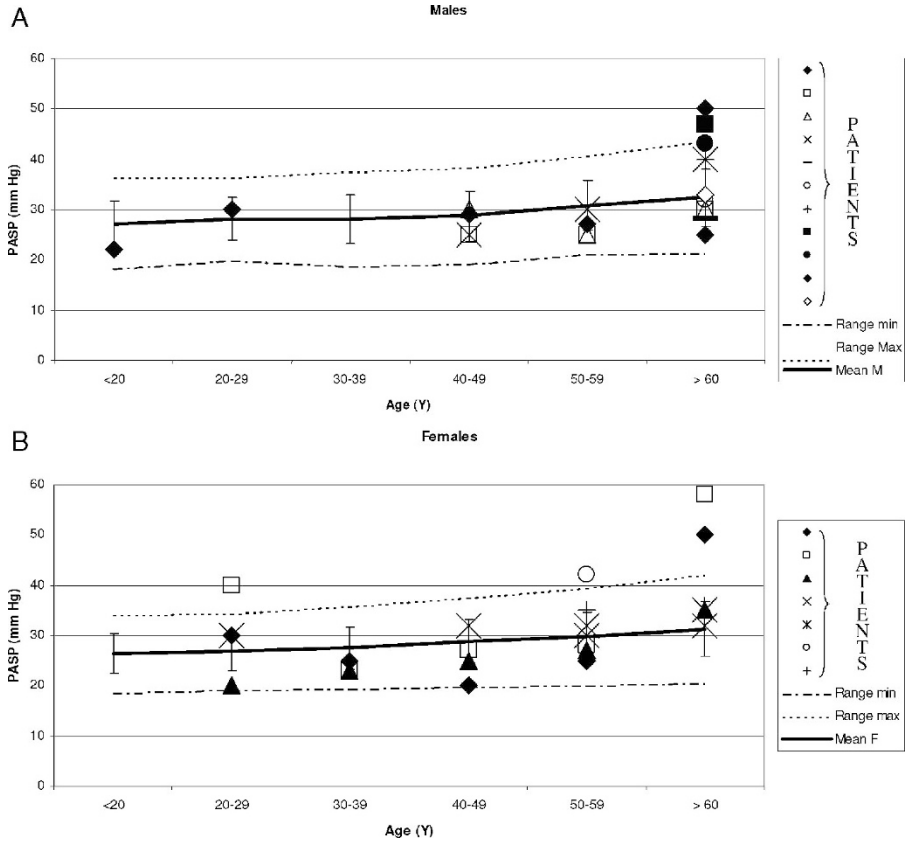

Fig. 1. Pulmonary artery pressure (PAP) distribution in our cohort of patients with HHT. **A:** males; **B:** females. Normal range limits, mean and 1 standard deviation (SD) values *(error bars)* as in McQuillan et al.[18]

mutations that have already been reported in patients with HHT and PAH (Table 1),[13–15] we tested PASP in six other patients with HHT carrying *ACVRL1* exon 10 mutations, all of whom showed normal findings (Table 4) (MB in Lyon).

Mutations found in either *ENG* or *ACVRL1* are listed in Table 3; in 27 subjects, a mutation in *ACVRL1* was found, whereas eight subjects (three of eight showing normal PASP values and five of eight with no tricuspid regurgitation, and thus nonmeasurable PASP) carried an *ENG* mutation.

Cases 10 to 16, in whom the R479X mutation in *ACVRL1* was found, are relatives of a patient (not included in the present report) who was diagnosed with PAH and who underwent lung transplantation at age 20 years; the diagnosis of HHT was made only several years later. She belongs to a very large family (>50 people in the pedigree), and no diagnosis of PAH was proposed for any of her other relatives. Cases 12 and 13 (Table 2) are at the upper limit for PASP for their age group, and case 10 is above 1 SD; other relatives are fully within normal limits (cases 11, 14, and 15) or not measurable (case 16); these data are in keeping with the reduced penetrance of PAH due to the *ACVRL1* mutation.

