Clinical Outcomes of Pulmonary Arterial Hypertension in Patients Carrying an *ACVRL1* (*ALK1*) Mutation

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***Rationale*: Activin A receptor type II-like kinase-1 (*ACVRL1*, also known as *ALK1*) mutation is a cause of hereditary hemorrhagic telangiectasia (HHT) and/or heritable pulmonary arterial hypertension (PAH).**

***Objectives*: To describe the characteristics of patients with PAH carrying an *ACVRL1* mutation.**

***Methods*: We reviewed clinical, functional, and hemodynamic characteristics of 32 patients with PAH carrying an *ACVRL1* mutation, corresponding to 9 patients from the French PAH Network and 23 from literature analysis. These cases were compared with 370 patients from the French PAH Network (93 with a bone morphogenetic protein receptor type 2 [*BMPR2*] mutation and 277 considered as idiopathic cases without identified mutation). Distribution of mutations in the *ACVRL1* gene in patients with PAH was compared with the HHT Mutation Database.**

***Measurements and Main Results*: At diagnosis, *ACVRL1* mutation carriers were significantly younger (21.8 $\pm$ 16.7 yr) than *BMPR2* mutation carriers and noncarriers (35.7 $\pm$ 14.9 and 47.6 $\pm$ 16.3 yr, respectively; $P < 0.0001$). In seven of the nine patients from the French PAH Network, PAH diagnosis preceded manifestations of HHT. *ACVRL1* mutation carriers had better hemodynamic status at diagnosis, but none responded to acute vasodilator challenge and they had shorter survival when compared with other patients with PAH despite similar use of specific therapies. *ACVRL1* mutations in exon 10 were more frequently observed in patients with PAH, as compared with what was observed in the HHT Mutation Database (33.3 vs. 5%; $P < 0.0001$).**

***Conclusions*: *ACVRL1* mutation carriers were characterized by a younger age at PAH diagnosis. Despite less severe initial hemodynamics and similar management, these patients had worse prognosis compared with other patients with PAH, suggesting more rapid disease progression.**

Keywords: bone morphogenetic protein receptor type 2, *BMPR2*; hemodynamic; hereditary hemorrhagic telangiectasia; pulmonary hypertension

(*Received in original form August 25, 2009; accepted in final form January 6, 2010*)

Supported by grants from the Ministère de l'Enseignement Supérieur et de la Recherche and the Université Paris-Sud.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 181. pp 851–861, 2010
Originally Published in Press as DOI: 10.1164/rccm.200908-1284OC on January 7, 2010
Internet address: www.atsjournals.org

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Patients with pulmonary arterial hypertension (PAH) carrying a bone morphogenetic protein receptor type 2 (*BMPR2*) mutation present approximately 10 years earlier than noncarriers and have a more severe hemodynamic compromise at diagnosis. Mutations in the activin A receptor type II-like kinase-1 (*ACVRL1*) gene are also recognized as a cause of heritable PAH. However, the influence of these mutations on clinical outcomes is unknown.

What This Study Adds to the Field

ACVRL1 mutation carriers are characterized by a younger age at PAH diagnosis. Despite a less severe initial hemodynamic compromise and similar management approach, these patients have a worse prognosis compared with other patients with PAH, suggesting more rapid disease progression.

Pulmonary arterial hypertension (PAH) is a severe disease affecting small pulmonary arteries, with progressive remodeling leading to elevated pulmonary vascular resistance and right ventricular failure (1, 2). PAH may occur in a number of different clinical contexts (3, 4). Idiopathic PAH corresponds to sporadic disease, without any family history of PAH or known triggering factor. When PAH occurs in a familial context, germline mutations in the bone morphogenetic protein receptor type 2 (*BMPR2*) gene are detected in approximately 70% of cases (5–10). *BMPR2* mutations can also be detected in 3.5 to 40% of apparently sporadic cases (5–8, 11). The term "heritable" PAH has been proposed to describe these genetic forms of the disease (4, 6, 7, 12–14).

Hereditary hemorrhagic telangiectasia (HHT) is characterized by mucocutaneous telangiectases, recurrent epistaxes, and macroscopic arteriovenous malformations, particularly in the pulmonary, hepatic, and cerebral circulation. When present, pulmonary arteriovenous malformations may create clinically significant right-to-left shunts, causing hypoxemia, paradoxical embolism, stroke, and cerebral abscesses (15–20). In patients with HHT, postcapillary pulmonary hypertension may develop as a consequence of a hyperkinetic state, resulting in high cardiac output heart failure. However, HHT is also associ-

ated with a precapillary pattern of pulmonary hypertension that is histologically indistinguishable from idiopathic PAH (20, 21).

HHT is inherited in an autosomal dominant fashion with late-onset penetrance and nearly complete penetrance (97%) by the age of 60 years (22). Several genes have been implicated in the pathogenesis of HHT, including activin receptor-like kinase-1 (*ACVRL1* or *ALK1*) located on chromosome 12, *endoglin* on chromosome 9, *MADH4* (encoding the protein mothers against decapentaplegic homologue 4 [SMAD4], mutations of which also lead to juvenile polyposis), and two new loci (*HHT3* and *HHT4*) mapped on chromosomes 5 and 7 (20, 23, 24). Data from several case series indicate that *ACVRL1* mutations may predispose to PAH (21, 25–28). In addition, rare cases of PAH in *endoglin* mutation patients have been reported, further supporting the probable involvement of the transforming growth factor (TGF)-β signaling pathway in the pathophysiology of both PAH and HHT (25, 27, 29).

Accumulated evidence indicates that patients with PAH carrying a *BMPR2* mutation present approximately 10 years earlier than noncarriers, with more severely compromised hemodynamic status at diagnosis, and are less likely to respond to acute vasodilator testing (6, 7, 30). We hypothesized that mutated *ACVRL1* status might be associated with distinct PAH phenotypes, as compared with patients with PAH without *ACVRL1* mutation. To test this hypothesis, the French PAH Network obtained data on consecutive patients displaying PAH in whom point mutations and large size rearrangements of *BMPR2* and *ACVRL1* were screened for, and reviewed data in the literature for patients with PAH carrying an *ACVRL1* mutation. Clinical, functional, and hemodynamic characteristics were compared between patients with PAH carrying an *ACVRL1* mutation, patients carrying a *BMPR2* mutation, and patients with PAH considered to be idiopathic and who were noncarriers of either a *BMPR2* or *ACVRL1* mutation.

METHODS

Patients

We reviewed data from all patients with PAH considered to be idiopathic and patients with a family history of PAH, who were tested for *BMPR2* and *ACVRL1* mutations, seen in the French PAH Network (Université Paris-Sud 11, Hôpital Antoine-Béclère, Clamart, France) between January 1, 2004 and April 1, 2009. In accordance with the guidelines of the American College of Chest Physicians (31), patients tested for *BMPR2* or *ACVRL1* mutations signed written informed consent and underwent genetic counseling. A diagnosis of PAH was defined by hemodynamic measurement during right-heart catheterization (*see below*). Manifestations of HHT were screened and reported in patients with PAH carrying an *ACVRL1* mutation. Patients with a family history of PAH without evidence of either *BMPR2* or *ACVRL1* mutation (n = 18) were excluded from the analysis to limit the risk of misclassification. All clinical characteristics at PAH diagnosis and follow-up were stored in the Registry of the French PAH Network (32). This registry was set up in agreement with French bioethics laws (French Commission Nationale de l'Informatique et des Libertés), and patients gave their consent to be included (7, 32). Additional detail is provided in the online supplement.

