Genetic analyses in a cohort of children with pulmonary hypertension

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ABSTRACT The prevalence of germline mutations in paediatric pulmonary hypertension (PH) is poorly documented. The objective of this study was to determine the mutation frequency in PH genes in a paediatric cohort and describe the clinical characteristics of mutation carriers.

The study involved 66 index cases with PH: 35 children with idiopathic pulmonary arterial hypertension (IPAH); five children with familial PAH (FPAH); three children with pulmonary veno-occlusive disease (PVOD); and 23 children with PAH associated with congenital heart disease (APAH-CHD).

No mutations were found in the 23 children with APAH-CHD. In the 40 children with IPAH or FPAH, 12 mutations were found: five on *BMPR2*; four on *ACVRL1*; and three on *TBX4*. In the three PVOD cases, two carried the *EIF2AK4* mutation. Mutation carriers had a more severe disease at diagnosis and more aggressive first-line therapy was required. The three patients with PVOD had a very severe disease at diagnosis and required a lung transplantation.

The genetic architecture of paediatric PAH is enriched in *ACVRL1* and *TBX4* mutations compared to adult PAH, but further studies are required to confirm these results. Childhood-onset PAH in children carrying a mutation in one of the genes tested has a more severe presentation at diagnosis but a similar outcome to that observed in non-carriers.

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Introduction

Pulmonary arterial hypertension (PAH) is a rare and devastating disease, resulting from progressive obliteration of small calibre pulmonary arteries by proliferating vascular cells and intimal fibrosis, and leading to cardiac failure, with an untreated mean survival <3 years in adults and <10 months in children [1–3]. PAH can be a complication of well-identified pathological conditions, it can occur in the context of family history or genetic mutations (heritable PAH; HPAH), or it is considered as idiopathic (IPAH) in the absence of an identified predisposing factor [4]. IPAH has an unknown origin and represents around 70% of PAH in adults [5]. In the prospective TOPP (Tracking Outcomes and Practice in Pediatric PH) registry, which collects data on paediatric and adolescent pulmonary hypertension, 88% of children had PAH, of which 57% were characterised as IPAH or HPAH, and 36% as PAH associated with congenital heart disease (APAH-CHD) [6]. HPAH is a genetically heterogeneous disease. The major gene associated with adult HPAH is *BMPR2*, a gene encoding a type 2 receptor of the bone morphogenetic protein signalling pathway. Low penetrance dominant *BMPR2* mutations were found in >70% of familial PAH (FPAH) and in 15% of IPAH [7, 8]. Penetrance of these mutations is different in the two sexes (14% in males *versus* 42% in females according to Larkin *et al.* [9]). Mutations in other genes of the same pathway, *ACVRL1* and in rare cases *ENG*, may cause PAH associated with hereditary haemorrhagic telangiectasia (HHT) in most cases [10, 11]. Few mutations in the *SMAD* family of genes, and in the *KCNK3* and *CAV1* genes have been reported [12–14]. Recently, *TBX4* mutations (small patella syndrome) have been identified as being associated with childhood-onset PAH [15]. Biallelic recessive mutations of the *EIF2AK4* gene have been found at the origin of heritable pulmonary veno-occlusive disease (PVOD) and in pulmonary capillary haemangiomatosis that present with closely related phenotypes predominating either on venous lesions or on capillary proliferation, respectively [16, 17].

A few studies were specifically dedicated to identifying gene mutations in paediatric PAH. Harrison *et al.* [18] identified two *BMPR2* mutations arising *de novo* and two HHT-provoking mutations in *ENG* and *ACVRL1* in 16 children with IPAH. Rosenzweig *et al.* [19] found 8 *BMPR2* mutation carriers (10%) in 78 children with PAH, most of them with FPAH (7/8). In a series of 29 FPAH and IPAH children, Pfarr *et al.* [20] found *BMPR2* mutations in four children with IPAH (14%), two mutations in *ACVRL1*, and one variant of unknown significance (VUS) in *ENG*. Fujiwara *et al.* [21] studied a series of 21 probands under 16 years of age with PAH and found four mutations in *BMPR2* (19%) and five mutations in *ACVRL1*. Identifying mutations in PAH genes has a prognostic significance as adult patients with *BMPR2* or *ACVRL1* mutations are younger at diagnosis and time to death is shorter in these two groups of mutation carriers *versus* non-carriers [10]. In paediatric forms of PAH, Chida *et al.* [22] showed a similar age at onset and a worse prognosis restricted to *BMPR2* carriers for whom time to death was significantly shorter, but this difference was not significant in children carrying the *ACVRL1* mutation.

