TGF-β receptor mutations and clinical prognosis in Chinese pediatric patients with idiopathic/hereditary pulmonary arterial hypertension

Xinyu Zhang | Chen Zhang | Qiangqiang Li | Hong Gu

Beijing Anzhen Hospital, Capital Medical University, Beijing, China

Correspondence
Hong Gu, Pediatric Cardiology
Department, Beijing Anzhen Hospital, Capital Medical University, No. 2 Anzhen Rd, Chaoyang District, Beijing 100029, China.
Email: koko_gu@hotmail.com

Funding information
National Natural Science Foundation of China, Grant/Award Number: 82070243

Abstract
The relationship between clinical prognosis and transforming growth factor-β (TGF-β) receptor mutations in Chinese pediatric patients with idiopathic/hereditary pulmonary arterial hypertension (IPAH/HPAH) remains unclear. We retrospectively studied the clinical characteristics and outcomes of pediatric patients with IPAH/HPAH who visited our Hospital from September 2008 to December 2020. One hundred and five pediatric patients with IPAH/HPAH were included, 46 of whom carried TGF-β receptor mutations with a mean age at diagnosis of 82.8 ± 52.7 months, and 67 of them underwent right cardiac catheterization examinations and acute vasodilator testing. The result showed that mutation carriers demonstrated higher pulmonary vascular resistance \((p = 0.012)\), higher right atrial pressure \((p = 0.026)\), and lower cardiac index \((p = 0.003)\). The 1-, 2-, and 3-year survival rates of mutation carriers were 79.4%, 61.5%, and 55.6%, respectively, compared with 96.6%, 91.1%, and 85.4% for nonmutation carriers \((p = 0.0001)\). The prognosis of mutation carriers was significantly worse than that of nonmutation carriers. TGF-β receptor gene mutation is an independent risk factor for death \((p = 0.049, \text{ odd ratio } = 3.809, 95\% \text{ confidence interval } 1.006–14.429)\). In conclusion, TGF-β receptor mutation is an important genetic factor for the onset of IPAH/PAH in Chinese pediatric patients. Those who carrying TGF-β receptor mutations have a poor clinical prognosis. Therefore, TGF-β receptor gene screening for pediatric patients with PAH and more aggressive treatment for mutation carriers are recommended.

KEYWORDS
gene mutation, pulmonary arterial hypertension, TGF-β receptor
INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rare and lethal pulmonary vascular disease characterized by persistent elevation of pulmonary artery pressure that can eventually lead to right heart failure and premature death. The annual incidence in Western countries is 15–50 per million, with a higher incidence in women. Without specific treatment, the disease progresses rapidly and leads to death in these patients, with a median survival of 2.8 years in adults. Even with effective vasodilator therapy, the mortality remains high, with a 5-year survival rate of 49%–67%. The median survival of pediatric patients with untreated PAH is 10 months. Although current diagnostic and therapeutic techniques have improved considerably, the exact pathogenesis of PAH remains unclear.

The main pathological alteration in pulmonary hypertension is pulmonary artery remodeling, in which transforming growth factor-β (TGF-β) receptor mutations play a crucial role. The TGF-β pathway is involved in the pathology of PAH and includes bone morphogenetic protein receptor type II (BMPR2), activin receptor-like kinase 1 (ACVRL1), and endoglin (ENG). ACVRL1 is a type I receptor, BMPR2 is a type II receptor, and ENG is a type III receptor. It has been proved that approximately 70%–80% of HPAH and 10%–20% of IPAH cases are caused by mutations in the BMPR2 gene. Some studies indicated that pediatric PAH patients are more susceptible to genetic factors, suggesting a greater role of genetic factors in the development of pediatric patients with PAH.

Adult patients with PAH carrying mutations in the BMPR2 and ACVRL1 genes have a poor clinical prognosis. A study by Chida et al. showed that pediatric patients carrying mutations in BMPR2 or ACVRL1 had a worse prognosis than nonmutation carriers. However, the sample size of their study was relatively small. Harrison et al. studied pediatric PAH patients with TGF-β receptor mutations but did not analyze the clinical characteristics and prognosis of the pediatric patients. Therefore, the relationship between TGF-β receptor mutations and the clinical prognosis of pediatric PAH patients is unclear. In particular, studies of Chinese pediatric PAH patients in this regard are lacking.

In this study, we retrospectively investigated the clinical characteristics and prognosis of 105 Chinese pediatric IPAH/HPAH patients to assess the characteristics and clinical features of TGF-β receptor mutations in patients and the correlation between the two features.