Hemodynamic Measurements and 6-Minute Walk Distance

PAH was defined as a mean pulmonary arterial pressure equal to or exceeding 25 mm Hg associated with a normal pulmonary capillary wedge pressure. Hemodynamic evaluation by right-heart catheterization was performed at baseline in all subjects according to our previously described protocol (33, 34). A nonencouraged 6-minute walk test was performed according to American Thoracic Society recommendations (35). Additional detail is provided in the online supplement.

Screening of Point Mutations and Large Rearrangements of *ACVRL1* and *BMPR2* Genes

Human genomic DNA was prepared from whole blood samples. Amplification of the entire coding sequence and intronic junctions of the *ACVRL1* and *BMPR2* genes was performed on 50 ng of genomic

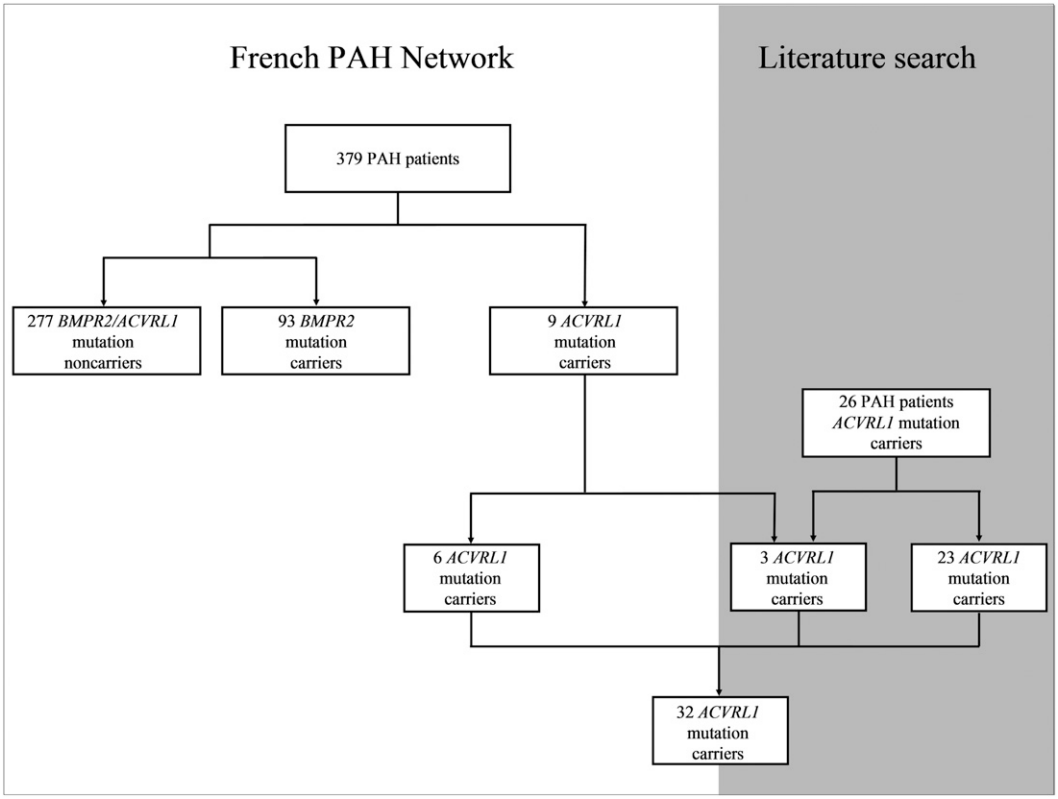


Figure 1. Patient disposition. *ACVRL1* = gene encoding activin A receptor type II-like kinase-1; *BMPR2* = gene encoding bone morphogenetic protein receptor type 2; PAH = pulmonary arterial hypertension.

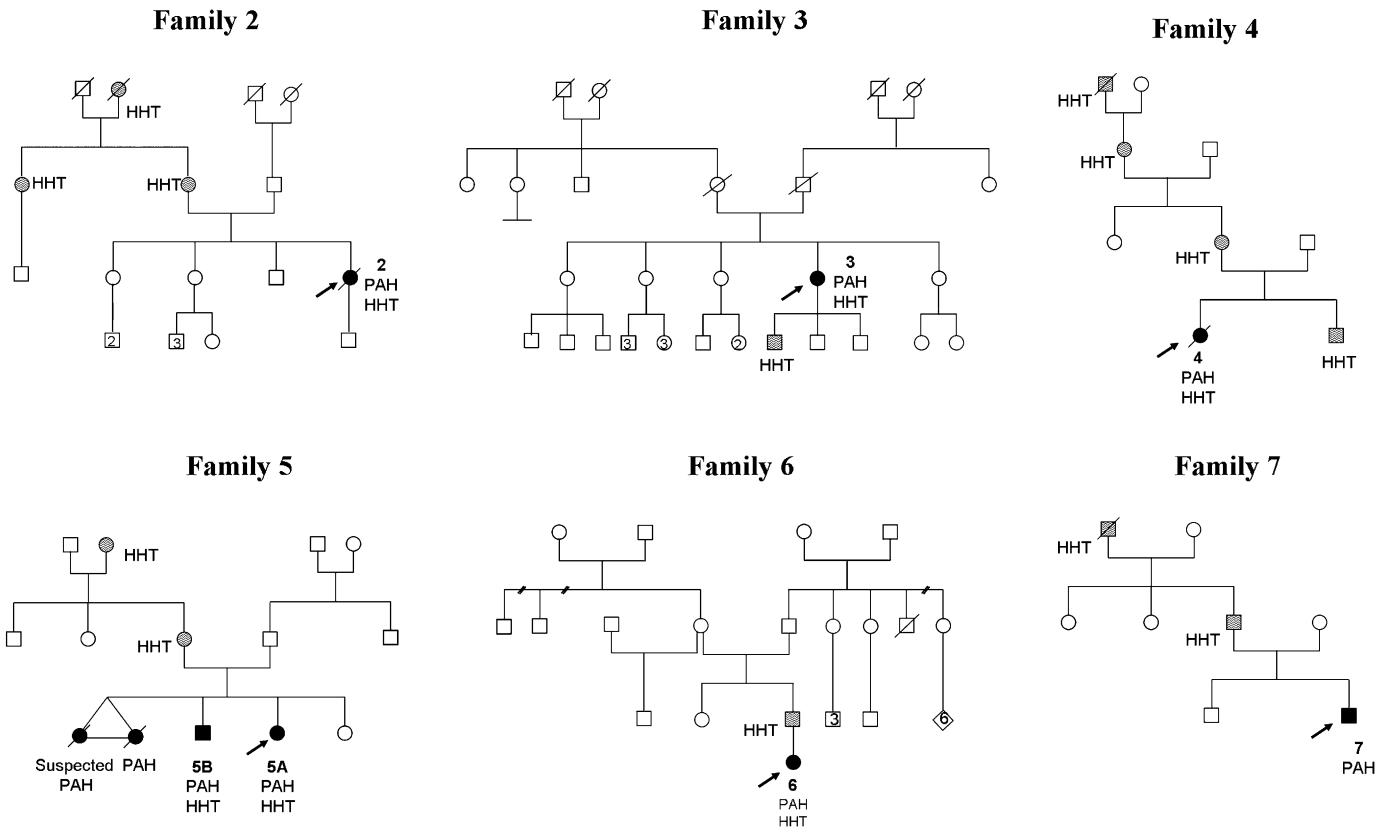


Figure 2. Family trees of patients with pulmonary arterial hypertension (PAH) and carrying an activin A receptor type II-like kinase-1 (*ACVRL1*) mutation from the French PAH Network. In family 1, no familial history was available (adoption). HHT = hereditary hemorrhagic telangiectasia.