In APAH-CHD, the causal relationship between the left-to-right shunt and PAH is considered obvious in the majority of cases. The last international classification defined type 3 APAH-CHD as PAH with coincidental CHD that includes small shunt defects not causing severe PAH and following a course similar to IPAH [23]. The role of PAH genes mutations in these patients is unknown, nor is it known in children with post-operative PAH (type 4) after CHD repair of any type who go on to develop unexpected pulmonary vascular disease. Limited genetic data is available for paediatric patients with APAH-CHD. Pfarr *et al.* [20] described one *ENG* unlikely pathogenic VUS in a patient with type 2 APAH-CHD (left-to-right shunt) and one *BMPR2* mutation in a familial case of PAH with CHD. Harrison *et al.* [18] did not identify any mutation in two cases of APAH-CHD. Roberts *et al.* [24] described three *BMPR2* VUS in three children with type 2 APAH-CHD.

Limited data are available on the genetic architecture of childhood-onset PAH, taking into account the investigation of recently identified genes. This study sought to determine the proportion of pulmonary hypertension (PH) gene mutations in children with FPAH, IPAH, PVOD and in type 3 and 4 APAH-CHD, and describe their baseline clinical characteristics at time of diagnosis and their respective outcomes.

Methods

Study population

From January 2007 to December 2013, index paediatric patients (n=66) were included in the study; 66 unrelated patients aged 6.8±5.13 years (mean±SD) with a PH diagnosis were included after their parents gave written informed consent for this study. All patients were studied in the same paediatric cardiac department. PH diagnosis was determined during right-heart catheterisation, and defined as mean pulmonary arterial pressure >25 mmHg at rest, pulmonary vascular resistance index (PVRI) >3 Wood units·m^2, and mean pulmonary capillary wedge pressure >12 mmHg. A complete diagnostic examination was performed in all children to detect comorbidities that may predispose to the development of PAH. HHT symptoms were searched for in all cases and their parents. Patients were classified according to the international classification agreed in Nice, France, in 2013 [4]. PAH was considered to be idiopathic after

clinical and biological investigation eliminated all known causes. Cases were considered as FPAH when at least two cases were reported in the family extending up to third-degree relatives. Patients with type 1 (Eisenmenger's syndrome) and type 2 (left-to-right shunt) APAH-CHD [23] were not included because haemodynamic status was considered sufficient to explain PAH occurrence. Patients with type 3 (n=13) and 4 (n=10) APAH-CHD were included. Dysmorphic syndromes or inborn errors of metabolism, known aneuploidies (trisomy 21) and other cytogenetic anomalies (on chromosomes 1 and 14) were excluded (n=18). Patients had an echocardiography, and N-terminal pro-brain natriuretic peptide (NT-proBNP) and circulating endothelial cells measurements at diagnosis (tables 1 and 2) [25]. Incident cases of PAH were those genotyped at diagnosis within the period of inclusion (33/40), whereas prevalent cases (7/40) were alive at inclusion but genotyped after a variable delay (7±4.5 years; mean±SD). Similarly, patients with APAH-CHD were both incident (11/23) and prevalent (12/23).

For children under the age of 18 years, genetic testing was proposed to symptomatic individuals after parent approval (no case of parent refusal). When a mutation was identified, genetic testing was performed in parents to differentiate inherited from *de novo* mutations. Genetic testing and detailed clinical examination was proposed to all relatives for PH diagnosis. Unaffected siblings were genotyped after parent approval and according to French laws for screening unaffected children.

Our institutional review board and ethics committee approved the protocol.