METHODS

Patients

The pediatric patients who visited our Hospital from September 2008 to December 2020 were included. The diagnosis of PAH is determined by hemodynamic indices measured by right cardiac catheterization examinations. PAH was defined as a mean pulmonary arterial pressure (MPAP) ≥ 25 mmHg, with a pulmonary artery wedge pressure ≤ 15 mmHg and pulmonary vascular resistance (PVR) > 3 Wood units. An acute pulmonary vasodilatation test was performed during cardiac catheterization examinations, and according to current guidelines, a decrease in MPAP of more than 10 mmHg with an absolute value of less than 40 mmHg, along with stable cardiac output, was judged as a positive response. Pulmonary hypertension of patients who could not undergo right cardiac catheterization examinations was diagnosed by echocardiography (peak tricuspid regurgitation velocity > 2.8 m/s) combined with clinical symptoms and other signs of right heart overload while excluding combined congenital heart disease, pulmonary disease, pulmonary embolism, connective tissue disease, and portal hypertension. Clinical and hemodynamic data were collected from patients during diagnosis and follow-up. All patients or their guardians provided informed consent before blood collection. Moreover, the study procedures were approved by the Research Ethics Committee of Beijing Anzhen Hospital.

Genetic studies

Genomic DNA was isolated from peripheral venous blood. Exon and exon–intron linker sequences of 25 PAH-associated genes (see Table S1) were enriched using the GenCap custom enrichment kit (mygenetics) to capture exons of all candidate genes. The enriched libraries were sequenced on an Illumina HiSeq 2000 sequencer at a length of 100 bp. Bioinformatics analysis was performed with the method described by Liu et al. Four bioinformatic prediction software (PolyPhen, SIFT, PANTHER, Pmut) were used for mutational pathogenicity prediction.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation or quartiles. Categorical variables were expressed as percentages. Continuous variables were compared by independent sample t-tests. Categorical
variables were compared by the $\chi^2$ test. A Kaplan–Meier overall survival curve was constructed to demonstrate the overall survival difference between groups and compared using the log-rank test. Logistic regression analysis was performed to determine factors associated with an increased risk of death. Cox proportional hazard regression model was used to examine the relationship between mutations and all-cause mortality. In addition, $p \leq 0.05$ was considered statistically significant. All analyses were performed using SPSS software version 23.0.

**RESULTS**

**Genetic characteristics**

Forty-six of the 105 pediatric patients carried TGF-β receptor mutations, including 34 BMPR2 mutations, 10 ACVRL1 mutations, and two ENG mutations. Among all mutations, missense mutations were the most common type of mutation ($n = 38, 82.6\%$), followed by shift mutations ($n = 4, 8.7\%$), and splice mutations ($n = 4, 8.7\%$). Six patients had spontaneous mutations, three patients’ parents were not genetically screened, and the rest were inherited from the previous generation. Two children with ACVRL1 mutations had a family history of PAH.

Two previously unreported ACVRL1 mutations were found, c.665, A > C (p.His222Pro) and c.1217, G > T (p.Trp406Leu). One patient carried a heterozygous mutation of both ACVRL1 (c.601 C > A, p.Q201K) and NOTCH3 (c.224 G > A, p.Arg75Gln). One patient carried a heterozygous mutation of both ACVRL1 (c.665 A > C, p.H222P) and EIF2AK4 (c.2320-4 T > G, splicing). Both patients were at high-risk, with poor cardiac function (NYHA III–IV).

Other gene mutations related to PAH were also found during genetic screening, such as BMPR1B, SMAD4, SMAD9, NOTCH3, KCNK3, ABCA3, CPS1, and HTR2B.

**Clinical characteristics**

One hundred and five pediatric patients were included, 50 (47.6\%) of whom were female. The mean age at diagnosis of 105 pediatric patients was $82.8 \pm 52.7$ months. The median plasma BNP level was 321.0 (12–12,239) (normal range 0–100 pg/ml). Forty-six patients suffered from markedly decreased right cardiac function (NYHA Class III–IV). The patients were divided into two groups (mutation, nonmutation) according to whether they carried the TGF-β receptor mutation or not. The clinical indicators, plasma BNP and hemodynamic indicators of the two groups were compared (Table 1). The age at diagnosis of the mutation-carrying group ($95.2 \pm 48.5$ months) was higher than that of the nonmutation group ($73.1 \pm 54.2$ months) ($p = 0.032$).