DNA from each individual. Genetic variation of the *BMPR2* sequence was detected as previously described (7). Genetic variation of *ACVRL1* was assayed by polymerase chain reaction and sequencing. The SALSA multiplex ligation-dependent probe amplification (MLPA) P093 HHT probemix kit (MRC-Holland BV, Amsterdam, The Netherlands) was used to screen for rearrangements of one or more exons of the *ACVRL1* and *BMPR2* genes. Additional description of the method is provided in the online supplement.

Literature Review

We performed a MEDLINE (National Library of Medicine, Bethesda, MD) search for PAH in patients carrying an *ACVRL1*/*ALK1* mutation in articles published in the English language until April 2009. We recorded all data from patients with PAH carrying an *ACVRL1* mutation reported in the literature. Individual clinical, functional, and hemodynamic characteristics from patients with PAH carrying an *ACVRL1* mutation were available in five studies (21, 25–27, 36) and *ACVRL1* mutation status for patients with PAH were reported in six studies (21, 25–28, 36). The distribution and frequency of mutations in the *ACVRL1* gene in patients with PAH were compared with *ACVRL1* mutations reported in the HHT Mutation Database (http://www.hhtmutation.org) on August 1, 2008, after analysis of the literature and exclusion of *ACVRL1* mutations exclusively reported in patients with PAH.

TABLE 1. CLINICAL, FUNCTIONAL, AND HEMODYNAMIC CHARACTERISTICS OF *ACVRL1* MUTATION CARRIERS FROM THE FRENCH PULMONARY ARTERIAL HYPERTENSION NETWORK

	Patient								
	1	2	3	4	5A	5B	6	7	Mean ± SD
Age at PAH diagnosis, yr	33.2	29.5	40.4	28	4.2	8.1	1	15	
NYHA functional class	III	III	III	III	III	II	III	II	
6-MWD, m	315	412	455	380	307	N/A	N/A	572	407 ± 99
mPAP, mm Hg	54	50	51	70	61.2	39	82	61	58 ± 13
RAP, mm Hg	20	5	4	13	11	8	1	3	8 ± 6
PCWP, mm Hg	8	4	N/A	N/A	9	10	1	5	8 ± 6
CO, L/min	3.0	3.8	N/A	2.9	3.1	4.3	1.3	6.7	3.6 ± 1.7
CI, L/min/m^2	1.8	2.4	6.0	1.4	5.6	4.6	3.0	3.9	3.6 ± 1.7
PVRi, mm Hg/L/min/m^2	26.3	19.3	N/A	N/A	9.3	6.3	26.7	14.4	15.4 ± 9.0
TPRi, mm Hg/L/min/m^2	30.8	21.0	8.5	48.9	10.9	8.5	27.1	15.6	21.4 ± 13.9
$S\bar{v}_{O_2}$, %	57	68	81	N/A	N/A	N/A	56	79	68 ± 12
Hemoglobin, g/dl	13.5	13	13.9	17.5	13.5	13.8	N/A	N/A	14.2 ± 1.6
Acute vasodilator response	No	No	No	No	No	No	No	No	All negative

Definition of abbreviations: 6-MWD = 6-minute walk distance; CI = cardiac index; CO = cardiac output; mPAP = mean pulmonary artery pressure; N/A = not available at PAH diagnosis; NYHA = New York Heart Association; PAH = pulmonary arterial hypertension; PCWP = pulmonary capillary wedge pressure; PVRi = indexed pulmonary vascular resistance; RAP = right atrial pressure; $S\bar{v}_{O_2}$ = mixed venous oxygen saturation; TPRi = indexed total pulmonary resistance.

TABLE 2. AGE AT FIRST SYMPTOMS AND DIAGNOSIS OF PULMONARY ARTERIAL HYPERTENSION AND HEREDITARY HEMORRHAGIC TELANGIECTASIA

	Patient	Sex	Age at PAH Diagnosis (*yr*)	Family History of PAH	Family History of HHT	HHT Manifestations	Age at HHT Diagnosis (*yr*)	Age at Death (*yr*)
French PAH Network	1	F	33.2	N/A	N/A	AVMs (pulmonary, ovarian, hepatic)	33.2	39.3
	2	F	29.5	No	Yes	AVMs (pulmonary hepatic) Telangiectases Epistaxes	31.3	31.5
	3	F	40.4	No	Yes	AVMs (splenic, hepatic) Epistaxes	37.8	—
	4	F	28	No	Yes	N/A	32.7	33
	5A	F	4.2	Yes	Yes	Epistaxes	10.1	—
	5B	M	8.1	Yes	Yes	Telangiectases Epistaxes	12.1	—
	5C	F	1.1	Yes	Yes	N/A	N/A	1.1
	6	F	1	No	Yes	No (postmortem diagnosis)	—	1.1
	7	M	15	No	Yes	No	—	—
Trembath and colleagues (21)	F1-III1	M	6	Yes	Yes	No	—	7
	F1-III2	M	1.5	Yes	Yes	No	—	2
	F1-III3	F	1.5	Yes	Yes	Telangiectases Epistaxes	N/A	9
	F2	M	29	No	Yes	Telangiectases Epistaxes	N/A	46
	F3-II1	F	45	Yes	Yes	AVMs (pulmonary) Telangiectases Epistaxes	N/A	50
	F3-II4	F	31	Yes	Yes	Telangiectases	N/A	34
Harrison and colleagues (25)	7685	F	51	No	No	No	—	54
	7340	F	50	No	Yes	Telangiectases Epistaxes	N/A	—
	7242	F	39	No	Yes	AVMs (pulmonary) Telangiectases Epistaxes	N/A	—
	7253	F	19	No	Yes	Telangiectases Epistaxes	N/A	N/A
	8261	F	39	Yes	Yes	Telangiectases Epistaxes	N/A	—
	7682	F	17	No	Yes	No	—	18
	8259	F	27	No	Yes	Telangiectases Epistaxes	N/A	—
	7252	F	21	No	Yes	Telangiectases Epistaxes	N/A	—
	7214	M	46	No	No	Telangiectases Epistaxes	N/A	—
Abdalla and colleagues (26)	60	F	8	Yes	Yes	Telangiectases Epistaxes	N/A	—
	82	M	0.4	No	Yes	Telangiectases Epistaxes	N/A	29
	91	F	43	No	Yes	AVMs (hepatic, gastrointestinal) Telangiectases Epistaxes	N/A	51
	100	F	18	No	Yes	AVMs (pulmonary, hepatic, gastrointestinal) Telangiectases Epistaxes	N/A	20
Harrison and colleagues (27)	7912	F	1.7	No	No	No	—	—
Smoot and colleagues (36)	K1	F	4	Yes	Yes	AVMs (pulmonary) Epistaxes	N/A	—
	K2	F	16	No	Yes	AVMs (pulmonary) Telangiectases Epistaxes	N/A	—
	K3	F	17	No	Yes	AVMs (pulmonary) Telangiectases Epistaxes	N/A	—

Definition of abbreviations: AVMs = arteriovenous malformations; F = female; HHT = hereditary hemorrhagic telangiectasia; M = male; N/A = not available; PAH = pulmonary arterial hypertension.