Genetic analysis

All patients were screened for *BMPR2 and ACVRL1* point mutations and large rearrangements. Patients with IPAH or FPAH, and without a mutation in *BMPR2* or *ACVRL1*, were further screened for *EIF2AK4, TBX4* and *KCNK3* point mutations. *ENG* and *SMAD9* screening was performed for patients with FPAH for whom no mutation was identified in the other genes (figure 1). Since *TBX4*, *KCNK3* and *EIF2AK4* were not known to be involved in PH at the beginning of the study, included patients were retrospectively tested for these additional genes.

Screening of point mutations and large rearrangements was performed as described in Sztrymf *et al.* [7]. Primers used for *BMPR2*, *ACVRL1*, *ENG*, *EIF2AK4*, *SMAD9* and *KCNK3* have been previously described [7, 10–12, 14, 16]. Primers used for *TBX4* screening are provided in supplementary table S1.

TABLE 1 Clinical and haemodynamic characteristics of children with IPAH and FPAH at diagnosis according to the presence or the absence of mutation in the PAH genes

	Mutations carriers[#,+,§]	Non-carriers[¶,+]	p-value
Age at diagnosis years mean±SD	8.3±5.9	8.5±5.8	NS
NYHA I–II	2 (17%)	18 (63%)	0.006
NYHA III–IV	10 (83%)	10 (37%)	
Syncope	8 (67%)	8 (29%)	0.02
Increased NT-proBNP[§]	5 (41%)	8 (29%)	NS
Elevated CEC[f]	8 (66%)	12 (44%)	NS
TAPSE mm	15±2.8	19.3±3.7	NS
Mean PAP mmHg	69.2±19.2	63±14.2	NS
RAP mmHg	7.5±2.7	5.7±1.5	0.043
PVRI Wood units·m^2	23±12.4	18.9±10.6	0.07
CI L·min^{-1}·m^{-2}	3.4±1.3	3.5±1.1	NS
AVT responder	1 (6%)	8 (25%)	NS
First-line therapy			
Calcium channel blockers	1	8	NS
Oral ERA or PDE5 inhibitor	3	13	NS
Oral combination therapy with ERA and PDE5 inhibitor	3	4	NS
Oral combination + *i.v./s.c.* prostanoids	5 (41%)	3 (11%)	0.025

Data are presented as mean±SD, unless otherwise stated. PAH: pulmonary arterial hypertension; IPAH: idiopathic PAH; FPAH: familial PAH; NYHA: New York Heart Association (classes I–IV); NT-proBNP: N-terminal pro-brain natriuretic peptide; CEC: circulating endothelial cells; TAPSE: tricuspid annular plane systolic excursion; PAP: pulmonary arterial pressure; RAP: right atrial pressure; PVRI: pulmonary vascular resistance index; CI: cardiac index; AVT: acute pulmonary vasodilator testing; ERA: endothelin receptor antagonist; PDE5: phosphodiesterase type 5; NS: nonsignificant. [#]: n=12; eight IPAH cases and four FPAH cases; nine females and three males; [¶]: n=28; 24 IPAH and four FPAH; 18 females and 10 males; [+]: pulmonary veno-occlusive disease excluded; [§]: one patient underwent right-heart catheterisation after treatment initiation; [f]: CEC >300 pg·mL^{-1}.

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TABLE 2 Characteristics at diagnosis and treatment initiation according to mutation type

	BMPR2	*ACVRL1*	*TBX4*	*EIF2AK4*	No mutation
Patients	5	4	3	2	28
Female/male	3/2	3/1	3/0	1/1	18/10
NYHA I–II	0	2	1	1	18
NYHA III–IV	5	2	2	1	10
Syncope	4	1	3	1	8
Elevated NT-ProBNP	2	2	0	1	8
Elevated number of CEC	4	2	2	1	9
TAPSE mm	17±22	14±10	13±1	21–20	19.3±4.6
Mean PAP mmHg	76±19	61±23	65±19	25–54	62±22
RAP mmHg	7.5±2.5	5.6±0.6	3±1	6–7	5.7±2.2
PVRI Wood units·m^2	29±23	14.3±5.7	13.5±19	5–28	19.2±9.6
CI L·min^{-1}·m^{-2}	3.3±1.4	4.5±1.6	8±4	-	3.5±1.3
AVT responder	0	0	1	0	8
First-line therapy					
Calcium channel blockers	0	0	1	0	8
Oral ERA or PDE5 inhibitor	1	2	1	0	13
Oral combination therapy with ERA and PDE5 inhibitor	1	1	1	1	4
Oral combination + *i.v./s.c.* prostanoids	3	1	1	1	3