| Variables                                      | Carriers $n = 46$ | Noncarriers $n = 59$ | $p$ value |
|------------------------------------------------|------------------|----------------------|-----------|
| Female, $n$ (%)                               | 20 (43.5)        | 30 (50.8)            | 0.458     |
| Age at diagnosis, months                      | $95.2 \pm 48.5$  | $73.1 \pm 54.2$      | 0.032     |
| NYHA FC, I–II/III–IV                         | 26/20            | 33/26                | 0.817     |
| BNP, pg/ml                                    | 364.5 (76.5–903.5) | 314.0 (70.5–887.8) | 0.775     |
| PASP (mmHg cardiac echocardiography)          | 87.4 ± 23.4      | 80.8 ± 22.5          | 0.157     |
| RHC parameters                                | $n = 32$         | $n = 35$             |           |
| MPAP, mmHg                                    | 73.0 ± 19.6      | 65.1 ± 25.0          | 0.156     |
| RAP, mmHg                                     | 9.5 (7.25–13.75) | 8.0 (6.0–9.0)        | 0.026     |
| PVR, WU                                       | 23.05 ± 13.39    | 16.68 ± 11.23        | 0.012     |
| CI, L/min/m²                                   | 2.8 (2.33–3.22)  | 3.9 (2.9–4.32)       | 0.003     |
| AVT, $n$ (%)                                   | 2/32 (6.3\%)     | 11/35 (31.4\%)       | 0.01      |

Note: Bold values indicate Statistically significant at $p < 0.05$.

Abbreviations: AVT, acute pulmonary vasodilatation test; BNP, brain natriuretic peptide; CI, cardiac index; MPAP, mean pulmonary arterial pressure; NYHA FC, New York Heart Association function class; PASP, pulmonary artery systolic pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; WU, wood unit.
There were 38 patients with PAH who did not undergo right heart catheterization, including 14 mutation carriers. The reason was mainly due to the patient's severe condition or their refusal. Thirty-two mutation carriers and 35 noncarriers underwent right cardiac catheterization examinations and acute vasodilator testing (Table 1). Right heart catheterization is an examination method in which a heart catheter is inserted through a vein to the right heart cavity and large blood vessels under the guidance of X-ray fluoroscopy. Mainly used for the determination of central venous pressure, right ventricular pressure, cardiac output, pulmonary artery pressure, etc. The results showed that pediatric patients with mutations had higher PVR \((p = 0.012)\), higher right atrial pressure (RAP) \((p = 0.026)\), and lower cardiac index (CI) \((p = 0.003)\) than noncarriers. In addition, pulmonary artery systolic pressure (PASP) and MPAP of mutation carriers trended higher than that of nonmutation carriers.

Acute pulmonary vasodilator testing was positive in 13 patients, 11 of whom were nonmutation carriers \((p = 0.01)\). Two mutation carriers were responders to acute vasodilator testing. One was an ENG mutation carrier \((c.727\ G > A,\ p.A243T)\), and the MPAP decreased from 92 to 36 mmHg during the test. The other was an ACVRL1 mutation carrier \((c.77\ C > T,\ p.P26L)\), and the MPAP decreased from 78 to 39 mmHg.

**Treatment**

One hundred and two (97.2%) pediatric patients received targeted drugs for PAH. Three pediatric patients did not receive targeted drug therapy because of financial reasons and mild PAH disease with light symptoms. Meanwhile, targeted drug regimens were developed according to treatment guidelines. Risk stratification was carried out according to the patient’s condition. Low-risk patients were given oral monotherapy, intermediate-risk patients were given oral dual-drug combination therapy, and high-risk patients were given oral dual-drug therapy with intravenous or subcutaneous pumping of prostacyclin analogs (such as Epoprostol and Treprostinil). However, there were more high-risk patients in mutation carrier group, so it looks like carriers were on triple therapy 2.5 times more often than noncarriers. The targeted drug regimens of the patients are shown in Table 2. Thirty-one mutation carriers (67.4%) and 29 (49%) nonmutation carriers received combination targeted drug therapy.

**Patients outcomes**

The median follow-up time was 45.5 months (2–147 months). Two pediatric patients underwent lung transplants. One underwent a double lung transplant at the age of 16 after 4 years of continuous treatment. One underwent a double lung transplant at the age of 17 after 6 years of continuous treatment. Thirty-eight pediatric patients died, including 18 BMPR2 mutation carriers, 4 ACVRL1 mutation carriers, 1 ENG mutation carrier, and 15 noncarriers. The 1-year survival rate for all pediatric patients was 89.3%, the 2-year survival rate was 79.7%, and the 3-year survival rate was 72.9%, as shown in Figure 1. The median survival time for mutation carriers was 18 months (2–120 months). The prognosis of mutation carriers was significantly worse than that of nonmutation carriers. The 1-, 2-, and 3-year survival rates were 90.7%, 79.2%, and 72.9% respectively, while the corresponding rates for noncarriers were 93.2%, 89.4%, and 83.3% respectively.