TABLE 3. FOLLOW-UP OF FRENCH PAH NETWORK PATIENTS WITH PULMONARY ARTERIAL HYPERTENSION CARRYING AN *ACVRL1* MUTATION

Patient	PAH Therapy	Follow-up (*months after diagnosis*)	Cause of Death
1	Epoprostenol	Death at Month 73	Right heart failure
2	Bosentan plus iloprost	Death at Month 24	Rupture of PAVM
3	Bosentan plus sildenafil	Alive at Month 51	—
4	Eproprostenol	Death at Month 59	Right heart failure
5A	Atrioseptostomy Epoprostenol plus bosentan	Alive at Month 81	—
5B	Bosentan plus epoprostenol	Alive at Month 59	—
5C	None: sudden death before initiation of PAH therapy	Death during the first month	Right heart failure
6	None: sudden death before initiation of PAH therapy	Death at Month 2	Right heart failure
7	Bosentan	Alive at Month 9	—

Definition of abbreviation: PAH = pulmonary arterial hypertension.

Statistical Analysis

We compared demographic and clinical features between *ACVRL1* mutation carriers, *BMPR2* mutation carriers, and *ACVRL1*/*BMPR2* mutation noncarriers by chi square test, Fisher's exact test, Kruskal-Wallis test, or analysis of variance followed by Fisher's protected least significant difference test, as appropriate. A P value less than 0.05 was considered to indicate statistical significance.

RESULTS

Patient Population

Between January 1, 2004, and April 1, 2009, all patients with PAH considered to be idiopathic and patients with a family history of PAH underwent genetic counseling and were offered *BMPR2* and *ACVRL1* screening. During this period, 388 patients were seen, 379 patients had genetic testing (98%), and 8 patients declined genetic testing. We identified 277 *BMPR2*/*ACVRL1* mutation noncarriers, 93 *BMPR2* mutation carriers, and 9 *ACVRL1* mutation carriers (Figure 1).

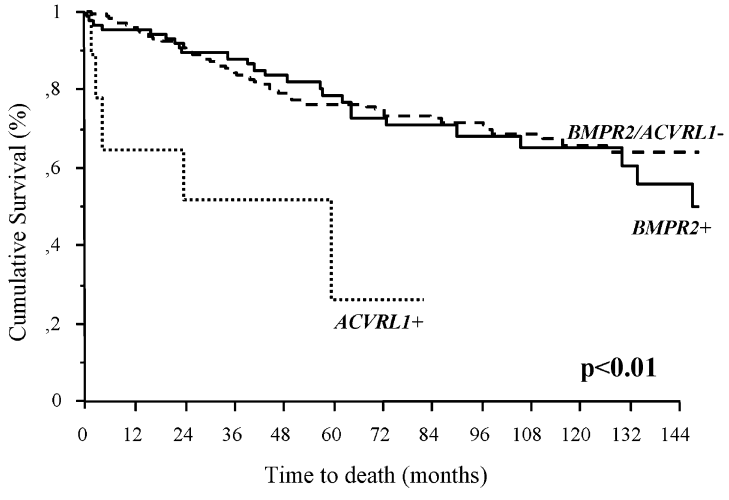
Characteristics of French PAH Network Patients Carrying an *ACVRL1* Mutation

The nine patients with PAH carrying an *ACVRL1* mutation were identified in seven different families. Six cases had not been described previously whereas three have been reported (21, 37) (Figure 1). Family trees of six families are presented in Figure 2. No familial data were available for family 1 (adoption).

Clinical, functional, and hemodynamic characteristics of the study population are presented in Table 1. Clinical manifestations of HHT, age at diagnosis of PAH and HHT, and age at death are presented in Table 2. As observed in patients with PAH considered as idiopathic or in patients with a family history of PAH, we observed a female predominance in *ACVRL1* mutation carriers who developed PAH (female-to-male ratio, 3.5). In four of nine patients, PAH was diagnosed before HHT, and two patients developed PAH without any clinical manifestations of HHT. Diagnoses of PAH and HHT were made at the same time in one patient. A personal HHT history was known before PAH diagnosis in only one patient. Epistaxes, telangiectases, and arteriovenous malformations were the most frequent manifestations of HHT observed in these patients. A familial PAH history was present in only one of the seven families reported (family 5). Patients were considered for PAH-specific therapy according to guidelines, but two of them died before initiation of this therapy (Table 3). In the nine patients with PAH and carrying an *ACVRL1* mutation, overall mortality was worse than in *BMPR2* mutation carriers or *BMPR2*/*ACVRL1* mutation noncarriers ($P < 0.01$) (Figure 3); the cause of death was directly related to PAH in all patients (Table 3).

Characteristics of French PAH Network Patients Carrying or Not Carrying a *BMPR2* Mutation

Outcome data relating to 223 patients with PAH from the French PAH Network, corresponding to 68 *BMPR2* mutation carriers and 155 *BMPR2*/*ACVRL1* mutation noncarriers, have previously been published by Sztrymf and colleagues (7). As of April 1, 2009, this series had been expanded to 379 patients with PAH, corresponding to 9 *ACVRL1* mutation carriers, 93 *BMPR2* mutations carriers, and 277 *BMPR2*/*ACVRL1* mutation noncarriers. A mutation was detected in 47 of 65 families (72.3%) with at least



BMPR2/ACVRL1-	277	225	193	166	134	114	99	84	73	56	40	33	26
BMPR2 +	91	82	70	63	50	44	37	30	25	23	18	13	9
ACVRLI +	9	5	4	4	3	1	1	0					

Figure 3. Outcomes of activin A receptor type II-like kinase-1 (*ACVRL1*) mutation carriers, bone morphogenetic protein receptor type 2 (*BMPR2*) mutation carriers and *BMPR2*/*ACVRL1* mutation noncarriers with pulmonary arterial hypertension from the French PAH Network.

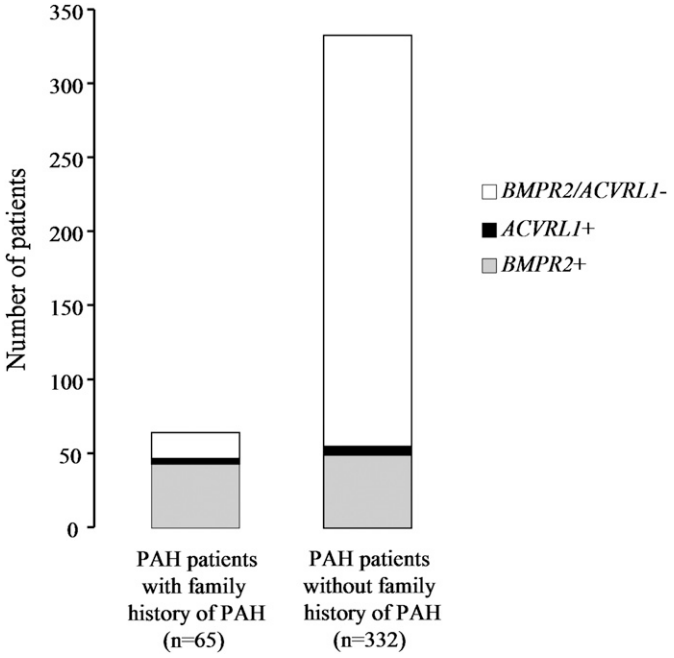


Figure 4. Distribution of bone morphogenetic protein receptor type 2 (*BMPR2*) and activin A receptor type II-like kinase-1 (*ACVRL1*) mutations in the French PAH Network. Nine of 379 patients with pulmonary arterial hypertension (PAH) with or without a family history of PAH were carriers of an *ACVRL1* mutation of whom three had a family history of PAH. In patients with PAH with a family history of PAH, 67.7% of patients carried a *BMPR2* mutation and 4.6% carried an *ACVRL1* mutation. In PAH considered to be idiopathic, 14.8% of patients were carriers of a *BMPR2* mutation and 1.8% were carriers of an *ACVRL1* mutation.

two cases of confirmed PAH, corresponding to 44 of 65 (67.7%) *BMPR2* mutations and 3 of 65 (4.6%) *ACVRL1* mutations. In those considered to have idiopathic PAH, 49 of 332 (14.8%) patients carried a *BMPR2* mutation and 6 of 332 (1.8%) carried an *ACVRL1* mutation (Figure 4). As previously demonstrated, we confirmed in this larger series that *BMPR2* mutation carriers were younger at diagnosis and at death ($P < 0.002$) (Figure 5). At diagnosis, *BMPR2* mutation carriers were more severely compromised in hemodynamic status, with a lower cardiac index and mixed venous oxygen saturation ($S\bar{v}_{O_2}$) and higher mean pulmonary arterial pressure and indexed pulmonary vascular resistance, as compared with *BMPR2*/*ACVRL1* mutation noncarriers (all $P < 0.02$) (Table 4).