Data are presented as n or mean±SD. NYHA: New York Heart Association (classes I–IV); NT-proBNP: N-terminal pro-brain natriuretic peptide; CEC: circulating endothelial cells; TAPSE: tricuspid annular plane systolic excursion; PAP: pulmonary arterial pressure; RAP: right atrial pressure; PVRI: pulmonary vascular resistance index; CI: cardiac index; AVT: acute pulmonary vasodilator testing; ERA: endothelin receptor antagonist; PDE5: phosphodiesterase type 5.

Nucleotide numbering follows the Human Genome Variation Society recommendations. All identified mutations were analysed with the Alamut software, version 2.2 (Interactive Biosoftware, Rouen, France).

Outcome

All patients were followed up with a standardised protocol at least twice a year, including World Health Organization functional class, NT-proBNP and echocardiography measurements (table 2). The occurrence of the following events was taken into account for analysis, either in isolation or in combination: death; lung or heart-lung transplantation; Potts shunt.

Statistics

Data are presented as mean±SD. The XLSTAT 2014 software (Addinsoft, New York, NY, USA) was used to perform a non-parametric Mann–Whitney U test, to compare patients with or without a mutation, or to calculate a Chi-squared test when appropriate; $p<0.05$ was considered statistically significant. The MedCalc statistical software, version 12.7.7 (MedCalc Software bvba, Ostend, Belgium) was used to construct Kaplan–Meier survival curves (figure 2) and calculate the log-rank test, which was used to test for a difference in the free probability distribution between mutation carriers and non-carriers (incident cases only) for three major events (death, transplantation, Potts shunt).

Results

Mutations in PH genes

Following pedigree analysis and clinical examination, 35 children were classified as IPAH, five as FPAH, 13 as type 3 APAH-CHD, 10 as type 4 APAH-CHD and three as PVOD. A mutation was identified in 14 of the 66 children: eight in IPAH patients (23% of IPAH), four in FPAH patients (80% of FPAH) and two in PVOD patients (67% of PVOD). No mutations in the *BMPR2* and *ACVRL1* genes were identified in children with type 3 and type 4 APAH-CHD (table 3).

Five *BMPR2* (three IPAH and two FPAH), four *ACVRL1* (three IPAH and one FPAH), three *TBX4* (two IPAH and one FPAH) and two biallelic *EIF2AK4* mutations (two PVOD) were identified (table 3).

Ten mutations have been previously described and classified as deleterious mutations. Four new mutations were identified: one large rearrangement of *BMPR2* (deletion of exons 2–7) and three *TBX4* mutations which are all non-sense mutations (all mutations are described in supplementary table S2).

No mutations were identified in the *ENG, KCNK3* or *SMAD9* genes. The four *ACVRL1* mutation carriers showed symptoms of HHT. In the 14 index cases with an identified mutation, parental genetic screening for 11 cases was performed. In two IPAH cases, the *BMPR2* mutations identified were *de novo* mutations.