| Protocols               | All \((n = 105)\) | Carriers \((n = 46)\) | Noncarriers \((n = 59)\) |
|------------------------|-------------------|----------------------|--------------------------|
| None, \(n\) (%)        | 3 (2.9)           | 2 (4.3)              | 1 (1.7)                  |
| Monotherapy, \(n\) (%) | 42                |                      |                          |
| ERAs                   | 22 (20.1%)        | 9 (19.6%)            | 13 (22%)                 |
| PDE-5is                | 16 (15.2%)        | 4 (8.7%)             | 12 (20.3%)               |
| CCBs                   | 4 (3.8%)          | 0                    | 4 (6.8%)                 |
| Combination therapy, \(n\) (%) | 60              | 31                   |                          |
| ERAs + PDE-5is         | 40 (38.1%)        | 17 (36.9%)           | 23 (38.9%)               |
| ERAs + prostanoids     | 4 (3.8%)          | 4 (8.7%)             | 0                        |
| CCBs + prostanoids     | 1 (1%)            | 0                    | 1 (1.7%)                 |
| ERAs + PDE-5is + prostanoids | 15 (14.3%) | 10 (21.7%) | 5 (8.5%)                 |

**Table 2** Targeted drug protocols of patients

| Protocols               | All \((n = 105)\) | Carriers \((n = 46)\) | Noncarriers \((n = 59)\) |
|------------------------|-------------------|----------------------|--------------------------|
| None, \(n\) (%)        | 3 (2.9)           | 2 (4.3)              | 1 (1.7)                  |
| Monotherapy, \(n\) (%) | 42                |                      |                          |
| ERAs                   | 22 (20.1%)        | 9 (19.6%)            | 13 (22%)                 |
| PDE-5is                | 16 (15.2%)        | 4 (8.7%)             | 12 (20.3%)               |
| CCBs                   | 4 (3.8%)          | 0                    | 4 (6.8%)                 |
| Combination therapy, \(n\) (%) | 60              | 31                   |                          |
| ERAs + PDE-5is         | 40 (38.1%)        | 17 (36.9%)           | 23 (38.9%)               |
| ERAs + prostanoids     | 4 (3.8%)          | 4 (8.7%)             | 0                        |
| CCBs + prostanoids     | 1 (1%)            | 0                    | 1 (1.7%)                 |
| ERAs + PDE-5is + prostanoids | 15 (14.3%) | 10 (21.7%) | 5 (8.5%)                 |

Abbreviations: CCBs, calcium channel blockers; ERAs, endothelin receptor antagonists; PDE-5is, phosphodiesterase-5 inhibitors.
rates of mutation carriers were 79.4%, 61.5%, and 55.6% for mutation carriers, while for nonmutation carriers, the proportions were 96.6%, 91.1%, and 85.4%, respectively \((p = 0.0001)\), as shown in Figure 2. The clinical and hemodynamic characteristics of the death and survival groups were further compared (Table 3). In comparison of patients who died with survivors, RAP and PVR trended in the direction of higher, CI trended in the direction of lower, but were statistically insignificant.

Logistic regression analysis showed that genetic mutation was an risk factor of death \((p = 0.049, \text{ odd ratio} = 3.809, 95\% \text{ confidence interval} 1.006–14.429)\) (Table 4). The prognosis of TGF-β receptor mutation carriers is significantly worse than that of noncarrier mutations (Table 5).

**DISCUSSION**

We retrospectively analyzed the clinical characteristics and prognosis of Chinese pediatric patients with PAH carrying TGF-β receptor mutations. Previous studies suggested that approximately 25%–30% of patients with IPAH have an underlying genetic cause.\(^{23}\) In this study, 43.8% of the patients carried TGF-β receptor mutations, which was higher than the result of previously reported European adult and pediatric cohorts. A large European study of adult PAH showed a BMPR2 carriage rate of 15.3%, ACVRL1 carriage rate of 0.9%, and ENG carriage rate of 0.6%.\(^{24}\) A European study of pediatric PAH showed that (12.5%) of 40 pediatric patients with IPAH/HPAH carried BMPR2 mutations, and 4 (10%) carried ACVRL1 mutations.\(^{25}\) Harrison et al.\(^{19}\) found that 4 (25%) of 16 pediatric patients with IPAH carried TGF-β receptor mutations, including 2 BMPR2 mutations, 1 ACVRL1 mutation, and 1 ENG mutation, indicating that TGF-β receptor mutations are the most common mutations in pediatric patients with IPAH/HPAH and TGF-β receptor mutations play an important role in the development of PAH in pediatric patients.