Literature Search

A literature search identified 23 additional patients with PAH carrying an *ACVRL1* mutation, with individual data in 20 different families (Figure 1) (21, 25, 36). Age at diagnosis of PAH, history of familial PAH, clinical manifestations of HHT, and age at death for *ACVRL1* mutation carriers (n = 32) are reported in Table 2.

Clinical, functional, and hemodynamic characteristics of the 32 *ACVRL1* mutation carriers from the French PAH Network (n = 9) and the literature search (n = 23) were compared with the characteristics of 93 *BMPR2* mutations carriers and 277 *BMPR2*/*ACVRL1* mutation noncarriers. At diagnosis, *ACVRL1* mutation carriers were significantly younger (21.8 ± 16.7 yr) than *BMPR2* mutation carriers and noncarriers (35.7 ± 14.9 and 47.6 ± 16.3 yr, respectively; $P < 0.0001$) (Figure 5). Age at death was lower in *ACVRL1* and *BMPR2* mutation carriers compared with noncarriers (26.6 ± 18.7, 35.2 ± 15.7, and 48.5 ± 17.5 yr, respectively; $P < 0.0019$) (Figure 5). Hemodynamic characteristics at diagnosis were less severe in *ACVRL1* mutation carriers, as compared with *BMPR2* mutation carriers, and were broadly similar to those observed in noncarriers (Table 4). In patients with PAH and who were carrying an *ACVRL1* mutation, cardiac index was significantly higher as compared with *BMPR2* mutation carriers (all $P < 0.0001$). No acute vasodilator responder was observed in our series or reported in the literature among *ACVRL1* mutation carriers.

ACVRL1 Mutations in Patients with PAH

Fujiwara and colleagues reported five other patients with PAH with an *ACVRL1* mutation (28). Thus, 36 patients with PAH carrying an *ACVRL1* mutation were identified in 32 different families in our series (n = 8) and in the literature search (n = 28) (21, 25–28, 36). In the context of PAH, we report 27 different *ACVRL1* exonic mutations. All mutations are described in Table 5. In our series of nine patients with PAH, we identified four *ACVRL1* mutations that were not previously reported: c.1280A > T (p.Asp427Val); c.1388delG (p.Gly463AlafsX2); c.602A > G (p.Gln201Arg); and c.1324G > A (p.Val442Met).

In the HHT Mutation Database (http://www.hhtmutation.org), we found 264 *ACVRL1* mutations in patients displaying HHT but no reported PAH. These corresponded to 242 exonic and 22 intronic mutations (with 5.3% splice defects). Twelve *ACVRL1* mutations (5% exonic mutations) were present in exon 10, of which 7 were localized in the nonactivating non–down-regulating (NANDOR) box (3%) in patients with HHT with no reported PAH (Table 6 and Figure 6). In patients with PAH carrying an *ACVRL1* mutation, we found 9 different mutations (in 14 patients with PAH from 11 distinct families) in exon 10. This represents 33.3% of reported *ACVRL1* mutations in patients with PAH, of which six (22.2%) were localized in the NANDOR box (Table 6 and Figure 6). Thus, compared with patients with HHT, *ACVRL1* mutations in exon 10 were more frequently observed in patients with PAH than in patients with HHT without reported PAH ($P < 0.0001$).

DISCUSSION

In this study, clinical, functional, and hemodynamic characteristics of *ACVRL1* mutation carriers, identified from the French PAH Network and from previously published cases, were compared with patients with PAH considered to be idiopathic or with patients with PAH carrying a *BMPR2* mutation. We demonstrated that age at PAH diagnosis and age at death of *ACVRL1* mutation carriers were significantly lower as compared with *BMPR2*/*ACVRL1* mutation noncarriers. In this series, we confirmed that *ACVRL1* mutation carriers may develop severe PAH without any clinical evidence of HHT. Our results on the distribution and frequency of mutations in the *ACVRL1* gene argue for a predominance of mutations in exon 10 in *ACVRL1* mutation carriers who develop PAH, as compared with *ACVRL1* mutation carriers with isolated HHT. The association between mutations in the *ACVRL1* gene and the development of severe PAH further supports the hypothesis that disruption of the TGF-β pathway is central to the development of PAH (5, 38). Furthermore, as previously described by Sztrymf and colleagues (7), we confirm that *BMPR2* mutation carriers were younger at diagnosis and at death, with more severely compromised hemodynamic status at diagnosis than *BMPR2*/*ACVRL1* mutation noncarriers. Interestingly, in our cohort, a *BMPR2* mutation was found in 44 patients (67.7%) with a family history of PAH, and in 49 patients (14.8%) without a family history of PAH. On the basis of our findings, systematic genetic counseling for idiopathic

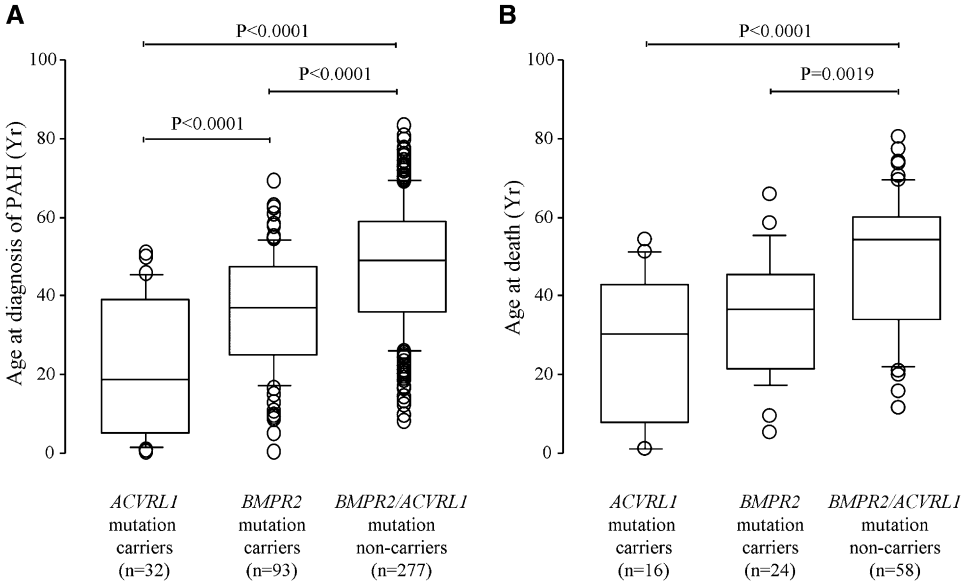


Figure 5. Age at diagnosis of pulmonary arterial hypertension (PAH) and age at death in activin A receptor type II-like kinase-1 (*ACVRL1*) mutation carriers, bone morphogenetic protein receptor type 2 (*BMPR2*) mutation carriers, and *BMPR2*/*ACVRL1* mutation noncarriers (7).

patients with PAH even in the absence of a family history of PAH should be considered.