The mother of the index case in this family (case 16) at age 56 years showed no tricuspid regurgitation and normal ECG; similarly, case 15 (the mother of cases 12 and 13) at age 52 years was fully normal, whereas her daughters have PASP at +1 SD compared with normal controls.

In our group of patients with HHT, we collected data from seven additional parent/child pairs: in five of them both parent and child were completely within normal limits or not measurable (cases 26 and 27; 36 and 37; 45 and 46; 48 and 49; 54 and 55; Table 2), whereas in two families (father/daughter and mother/daughter, cases 2 and 3, and 4 and 5, Table 2), the parent presented with a PASP value above the upper range limit, whereas their offspring showed PASP values within normal limits.

DISCUSSION

The association of PAH with HHT in the same family or the same subject was first reported by Trembath et al.[13] and subsequently confirmed by several authors[14,15,23] in 23 cases. All of the families in whom both PAH and HHT were present carried mutations in the *ACVRL1* gene, and mutations or linkage to *BMPR2* was consistently excluded. The 16 different mutations were scattered over several exons, but 11 of 16 were localized in exon 8 or 10 (Table 1).

Three patients who carried an *ENG* mutation and showed PAH were reported as having a known intake of dexfenfluramine, a drug that is known to possibly cause PAH, or as having other well-known causes for increased pulmonary artery pressure[14,24]; a further case carrying a branch site mutation of *ENG* was recently reported by Harrison et al.[25]

All cases or families reported up to now have been selected on the basis of a clinical diagnosis of PAH associated with symptoms of HHT.

In the course of a general clinical screening program on patients diagnosed with HHT according to Curaçao's criteria,[3] but who evidenced no clinical evidence of PAH, we obtained PASP data by using TTE in 44 of 68 patients.

This method is suitable for obtaining reliable estimates of PASP because the measurements obtained strictly correlate with those acquired by means of invasive procedures (concordance correlation coefficient 0.88, 95% CI 0.82–0.93).[16] Indeed, TTE has been considered a routine method for noninvasive PASP assessment by several other groups[26,27]; in addition, it makes it possible to obtain more general information on heart anatomy and function, including the right-sided chambers.

TTE proved useful in case 1, in whom a previously undiagnosed mitral valve disease was present; naturally, when the patient's cardiologic evaluation showed abnormalities that required further diagnostic steps, invasive procedures were also performed with consistent results (case 1, PASP 41 mm Hg by right-sided heart catheterization), as expected.

Overall, 8 of 44 patients (excluding case 1) (18.2%, three females) showed PASP values above 1 SD, and five of them were also above the upper 95% CI for age-related controls (Fig. 1A and B).

Our results indicate that PASP values exceeding control values may be found in a relevant proportion (5/44, 3 females, 11.4%) of patients selected solely on the basis of a diagnosis of HHT, without overt signs of PAH. Moreover, in the large reference study by McQuillan et al.,[18] 28% of healthy subjects, irrespective of age or other parameters, had PASP values greater than 30 mm Hg, whereas in our group this figure increases to 34.1% (15/44); indeed, if cases of PASP values 30 mm Hg or greater are considered, this statistic increases to 54.5% (24/44).

Among patients with increased PASP values, five of nine also showed hepatic arteriovenous fistulae. These were of limited size and number (grade 1 or 2 according to Buscarini et al.[28]) and did not cause a significant increase of right atrial flow as assessed both by normal appearance of the right atrial component of P wave on standard ECG and by absence of right atrial dilatation on TTE. Therefore, these fistulae do not seem to be related to the observed changes in PASP.

No data are currently available on the course of PASP levels in patients with HHT to assess whether these high values will increase over time to develop into a fully expressed PAH or not; on the basis of our results, we now recommend a yearly follow-up with TTE to all patients with PASP values at or above 1 SD over the mean; this is in agreement with the protocol suggested by Daniels et al.[27] for serendipitously diagnosed cases of mild asymptomatic pulmonary hypertension.