In this study, 34 (32.4%) pediatric patients carried BMPR2 mutation, 10 (9.5%) pediatric patients carried ACVRL1, and 2 (1.9%) pediatric patients carried ENG mutation. It is indicated that BMPR2 mutation is the most common mutation in pediatric patients with IPAH/HPAH, followed by ACVRL1 mutation, which is consistent with the results of studies in Western countries.\(^{19,25}\)

Mutations in ACVRL1 or ENG genes can lead to the development of hereditary hemorrhagic telangiectasia (HHT) and PAH.\(^{15}\) There were 10 ACVRL1 mutation carriers and 2 two ENG mutation carriers in our study. One ACVRL1 mutation carrier showed symptoms of recurrent rhinorrhea, but none of the 12 mutation carriers met the diagnostic criteria for HHT.\(^{26}\) However, whether pediatric patients carrying ACVRL1 and ENG mutations will develop HHT requires a long follow-up period. Unfortunately, 4 ACVRL1 mutation carriers and 1 ENG mutation carrier died during the follow-up period.

Sztrymf et al.\(^{27}\) showed that patients with PAH carrying the BMPR2 mutation were diagnosed about 10 years younger than those with nonmutation and had poorer hemodynamic indices. Girerd et al.\(^{17}\) showed that patients with PAH carrying the ACVRL1 mutation were diagnosed much younger than those with nonmutation and had poorer survival rates. Similar findings were obtained in studies of pediatric patients with IPAH and HPAH.\(^{28}\) In our study, the age of onset was slightly higher in pediatric patients with PAH carrying TGF-β receptor mutations than nonmutation carriers. This result was different from the research of western scholars, and the mainly possible reason was that these data were from a single center. Multicenter analysis of larger samples is required. The mutation carriers had poorer hemodynamic status at diagnosis than nonmutation carriers and responded less well to the acute pulmonary vasodilation test. There were no significant
Many patients with PAH have benefited from targeted drugs. The application of targeted drugs is still the main method of treatment for PAH. The application protocol of targeted drugs in this study was developed according to the treatment guidelines, the severity of the pediatric patients’ conditions, and economic conditions. Endothelin receptor antagonists are the most commonly used targeted drugs, and endothelin receptor antagonists and phosphodiesterase-5 inhibitors are the most commonly used combination.

In our study, patients carrying TGF-β receptor mutations

### TABLE 3  Characteristics of dead and surviving patients

| Variables                     | Deaths/LT n = 38 | Survivors n = 67 | p value |
|-------------------------------|-----------------|-----------------|---------|
| Female, n (%)                 | 16 (42%)        | 34 (50.7%)      | 0.396   |
| Age at diagnosis, months      | 97.2 ± 47.4     | 74.6 ± 54.0     | 0.034   |
| NYHA FC                       |                 |                 |         |
| I–II, n (%)                   | 21 (55.3%)      | 38 (56.7%)      | 0.886   |
| III–IV, n (%)                 | 17 (44.7%)      | 29 (43.3%)      |         |
| Gene mutation, n (%)          | 23 (60.5%)      | 23 (34.3%)      | 0.01    |
| BNP, pg/ml                    | 532.0 (75.0–1163.0) | 300.0 (71.75–795.0) | 0.197   |
| PASP, mmHg                    | 88.0 (69.0–105.0) | 81.0 (67.5–91.5) | 0.153   |
| RHC parameters                |                 |                 |         |
| MPAP, mmHg                    | 63.89 ± 28.26   | 60.78 ± 29.93   | 0.697   |
| RAP, mmHg                     | 10.0 (8.0–11.0) | 8.0 (6.0–12.5)  | 0.315   |
| PVR, WU                       | 17.0 (13.4–26.69) | 15.68 (9.08–23.22) | 0.142   |
| CI, L/min/m²                  | 2.9 (2.39–3.43) | 3.25 (2.61–4.19) | 0.094   |
| AVT, n (%)                    | 4 (21.1%)       | 8 (16.3%)       | 0.649   |
| PAH targeted drugs            |                 |                 | 0.343   |
| None, n (%)                   | 2 (5.2%)        | 1 (1.5%)        |         |
| Monotherapy, n (%)            |                 |                 |         |
| Carrier                       | 8 (21.1%)       | 5 (7.5%)        |         |
| Noncarrier                    | 8 (21.1%)       | 21 (31.3%)      |         |
| Dual therapy                  |                 |                 |         |
| Carrier                       | 8 (21.1%)       | 13 (19.4%)      |         |
| Noncarrier                    | 7 (18.4%)       | 17 (25.5%)      |         |
| Triple therapy                |                 |                 |         |
| Carrier                       | 4 (10.5%)       | 6 (8.9%)        |         |
| Noncarrier                    | 1 (2.6%)        | 4 (5.9%)        |         |

**Note:** Bold values indicate Statistically significant at p < 0.05.

Abbreviations: BNP, brain natriuretic peptide; CI, cardiac index; LT, lung transplantation; MPAP, mean pulmonary arterial pressure; NYHA FC, New York Heart Association Function Class; PASP, pulmonary artery systolic pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; WU, wood unit.