Our data indicate that *ACVRL1* mutation carriers are significantly younger at PAH diagnosis, as compared with *BMPR2* mutation carriers and noncarriers. More than one third of patients with PAH carrying an *ACVRL1* mutation were children or young teenagers (<16 yr). All causes of death were directly related to PAH, and, even if their baseline hemodynamic characteristics were less severe compared with those of *BMPR2* mutation carriers, age at death among patients with PAH who were carriers of an *ACVRL1* mutation was broadly similar to that of *BMPR2* mutation carriers and significantly less than that of patients without *BMPR2* or *ACVRL1* mutations.

Sztrymf and colleagues demonstrated that *BMPR2* mutation carriers and noncarriers had similar overall survival, but that *BMPR2* mutation carriers were more likely to undergo lung transplantation with a significantly shorter time to death or lung transplantation (7). Because lung transplantation is the only remaining therapeutic option for patients with severe PAH who cannot be managed medically, time to death or lung transplantation is an accepted indicator of disease severity in this patient population. Therefore, these data suggest that *BMPR2* mutation carriers have more severe disease, which is in accordance with the observation that this patient population is more severely compromised hemodynamically compared with noncarriers. However, none of the *ACVRL1* mutation carriers underwent lung transplantation, partly because of the shortage of donor lungs for pediatric PAH but also because of physician reluctance to propose lung transplantation in patients with evidence of HHT (including extrapulmonary arteriovenous malformations). Thus, to avoid a biased analysis due to different surgical treatment options in different subgroups we decided to

TABLE 4. CLINICAL, FUNCTIONAL, AND HEMODYNAMIC CHARACTERISTICS AT DIAGNOSIS OF PULMONARY ARTERIAL HYPERTENSION

	ACVRL1 Mutation Carriers (*n = 32*)	*BMPR2* Mutation Carriers (*n = 93*)	*BMPR2*/*ACVRL1* Mutation Noncarriers (*n = 277*)
Age at diagnosis, yr (mean ± SD)	21.8 ± 16.7*†	35.7 ± 14.9*	47.6 ± 16.3
Sex, female/male (ratio)	25/7 (3.6)	60/33 (1.8)	182/95 (1.9)
6-MWD, m	407 ± 99	346 ± 100	340 ± 114
mPAP, mm Hg	60 ± 17	63 ± 13*	56 ± 14
RAP, mm Hg	8 ± 6	8 ± 5	8 ± 5
PCWP, mm Hg	9 ± 4	8 ± 3	8 ± 3
CI, L/min/m^2	3.04 ± 1.33†‡	2.11 ± 0.64*	2.50 ± 0.71
PVRi, mm Hg/L/min/m^2	19.0 ± 10.0	23.8 ± 12.8§	20.6 ± 9.0
$S\bar{v}_{O_2}$, %	66 ± 13‖	59 ± 9¶	63 ± 10
Acute vasodilator responders, %	0/23	1/91	33/258

Definition of abbreviations: *ACVRL1* = activin A receptor type II-like kinase-1; *BMPR2* = bone morphogenetic protein receptor type 2; 6-MWD= 6-minute walk distance; CI = cardiac index; mPAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; PVRi = indexed pulmonary vascular resistance; RAP = right atrial pressure; $S\bar{v}_{O_2}$ = mixed venous oxygen saturation.

Results are expressed as means ± SD.

* $P < 0.0001$ compared with *BMPR2*/*ACVRL1* mutation noncarriers.

† $P < 0.0001$ compared with *BMPR2* mutation carriers.

‡ $P < 0.002$ compared with *BMPR2*/*ACVRL1* mutation noncarriers.

§ $P < 0.02$ compared with *BMPR2*/*ACVRL1* mutation noncarriers.

‖ $P < 0.05$ compared with *BMPR2* mutation carriers.

¶ $P < 0.005$ compared with *BMPR2*/*ACVRL1* mutation noncarriers.

TABLE 5. DETAILS OF *ACVRL1* MUTATIONS

	Patient	Mutation Location	Mutation Category	Nucleotide Change	Amino Acid Change
French PAH Network	1	Exon 2	Frameshift	c.37delC	p.Leu13CysfsX2
	2	Exon 9	Missense	c.1280A > T	p.Asp427Val
	3	Exon 10	Frameshift	c.1388delG	p.Gly463AlafsX2
	4	Exon 10	Nonsense	c.1468C > T	p.Gln490X
	5A	Exon 10	Missense	c.1450C > T	p.Arg484Trp
	5B	Exon 10	Missense	c.1450C > T	p.Arg484Trp
	6	Exon 5	Missense	c.602A > G	p.Gln201Arg
	7	Exon 9	Missense	c.1324G > A	p.Val442Met
Trembath and colleagues (21)	F1-III1	Exon 10	Missense	N/A	p.Arg484Trp
	F1-III2	Exon 10	Missense	N/A	p.Arg484Trp
	F1-III3	Exon 10	Missense	N/A	p.Arg484Trp
	F2	Exon 8	Missense	N/A	p.Arg411Trp
	F3-II1	Exon 6	Deletion	N/A	p.254delAsp
	F3-II4	Exon 6	Deletion	N/A	p.254delAsp
Harrison and colleagues (25)	7685	Exon 5	Missense	c.536A > C	p.Asp179Ala
	7340	Exon 6	Missense	c.632G > A	p.Gly211Asp
	7242	Exon 7	Missense	c.1031G > A	p.Cys344Tyr
	7253	Exon 7	Missense	c.1031G > A	p.Cys344Tyr
	8261	Exon 8	Missense	c.1120C > T	p.Arg374Trp
	7682	Exon 8	Missense	c.1121G > A	p.Arg374Gln
	8259	Exon 8	Missense	c.1196G > C	p.Trp399Ser
	7252	Exon 8	Missense	c.1232G > A	p.Arg411Gln
	7214	Exon 10	Missense	c.1460A > C	p.Lys487Thr
Abdalla and colleagues (26)	60	Exon 10	Insertion	c.1450C > T, 1450-1451insG	p.Arg484TrpfsX493
	82	Exon 10	Nonsense	c.1435C > T	p.Arg479X
	91	Exon 8	Missense	c.1120C > T	p.Arg374Trp
	100	Exon 10	Nonsense	c.1385C > G	p.Ser462X
Harrison and colleagues (27)	7912	Exon 10	Missense	c.1451G > A	p.Arg484Gln
Fujiwara and colleagues (28)	4	Exon 10	Missense	c.1436G > A	p.Arg479Gln
	9	Exon 8	Missense	c.1142T > C	p.Leu381Pro
	18	Exon 10	Missense	c.1451G > A	p.Arg484Gln
	20	Exon 9	Missense	c.1270C > A	p.Pro424Thr
	21	Exon 7	Missense	c.936C > G	p.His312Gln
Smoot and colleagues (36)	K1	Exon 7	Missense	c.T818C	p.Leu273Pro
	K2	Exon 8	Missense	c.C1055A	p.Ala352Asp
	K3	Exon 8	Missense	c.C1055A	p.Ala352Asp

n = 36 patients in 32 different families.

The mutation nomenclature follows current guidelines as recommended by the Human Genome Variation Society (www.hgvs.org/mutnomen/). Protein consequence for frameshift mutation: "the position of the stop in the new reading frame is calculated starting at the first amino acid that is changed by the frame shift, and ending at the first stop codon."

study only overall survival in the present analysis. This confirmed similar overall survival in *BMPR2* mutation carriers and noncarriers, but *ACVRL1* mutation carriers had significantly worse overall survival (Figure 3).