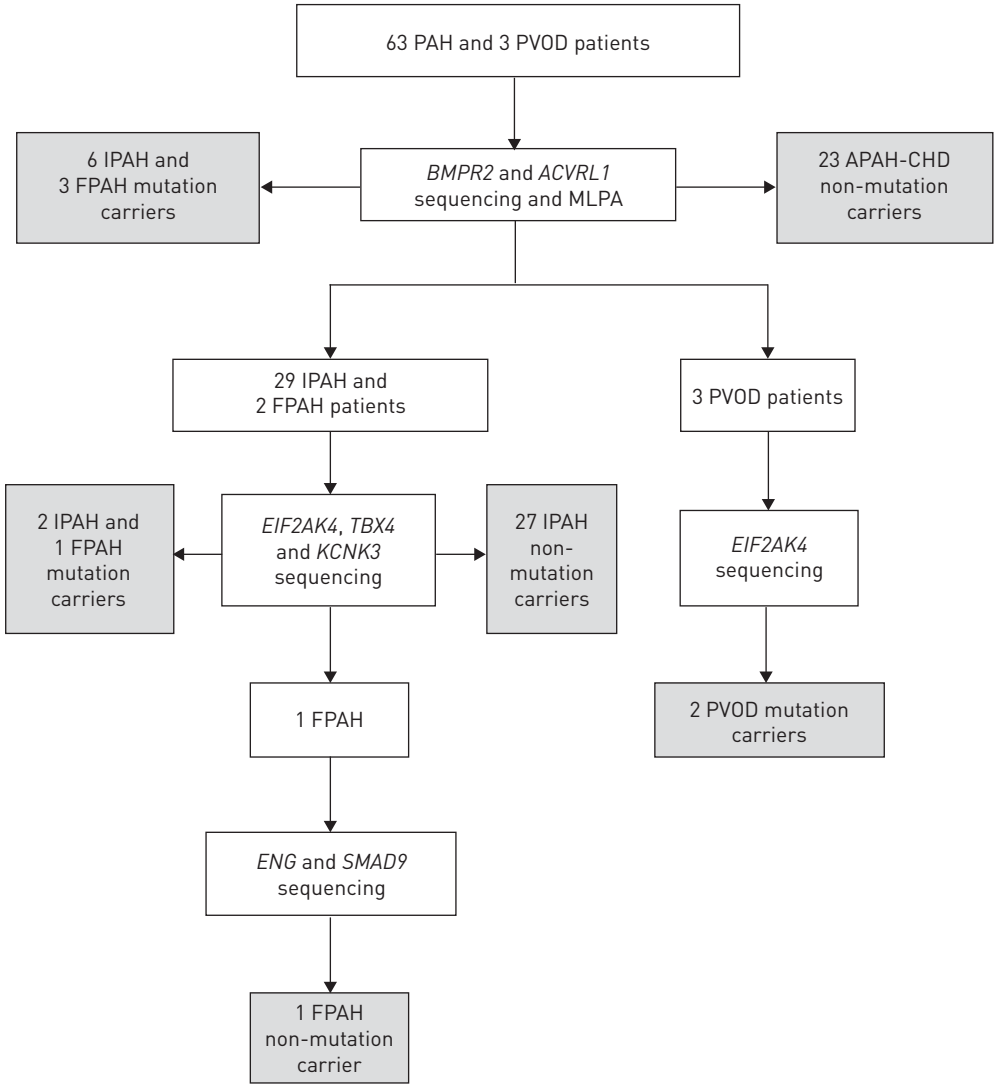

FIGURE 1 Flow chart of genetic analysis scheme. Each gene tested is indicated together with the technique used for detecting mutations or rearrangements by multiplex ligation-dependent probe amplification (MLPA®). The number of subjects carrying a mutation is indicated for each gene. PAH: pulmonary arterial hypertension; PVOD: pulmonary veno-occlusive disease; IPAH: idiopathic PAH; FPAH: familial PAH; APAH-CHD: PAH associated with congenital heart disease.

The remaining mutations were inherited and in all cases the parent carrying the mutation was asymptomatic for PAH or a single allele carrier for the *EIF2AK4* mutations for PVOD.

In the three families with the *TBX4* mutation, the mutation was inherited. The parent carrying the mutation was asymptomatic for PAH with normal pulmonary arterial pressure (PAP) at echocardiography, but symptomatic for bone abnormalities associated with *TBX4* mutations.

Mutations in the *EIF2AK4* gene were identified in two patients (previously reported in Eyries *et al.* [16]), who presented with the clinical, haemodynamic and chest computed tomography (CT) features of PVOD, while no *EIF2AK4* mutation was identified in children with IPAH or FPAH. An 8-year-old girl was suspected to have PVOD on chest CT at the time of diagnosis, but no mutation in *EIF2AK4* was found, nor in other genes tested. Subsequent CT scans, lung biopsy and evolution confirmed the diagnosis and her clinical status required transplantation during follow-up.

Clinical status at time of diagnosis in IPAH and FPAH patients

At time of diagnosis, IPAH and FPAH patients who had a mutation in one of the PAH genes were in a worse functional class, had more frequently associated signs of right ventricular dysfunction (high right atrial pressure) and had more frequent syncope (table 1). Although statistically they did not have different mean PAP, PVRI and cardiac index, values for these parameters tended to be worse in mutation carriers.