### TABLE 4  Univariate logistic regression analysis for death in patients with pulmonary arterial hypertension

| Characteristic               | Odds ratio | 95% confidence interval | p value |
|-----------------------------|------------|-------------------------|---------|
| TGF-β receptor mutation     | 3.809      | 1.006–14.429            | 0.049   |
| MPAP                        | 0.991      | 0.952–1.031             | 0.650   |
| RAP                         | 1.093      | 0.901–1.326             | 0.365   |
| PVR                         | 0.996      | 0.934–1.063             | 0.911   |
| CI                          | 0.775      | 0.439–1.369             | 0.381   |

Abbreviations: CI, cardiac index; MPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure.

differences in cardiac function and plasma BNP levels between the two groups.

Many patients with PAH have benefited from targeted drugs. The application of targeted drugs is still the main method of treatment for PAH. The application protocol of targeted drugs in this study was developed according to the treatment guidelines, the severity of the pediatric patients’ conditions, and economic conditions. Endothelin receptor antagonists are the most commonly used targeted drugs, and endothelin receptor antagonists and phosphodiesterase-5 inhibitors are the most commonly used combination. In our study, patients carrying TGF-β receptor mutations
responded poorly to AVT, which is similar to the findings of studies in Western countries.30 During the 12-year follow-up, with the abundance of targeted drugs, the number of cases using triple therapy gradually increased. After Treprostinil entered the Chinese market in 2014, more and more high-risk children began to use triple therapy (in addition to oral dual-drug therapy plus intravenous or subcutaneous continuous pumping of Treprostinil), and benefited from it. The mortality rate was lower than before (see Figure 1).

It was indicated that TGF-β receptor mutation carriers have a worse prognosis than nonmutation carriers. The survival rate of mutation carriers at 3 years was 55.6%, significantly lower than that of nonmutation carriers (85.4%). RAP and PVR were considerably higher than those of nonmutation carriers, and the CI was significantly lower than that of nonmutation carriers, indicating that TGF-β receptor mutation carriers had significantly worse right heart function than those nonmutation carriers. The TGF-β receptor gene mutation was an risk factor for death in this study, indicating that the gene mutation greatly affected the prognosis of pediatric patients with IPAH/HPAH.

There are some limitations in our study. As a single-center retrospective study, our study has a relatively small size, and some patients did not undergo cardiac catheterization. Larger sample sizes, multicenter data, and long-term follow-up are needed to characterize better the clinical features and prognosis of pediatric patients with IPAH/HPAH carrying TGF-β receptor mutations.

In conclusion, TGF-β receptor mutation is an important genetic factor for the onset of IPAH/PAH in Chinese pediatric patients. In our cohort of Chinese pediatric patients with IPAH/HPAH, those carrying TGF-β receptor mutations have a worse prognosis. Genetic screening for TGF-β receptors and more aggressive treatment of mutation carriers are recommended for pediatric patients with IPAH or HPAH and their families.

ACKNOWLEDGMENT
We are grateful to the patients and their families, whose generosity and cooperation have made this study possible. Professor Hong Gu makes substantial contributions to the conception and design, and revising article critically for important intellectual content. Xinyu Zhang is responsible for acquisition of data, analysis of data, and drafting the article. Chen Zhang and Qiangqiang Li are responsible for enrolling patients with clinically characterizes and performing right heart catheterization, and interpretation of data. All authors contributed to and discussed the results and critically reviewed the manuscript. All authors read and approve the final version to be published. This study was supported by the fund of National Natural Science Foundation of China (82070243).

CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

ETHICS STATEMENT
This study was approved by the Human Research Ethics Committee of Beijing Anzhen Hospital (no.2021106X) and was performed in accordance with the Declaration of Helsinki and the ethical standards of the institutional committee on human experimentation.

ORCID
Qiangqiang Li http://orcid.org/0000-0002-2649-6775
Hong Gu http://orcid.org/0000-0002-3557-7147

REFERENCES
1. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk-Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. Rev Esp Cardiol (Engl Ed). 2016;69(2):177.
2. Peacock AJ, Murphy NF, McMurray JJ, Caballero L, Stewart S. An epidemiological study of pulmonary arterial hypertension. Eur Respir J. 2007;30(1):104–9.
3. D’Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Golding RM, Groves BM, Kernis JT. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. Ann Intern Med. 1991;115(5):343–9.
4. Humbert M, Sitbon O, Yaïci A, Montani D, O’Callaghan DS, Jaïs X, Parent F, Savale L, Natali D, Günther S, Chaouat A, Chabot F, Cordier JF, Habib G, Gressin V, Jing ZC, Souza R, Simonneau G, French Pulmonary Arterial Hypertension N. Survival in incident and prevalent cohorts of patients with pulmonary arterial hypertension. Eur Respir J. 2010;36(3):549–55.
5. Runo JR, Loyd JE. Primary pulmonary hypertension. Lancet. 2003;361(9368):1533–44.