Patients with *ACVRL1* mutations may also have HHT (diagnosed either before or after PAH), which has a known association with comorbidities including visceral hemorrhage. In patients from the French Pulmonary Hypertension Referral Center, anemia was excluded as a cause of worse prognosis in our population. In addition, the hypothesis of a worse prognosis directly or indirectly related to comorbidities of HHT was not supported by the extensive analysis of the patients of the French Pulmonary Hypertension Referral Center. Indeed, *ACVRL1* mutation carriers with PAH clinically deteriorated more quickly and they ultimately died of pulmonary hypertension–related causes, indicating that these individuals had more rapid progression, as compared with other patients with PAH, despite similar therapeutic approaches. Only one patient died of a partly HHT-related complication, namely rupture of a pulmonary arteriovenous malformation, but this was due at least in part to the severity of underlying PAH (37).

These observations suggest more rapid disease evolution in *ACVRL1* mutation carriers compared with *BMPR2*/*ACVRL1* mutation noncarriers, as observed in *BMPR2* mutation carriers (7). As previously reported in patients with PAH carrying a *BMPR2* mutation (7, 30), acute vasodilator response was uncommon in heritable PAH and no acute responders were identified among patients with PAH carrying an *ACVRL1* mutation.

TABLE 6. DISTRIBUTION OF THE VARIOUS *ACVRL1* MUTATIONS IN PATIENTS WITH PULMONARY ARTERIAL HYPERTENSION* AND IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA WITHOUT REPORTED PULMONARY ARTERIAL HYPERTENSION†

Exon	PAH patients with ACVRL1 Mutation*	HHT without Reported PAH†
2	1 (3.7%)	2 (0.8%)
3	0 (0%)	61 (25.2%)
4	0 (0%)	22 (9.1%)
5	2 (7.4%)	9 (3.7%)
6	2 (7.4%)	20 (8.3%)
7	3 (11.1%)	53 (21.9%)
8	7 (25.9%)	40 (16.5%)
9	3 (11.1%)	23 (9.5%)
10	9 (33.3%)	12 (5.0%)

Definition of abbreviations: *ACVRL1* = activin A receptor type II-like kinase-1; HHT = hereditary hemorrhagic telangiectasia; PAH = pulmonary arterial hypertension.

* n = 27 different mutations in 36 patients.

† Mutation Database: http://www.hhtmutation.org (n = 242 exonic mutations).

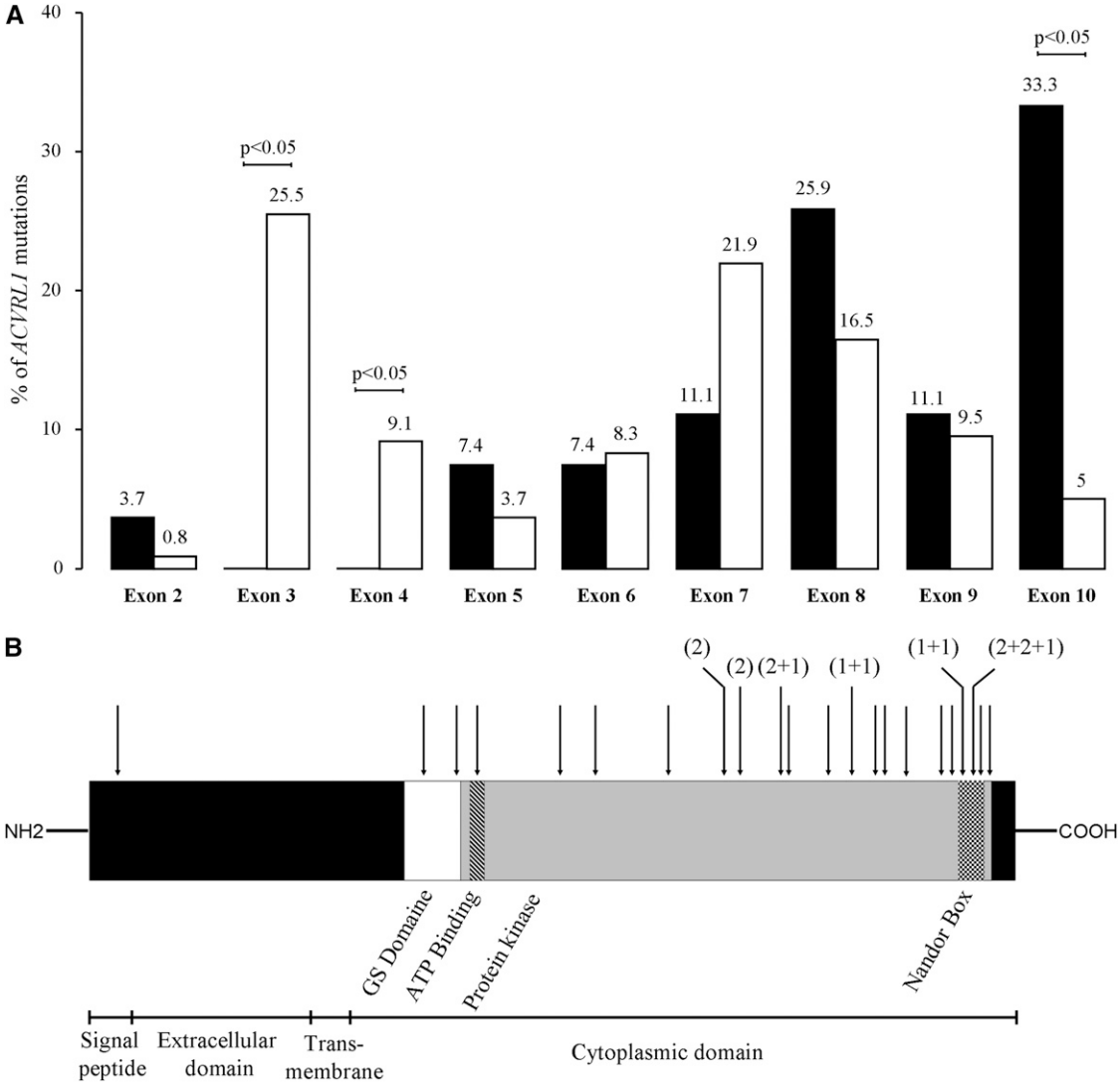


Figure 6. (*A*) Distribution of the various activin A receptor type II-like kinase-1 (*ACVRL1*) mutations in patients with pulmonary arterial hypertension (PAH) (*solid columns*) (n = 27 different mutations in 36 patients) and in patients with hereditary hemorrhagic telangiectasia (HTT) without reported PAH (*open columns*) (Mutation Database; http://www.hhtmutation.org) (n = 242 exonic mutations). (*B*) Repartition of *ACVRL1* exonic mutations in patients with PAH. GS = glycine/serine.

HHT is an autosomal dominant disease with nearly complete penetrance by the age of 60 years, with a female-to-male ratio of 1:1 (15). Interestingly, as previously demonstrated in patients with PAH considered to be idiopathic and in patients with a family history of PAH, we report a female predominance among patients with PAH carrying an *ACVRL1* mutation. It has been demonstrated that in HHT, pulmonary arteriovenous malformations and possibly hepatic involvement and cerebral hemorrhage are more frequent in women (16, 39). It was hypothesized that such female predominance may reflect an effect of female sex hormones on the remodeling observed in HHT vasculature or relate to hemodynamic changes during pregnancy (16).