Estimated PASP values and mutation analysis in the family to which cases 10 to 16 belong (see "Results") are in keeping with the reduced penetrance of PAH due to *ACVRL1* mutation and with previous observations of some families in whom the appearance of PAH may show age anticipation between generations.[29]

On the basis of familial recurrence of PAH in families with HHT, we believe that patients with HHT such as cases 3 and 5 (with normal PASP values but one parent with increased values), should be offered the same follow-up as for subjects with confirmed increase of PASP values.

We also selected six additional patients solely on the basis of presence of mutations in exon 10 from the cohort of HHT cases reported by Lesca et al.[22] because this exon has frequently been reported (Table 1) to bear PAH-related mutations; however, in these additional cases TTE failed to demonstrate increased values of PASP (Table 4).

Overall, we found more mutations in the *ACVRL1* gene than in *ENG* (Table 3); this irregular distribution is in keeping with similar data provided by Lesca et al.[22] in the French population, and with our unpublished data on more than 100 index cases from among the Italian population.

The distribution of mutations of the *ACVRL1* gene in HHT/PAH is peculiar in the sense that 11 of 16 reported mutations (68.8%) are localized in exons 8 and 10; these two exons contain only 30% to 35% of the variously reported mutations when taking into consideration the reviews by Abdalla and Letarte,[30] and van den Driesche et al.,[31] the data by Lesca et al.,[22] and our unpublished results.

Among the mutations we found in patients with HHT with increased PASP (Table 3), two of six are in exon 10 and have already been described in patients with PAH and HHT; the others are in exon 8, and one of them was previously unreported.

Mutations occurring in exon 8 frequently cause the modification of an arginine residue; R437 and R411 have so far been involved five times and are thus likely to be mutation hot spots. The possible mechanism to explain the frequent involvement of arginine residues has been discussed by Abdalla et al.[32]

Exon 10 contains the NANDOR BOX, relevant for the regulation of TGFbeta signaling,[15,33] and four of six mutations fell into this region, as did the two mutations we observed where codon 479 was more frequently involved.

The only known mutation of exon 5 of *ACVRL1* was observed in a patient with PAH who did not, however, show any clinical signs or have a family history of HHT.[14]

At present, PAH was observed only in association with HHT2; this observation, if confirmed, would be a defined genotype–phenotype correlation for *ACVRL1* mutations.

It is clearly essential to document more cases to verify whether mutations in exons 8 and 10 in general, or those previously discussed in particular, possibly constitute a specific genetic risk factor for developing PAH.

In our group of patients with HHT, if we exclude all the cases from the family with a single known patient affected with PAH together with case 1, we still have 5 of 37 (13.5%) cases with PASP values of the normal range; this suggests that this seemingly abnormal finding may in fact be much more common than previously thought. Thus our data add confirmatory evidence to recent reports by Harrison et al.[14] and Abdalla et al.[15] demonstrating that

PAH should be considered a possible severe complication in the course of HHT.

Presently, TTE is being used in the routine clinical workup of patients affected by HHT to assess the presence of lung AVMs. We believe that PASP measurement should also be attempted in all HHT cases, and certainly in those patients carrying mutations previously demonstrated to be associated with PAH.

TTE is a noninvasive test, well tolerated by patients, that can even be safely performed in children, a relevant point in view of the increasing number of cases of PAH observed in pediatric age,[25,13,29] and in light of the possible anticipation of symptoms.

In conclusion, the pathway by which *ENG* and *ACVRL1* regulate transforming growth factor-beta signaling includes a large number of genes and proteins; it is therefore highly likely that other unreported associations between HHT and apparently unrelated diseases may be discovered in the future by means of a more careful and focused clinical examination of large series of patients.

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