6. Tuder RM, Archer SL, Dorfmüller P, Erzurum SC, Guignabert C, Michelakis E, Rabinovitch M, Schermuly R, Stenmark KR, Morrell NW. Relevant issues in the pathology and pathobiology of pulmonary hypertension. J Am Coll Cardiol. 2013;62(25, Suppl):D4–12.

7. Morrell NW, Adnot S, Archer SL, Dupuis J, Lloyd Jones P, MacLean MR, McMurry IF, Stenmark KR, Thistlethwaite PA, Weissmann N, Yuan JX, Weir EK. Cellular and molecular basis of pulmonary arterial hypertension. J Am Coll Cardiol. 2009;54(1, Suppl):S20–31.

8. International PPH Consortium, Lane KB, Machado. RD, Morrell NW, Adnot S, Archer SL, Dupuis J, Lloyd Jones P, MacLean MR, McMurry IF, Stenmark KR, Thistlethwaite PA, Weissmann N, Yuan JX, Weir EK. Cellular and molecular basis of pulmonary arterial hypertension. J Am Coll Cardiol. 2009;54(1, Suppl):S20–31.

9. Machado RD, Pauciullo MW, Thomson JR, Lane KB, Morgan NV, Wheeler L, Phillips JA, Newman J, Williams D, Gallié N, Manes A, McNeil K, Yacoub M, Mikhail G, Rogers P, Corris P, Humbert M, Donnai D, Martensson G, Tranebjaerg L, Loyd JE, Trembath RC. Haplinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. Am J Hum Genet. 2001;68(1):92–102.

10. Trembath RC, Thomson JR, Machado RD, Morgan NV, Atkinson C, Winship I, Simonneau G, Gallié N, Loyd JE, Humbert M, Nichols WC, Morrell NW, Berg J, Manes A, McGaughran J, Pauciullo M, Wheeler L. Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. N Engl J Med. 2001;345(3):325–34.

11. Chaouat A, Coulet F, Favre C, Simonneau G, Weitenblum E, Soubrier F, Humbert M. Endoglin germline mutation in a patient with hereditary hemorrhagic telangiectasia and dexfenfluramine associated pulmonary arterial hypertension. Thorax. 2004;59(5):446–8.

12. Tielemans B, Delcroix M, Belge C, Quarc R. TGFbeta and BMPR2 signalling pathways in the pathogenesis of pulmonary arterial hypertension. Drug Discov Today. 2019;24(3):703–16.

13. Evans JD, Girerd B, Montani D, Wang XJ, Gallié N, Austin ED, Elliott G, Asano K, Grünig E, Yan Y, Jing ZC, Manes A, Palazzini M, Wheeler LA, Nakayama I, Satoh T, Eichstaedt C, Hinderhofer K, Wolf M, Rosenzweig EB, Chung WK, Soubrier F, Simonneau G, Sibton O, Gräf S, Kaptoge S, Di Angelantonio E, Humbert M, Morrell NW. BMPR2 mutations and survival in pulmonary arterial hypertension: an individual participant data meta-analysis. Lancet Respir Med. 2016;4(2):129–37.

14. Hansmann G, Hoepner MM. Registries for paediatric pulmonary hypertension. Eur Respir J. 2013;42(3):580–3.

15. Berger RM, Beghetti M, Humph T, Raskob GE, Ivy DD, Jing ZC, Bonnet D, Schulze-Neick I, Barst RJ. Clinical features of paediatric pulmonary hypertension: a registry study. Lancet. 2012;379(9815):537–46.

16. Sztrymf B, Coulet F, Girerd B, Yaici A, Jais X, Sibton O, Montani D, Souza R, Simonneau G, Soubrier F, Humbert M. Clinical outcomes of pulmonary arterial hypertension in carriers of BMPR2 mutation. Am J Respir Crit Care Med. 2008;177(12):1377–83.

17. Girerd B, Montani D, Coulet F, Sztrymf B, Yaici A, Jais X, Tregouet D, Reis A, Drouin-Garraud V, Fraise S, Sitbon O, O’Callaghan DS, Simonneau G, Soubrier F, Humbert M. Clinical outcomes of pulmonary arterial hypertension in patients carrying an ACVRL1 (ALK1) mutation. Am J Respir Crit Care Med. 2010;181(8):851–61.