ACVRL1 mutation carriers from the French PAH Network had a diagnosis of PAH preceding HHT diagnosis in the majority of cases (seven of nine cases). Epistaxes, telangiectases, and arteriovenous malformations were the most frequent signs of HHT observed in the course of the disease. History of familial PAH was present in only one of the seven families reported. This is in agreement with previous reports indicating that signs of PAH may be the first or indeed the only manifestation in *ACVRL1* mutation carriers (25, 27, 28). For instance, Fujiwara and colleagues found 5 *ACVRL1* mutations in 21 pediatric patients with PAH without any sign of HHT (28). In addition, Harrison and colleagues reported a case of a patient with PAH carrying an *ACVRL1* mutation, with severe PAH diagnosed at the age of 51 years, an age that is considered to have nearly complete penetrance of HHT (25). This patient died at the age of 54 years and necropsy found no pathological features of HHT (25). Furthermore, some characteristic signs of HHT, and particularly epistaxes, are nonspecific and relatively common, and usually not spontaneously reported by the patient or not considered as informative by the medical team in the context of PAH. Therefore, better awareness of the possibility of HHT in patients with PAH should lead to a systematic search for clinical features of HHT in this patient population. In addition, a detailed family history and careful examination of first-degree relatives for subtle manifestations of HHT are required, although phenotypic variability and the possibility of *de novo* mutation mean that the diagnosis cannot be excluded even if no abnormalities are identified within the family.

The observation that *ACVRL1* mutation carriers develop PAH earlier may illustrate the constitutive susceptibility conferred by the mutation on the course of the disease. The formation of an heteromeric complex with BMPR-II and ALK1 has been proposed (5, 21, 40, 41) and this model could at least in part explain why subjects carrying either a *BMPR2* or

ACVRL1 mutation are predisposed to PAH. Furthermore, this complex may be associated with an accessory receptor such as endoglin, and heretofore, only four cases of PAH associated with an *endoglin* mutation have been reported in the literature (25, 27, 29). Thus, patients with *endoglin* mutation seem to be at lower risk of developing PAH compared with *ACVRL1* mutation carriers. *ACVRL1* haploinsufficiency may lead to a pulmonary vascular status that predisposes to the development of PAH. Interestingly, our data confirm that *ACVRL1* mutation carriers with PAH have a predominance of mutations in exon 10 and particularly in the NANDOR box. Of note, the NANDOR box, located from codon 479 to 489, is necessary for regulation of TGF-β signaling (25, 27, 42). Thus, mutations in this region may critically affect the regulation of the TGF-β signalization pathway. Dysregulation of this pathway may promote pulmonary endothelial and/or smooth muscle cell dysfunction and proliferation characteristic of PAH (21). The high frequency of mutations in exon 10 underscores the need for increasing PAH awareness in this subgroup of patients. However, there are several reported patients with HHT carrying a mutation in the NANDOR box in exon 10, but with no evidence of PAH. Furthermore, most *ACVRL1* mutation carriers in a given family will not develop PAH, indicating that an *ACVRL1* mutation is not sufficient to induce PAH. These results reinforce the hypothesis that an additional genetic or environmental hit is necessary in order to trigger a pulmonary vascular disease in predisposed subjects (43, 44).

Our study included literature-based subjects, which could induce a bias in the analysis of *ACVRL1* mutation carriers. However, all published cases of *ACVRL1* mutation carriers in the literature and all *BMPR2* mutation carriers and noncarriers from the French PAH Network were reported in our study. Moreover, age at diagnosis and age at death of *ACVRL1* mutation carriers from the French PAH Network and from the literature search were similar (age at diagnosis: 18.5 ± 16.0 and 23.1 ± 17.2, respectively [$P = 0.50$]; age at death: 21.2 ± 18.6 and 29.1 ± 19.2, respectively [$P = 0.45$]). Furthermore, to avoid a potential survival bias because of different management strategies adopted for the cases from the literature, our survival analysis focused only on patients from the French Pulmonary Hypertension Referral center, where all patients with PAH have a similar therapeutic approach irrespective of the genetic or familial background.

Another possible limitation of this study relates to familial clustering. Indeed, familial clustering could impact our data in terms of both gene and environmental interactions that may alter disease expression beyond that of a single gene mutation. However, the majority of PAH cases we studied were the only reported cases from their families and familial clustering represented only a minority of reported cases. In the French PAH Network, six of nine *ACVRL1* mutation carriers were the only PAH cases reported in their families, with three belonging to the same family. In the literature search, 18 of 23 *ACVRL1* mutation carriers were the only family members with PAH, with two families having 3 and 2 cases reported, respectively. Thus the majority of cases were the only reported PAH in their families and familial clustering represented a minority of reported cases. A similar proportion of multiple reported cases from single families was identified in *BMPR2* mutation carriers (24 of 93 had an affected family member, 26%). On the basis of these findings, it was concluded that a familial influence was similar in *ACVRL1* and *BMPR2* mutation carriers, and we have thus not performed any family-based approach in our analysis.

In conclusion, our study served to describe clinical characteristics, hemodynamic features, and outcomes for patients with PAH carrying an *ACVRL1* mutation. Of note, these patients had a poor clinical outcome, and patients with PAH carrying an *ACVRL1* mutation were characterized by a younger age at diagnosis and death, as compared with patients with PAH without *BMPR2* and *ACVRL1* mutation. Although PAH is a rare complication in *ACVRL1* mutation carriers, our data emphasize the poor prognosis of this patient population, and argues in favor of screening for clinical signs of PAH in such patients to detect PAH earlier when medical management may be more efficacious. Furthermore, because PAH may develop in *ACVRL1* mutation carriers without obvious manifestations of HHT, a detailed family history and a careful examination of patients with PAH and first-degree relatives for stigmata of HHT may help detect these patients.

Conflict of Interest Statement: B.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.M. received lecture fees from Actelion ($1,001–$5,000), Pfizer (up to $1,000), and GlaxoSmithKline (GSK) ($1,001–$5,000). F.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. B.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. A.Y. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. X.J. served on the board or advisory board for Actelion and Pfizer ($1,001–$5,000), and received lecture fees from Actelion ($5,001–$10,000), Pfizer (up to $1,000), and GSK ($1,001–$5000). D.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. A.R. served on the board or advisory board for Sanofi Aventis ($5,001–$10,000) and for Actelion ($1,001–$5,000). He received lecture fees from Bayer Schering Pharma ($1,001–$5,000), Actelion ($5,001–$10,000), and Praxis ($1,001–$5,000). V.D.-G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. A.F. received lecture fees and grant support from Actelion ($1,001–$5,000). O.S. served on the board or advisory board for Actelion ($5,001–$10,000) and for GSK and Pfizer ($1,001–$5,000). He received lecture fees from Actelion ($5,001–$10,000), Bayer Schering ($1,001–$5,000), GSK and Pfizer ($1,001–$5,000), and United Therapeutics (up to $1,000). D.S.O. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. G.S. served on the board or advisory board for Actelion ($5,001–$10,000), Merck Sharp & Dohme (MSD), and GSK ($1,001–$5,000). He received lecture fees from Actelion ($5,001–$10,000), Bayer Schering, GSK, and Pfizer ($1,001–$5,000), and United Therapeutics (up to $1,000). F.S. received lecture fees from Actelion Pharmaceuticals (up to $1,000). M.H. served on the board or advisory board for Actelion ($5,001–$10,000) and Novartis, GSK, and MSD ($1,001–$5,000). He received lecture fees from Actelion ($5,001–$10,000), Bayer Schering and GSK ($1,001–$5,000), Pfizer ($1,001–$5,000), and United Therapeutics (up to $1,000).

Acknowledgment: The authors thank the patients, their families, and healthcare providers for agreeing to collaborate.

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