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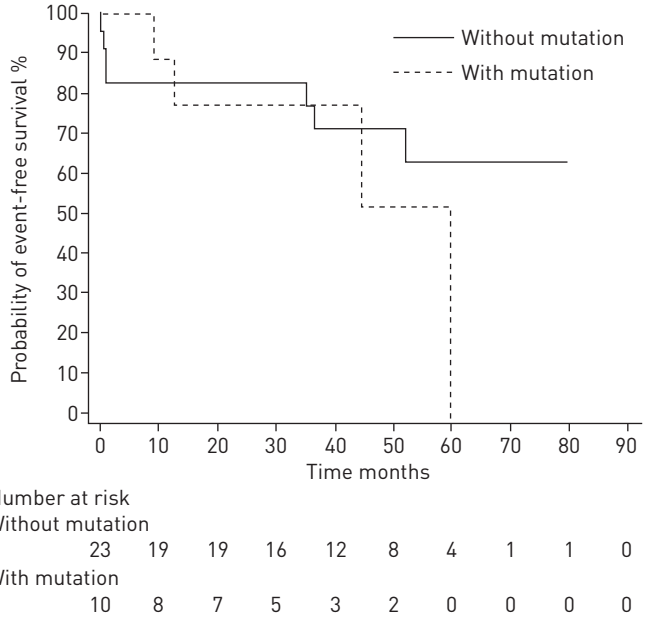

FIGURE 2 Distribution of probability of event-free survival according to mutation carrier status in pulmonary arterial hypertension patients (incident cases only). Events taken into account: death; transplantation; Potts shunt. The log-rank test was used to compare the two distribution curves: Chi-squared=0.6680; d.f.=1; p=0.4137.

Only one *TBX4* mutation carrier was considered a responder at the acute vasodilation test according to the Sitbon criteria [26]. Consequently, upfront combination therapy (oral combination of endothelin receptor antagonists and phosphodiesterase type 5 inhibitors) or triple therapy with a prostanoid analogue, was more frequently used as the first-line therapy in this group. Of note, female predominance was observed as in the adult population, but the sex ratio was similar in mutation carriers and non-carriers. There was no difference in age at diagnosis between these two groups.

The more severe clinical and haemodynamic status at diagnosis in the two groups was overrepresented in the group of children younger than 2 years (four with mutation and eight without).

Table 2 shows the baseline characteristics of patients for each type of mutation.

Outcomes

The mean duration of follow-up of the mutation carriers and non-carriers was similar (table 4). In IPAH and FPAH mutation carriers, 11/12 children were in functional class I or II and PVRI was significantly improved by treatment at the last follow-up. None of the mutation carriers died during follow-up. By contrast, in the non-carrier group, four patients died, two immediately after diagnosis despite upfront triple therapy, and the other two 4 years after diagnosis from class III heart failure. The three patients with PVOD had rapid functional deterioration and underwent a transplant.

The number of patients in both groups who had events and were on triple therapy during follow-up was similar in the two groups (table 4). The event-free (death, transplantation, Potts anastomosis) survival

TABLE 3 Mutations in pulmonary hypertension genes

	IPAH	PVOD	FPAH	Type 3 APAH-CHD	Type 4 APAH-CHD	Total
Cases (female/male)	35 (23/12)	3 (2/1)	5 (4/1)	13 (9/4)	10 (5/5)	66 (43/23)
BMPR2	3	0	2	0	0	5
ACVRL1	3	0	1	0	0	4
TBX4	2	0	1	0	0	3
KCNK3	0	0	0	0	0	0
EIF2AK4	0	2	0	0	0	2
Total	8	2	4	0	0	14

IPAH: idiopathic pulmonary arterial hypertension; PVOD: pulmonary veno-occlusive disease; FPAH: familial PAH; APAH-CHD: PAH associated with congenital heart disease.

TABLE 4 Clinical characteristics and major events at last follow-up in patients with idiopathic and familial pulmonary arterial hypertension

	Mutation carriers[#]	Non-carriers[#]	p-value
Patients	12	28	N/A
Mean±SD follow-up years	5.5±3.6	5±2.3	NS
NYHA I–II	11 (91%)	19 (68%)	NS
NYHA III–IV	1 (9%)	9 (32%)	
Oral combination + prostanoid *i.v./s.c.* (triple therapy)	6 (50%)	8 (30%)	NS
Potts anastomosis	2 (15%)	5 (17.5%)	NS
Transplantation	1 (8%)	0	NS
Death	0	4 (14%)[¶]	NS

NYHA: New York Heart Association (classes I–IV); N/A not applicable; NS: nonsignificant. [#]: pulmonary veno-occlusive disease excluded; [¶]: two triple therapy; one combined therapy; and one calcium channel blocker.

curve in the two groups showed that the occurrence rate in mutation carriers and non-carriers was not significantly different (figure 2).

Discussion

This study looked at the genetic status of a cohort of children with various forms of PH to better define the genetic architecture of PAH in childhood and evaluate the clinical conditions associated with different gene mutations. Patients with IPAH, FPAH, PVOD and type 3 and 4 APAH-CHD were included and screened for mutations in known PAH and PVOD genes, including recently identified genes (*TBX4*, *KCNK3* and *EIF2AK4*). Patients for whom PAH was part of a systemic disease or associated with chromosomal anomalies were not included in the study.

No mutation in PAH coincidental with CHD (type 3 APAH-CHD) and in post-operative PAH (type 4 APAH-CHD) in the *BMPR2* and in *ACVRL1* genes was found, suggesting that the genetic background is different from IPAH. In these two groups, the absence of a predisposing mutation suggests either that haemodynamic factors are sufficient to induce PAH, or that other, yet-to-be identified genetic factors are involved in this setting. A sample of this size had a 94% power (data not shown) to detect any susceptibility variant in the genes tested if the genetic background was the same. Previously published results on a small series of patients with APAH-CHD did not detect pathogenic mutations in either the *BMPR2* or *ACVRL1* genes; only missense VUS (most of them probably benign according to *in silico* tools) were found but no deleterious mutations, in the same context [18, 20, 24]. Therefore, both the results of this study and those already published do not justify screening these patients with the genes used in this study.

In FPAH, *BMPR2* mutations were identified in less than half of index cases, whereas *BMPR2* mutations are found in more than 80% of adult familial cases [27] and other mutations were found in *ACVRL1* and *TBX4* that represented nearly 50% of mutations found in children. Similarly, in IPAH, beside the *BMPR2* mutations found in similar proportions in the adult PAH population, mutations were also found in the *ACVRL1* and *TBX4* genes that were as frequent as *BMPR2* mutations if considered together. This study lacks the statistical power to reach significant differences in mutation distribution in adults with FPAH because the sample size was small; further studies are needed to confirm these differences. *ACVRL1* missense mutations found in this study have already been reported in HHT patients and were found in children from families where HHT symptoms were present either in affected children or in the carrier parent since PAH can precede other symptoms of HHT in young children but are usually present in the carrier parent [10]. Indeed, Girerd *et al.* [10] previously showed that, in adults, the age of onset of PAH associated with a mutation in *ACVRL1* was earlier than in *BMPR2* mutation carriers and this age of onset was earlier than in adults without a detected mutation.

TBX4 mutations were detected in 3/40 (7.5%) of IPAH/FPAH patients in our study, a proportion lower than that reported by Kerstjens-Frederikse *et al.* [15] where *TBX4* mutations were detected in 21% of PAH children without developmental anomalies. Routine testing of this gene in paediatric and adult PAH will help identify the importance of *TBX4*.

TBX4 mutations were transmitted from parents presenting with skeletal malformations typical of small patella syndrome, but without symptoms of PAH. Indeed, a mutation in the *TBX4* gene rarely causes PAH in adults (<2% according to Kerstjens-Frederikse *et al.* [15]).

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Currently, the major change in lung penetrance of *TBX4* mutations from one generation to the following and the severity of PAH observed in some children cannot be explained. Interaction with a genetic factor inherited from one parent and/or involvement of epigenetic mechanisms are possible hypotheses that require further studies. The absence of adults affected by PAH and carrying *TBX4* mutations could be because of high mortality in this type of childhood-onset PAH, but no follow-up and family data are currently available to support such a hypothesis. Our limited experience (n=73) in routinely searching for *TBX4* mutations in adult PAH patients shows a frequency of mutations similar to what has been already reported (data not shown) [15].

All three children with PVOD had very rapid clinical worsening and underwent a lung transplant. Although the three patients had a similar evolution leading to lung transplantation, no mutation of the *EIF2AK4* gene was found in one of the three patients who had no affected siblings. No genetic heterogeneity was detected in the study by Eyries *et al.* [16], but other unidentified genetic factors might have been involved in this patient.

In this study, mutation carriers had a more severe clinical presentation at diagnosis and this is in accordance with previously reported experience in adult patients with *BMPR2* and *ACVRL1* mutations [7, 10]. However, differences with regard to haemodynamic status did not reach significance, probably because of the small number of patients involved. Because of this worse clinical presentation at diagnosis, initial therapy was more aggressive in this group (table 2) but this difference was no more significant at the outcome (table 4). Interestingly, in adult and paediatric PAH, *BMPR2* mutation carriers are nearly never responders to calcium channel blockers. This is also the case in the series of childhood-onset PAH in this study, except for one patient with a *TBX4* mutation (tables 2 and 3) [19, 28, 29].

In this study, identification of the mutation did not influence the initial treatment; this was based on the usual criteria before the results of the genetic screening were obtained. This holds true if patients with PVOD, in whom lung transplantation was indicated in all cases, were excluded.

In adults, *BMPR2* and *ACVRL1* mutation carriers also have a more severe presentation at diagnosis and die younger than mutation non-carriers [7, 10, 29]. The sample size in this study does not allow for any significant difference in outcome to be detected according to mutation carrier status (table 4 and figure 2) Patients with a more severe disease presentation, who required lung transplantation or Potts anastomosis immediately after diagnosis, were PVOD patients (*EIF2AK4* mutation) or *ACVRL1* mutation carriers as also observed in adults [10]. In the study by Kerstjens-Frederikse *et al.* [15], there was no increased mortality in *TBX4* carriers compared to other childhood-onset PAH patients. This was also the case in this study.

This study has some limitations because it involves a rare disease and the sample size is small. The lack of power might explain the absence of significant differences between groups. Allowing prevalent cases, who can be considered as survivors, within the inclusion period could have modified the mutation carrier frequency. However, the mutation carrier frequency was similar in prevalent (2/7; 29%) and in incident cases (10/33; 30%). This is not relevant for APAH because no mutation was detected in the whole group. Although unlikely, some mutations may have been missed because some patients were not included or because some genes were not screened in all the patients included in the study (figure 1).

In conclusion, this study shows that screening patients with type 3 and 4 APAH-CHD for *BMPR2* and *ACVRL1* is not advantageous and this result corroborates the absence of deleterious mutation found in other studies [18, 20, 24]. The proportion of PAH patients with a mutation is comparable to that of adults but with a very different distribution of the involved genes. The important contribution of TBX4 mutations points out that the penetrance of these mutations is different between adults and children.

Finally, more severe clinical status at diagnosis in patients with a mutation appears to be comparable to what has been previously observed in the adult population.

The series in this study does not allow specific recommendations for initial therapy in the population of children with a mutation. The presence of a mutation in one of the genes tested did not predict clinical outcome in our study, except for the *EIF2AK4* gene. Screening for these mutations in a large population of paediatric IPAH and FPAH patients may help to further define the predictive value of these mutations with regard to clinical outcome.

One key issue of genetic diagnosis in paediatric PAH is the possibility to test relatives for their carrier or non-carrier status, such as siblings in the case of PVOD transmitted as a recessive disease. The benefit of an early diagnosis and careful follow-up should exceed the prejudice of a diagnosis given for a severe disease before symptoms have appeared, and for which the probability of appearance has not been determined precisely. Knowing the poor prognosis of some forms of paediatric HPAH will be a source of high emotional stress for parents, but one that would help them in their parental decisions. In these cases, genetic counselling delivered by geneticists and genetic counsellors should help parents to clearly understand the genetic situation and inform their decision-making.

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