18. Chiha A, Shintani M, Yagi H, Fujiwara M, Kojima Y, Sato H, Imamura S, Yokozawa M, Onodera N, Horigome H, Kobayashi T, Hatai Y, Nakayama T, Fukushima H, Nishiyama M, Doi S, Ono Y, Yasukouchi S, Ichida F, Fujimoto K, Ohtsuki S, Teshima H, Kawano T, Nomura Y, Gu H, Ishiwata T, Furutani Y, Inai K, Saji T, Matsuoka R, Nonoyama S, Nakashima T. Outcomes of childhood pulmonary arterial hypertension in BMPR2 and ALK1 mutation carriers. Am J Cardiol. 2012;110(4):586–93.

19. Harrison RE, Berger R, Haworth SG, Tulloh R, Mache CJ, Morrell NW, Aldred MA, Trembath RC. Transforming growth factor-beta receptor mutations and pulmonary arterial hypertension in childhood. Circulation. 2005;111(4):435–41.

20. Sitbon O, Humbert M, Jais X, Ios V, Hamid AM, Provencher S, Garcia G, Parent F, Hervé P, Simonneau G. Long-term response to calcium channel blockers in idiopathic pulmonary arterial hypertension. Circulation. 2005;111(23):3105–11.

21. Piao C, Zhu Y, Zhang C, Xi X, Liu X, Zheng S, Li X, Guo J, Jia L, Nakashima T, Cai T, Gu H, Du J. Identification of multiple ACVRL1 mutations in patients with pulmonary arterial hypertension by targeted exome capture. Clin Sci (Lond). 2016;130(17):1559–69.

22. Liu X, Jiang T, Piao C, Li X, Guo J, Zheng S, Zhang X, Cai T, Du J. Screening mutations of MYBPC3 in 114 unrelated patients with hypertrophic cardiomyopathy by targeted capture and next-generation sequencing. Sci Rep. 2015;5:11411.

23. Morrell NW, Aldred MA, Chung WK, Elliott CG, Nichols WC, Soubrier F, Trembath RC, Loyd JE. Genetics and genomics of pulmonary arterial hypertension. Eur Respir J. 2019;53(1):1801899.

24. Gräf S, Haimel M, Bleda M, Hadinnappola C, Southgate L, Li W, Hodgson J, Liu B, Salmon RM, Southwood M, Machado RD, Martin JM, Treacy CM, Yates K, Daugherty LC, Shamardina O, Whitehorn D, Holden S, Aldred M, Bogaard HJ, Church C, Coghlan G, Condiffe R, Corris PA, Danesino C, Eyriss M, Gall H, Ghio S, Ghofrani HA, Gibbs J, Girerd B, Houweling AC, Howard L, Humbert M, Kiely DG, Kovacs G, MacKenzie Ross RV, Moledina S, Montani D, Newnham M, Olschewski A, Olschewski H, Peacock AJ, Pepke-Zaba J, Prokopenko I, Rhodes JC, Scelsi L, Seeger W, Soubrier F, Stein DF, Suntharalingam J, Swietlik EM, Toshner MR, vanHeel DA, Vonk Noordegraaf A, Waisfisz Q, Wharton J, Wort SJ, Wrona WH, Soranzo N, Lawrie A, Upton PD, Wilkins MR, Trembath RC, Morrell NW. Identification of rare sequence variation underlying heritable pulmonary arterial hypertension. Nat Commun. 2018;9(1):1416.

25. Levy M, Eyriss M, Szepesanski I, Ladouceur M, Nadaud S, Bonnet D, Soubrier F. Genetic analyses in a cohort of children with pulmonary hypertension. Eur Respir J. 2016;48(4):1118–26.
26. Shovlin CL, Guttmacher AE, Buscarini E, Faughnan ME, Hyland RH, Westermann CJ, Kjeldsen AD, Plauchu H. Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu–Osler–Weber syndrome). Am J Med Genet. 2000; 91(1):66–7.

27. Sztrymf B, Coulet F, Girerd B, Yaici A, Jais X, Sitbon O, Montani D, Souza R, Simonneau G, Soubrier F, Humbert M. Clinical outcomes of pulmonary arterial hypertension in carriers of BMPR2 mutation. Am J Respir Crit Care Med. 2008;177(12):1377–83.

28. Chida A, Shintani M, Yagi H, Fujiwara M, Kojima Y, Sato H, Imamura S, Yokozawa M, Onodera N, Horigome H, Kobayashi T, Hatai Y, Nakayama T, Fukushima H, Nishiyama M, Doi S, Ono Y, Yasukouchi S, Ichida F, Fujimoto K, Ohtsuki S, Teshima H, Kawano T, Nomura Y, Gu H, Ishiwata T, Furutani Y, Inai K, Saji T, Matsuoka R, Nonoyama S, Nakanishi T. Outcomes of childhood pulmonary arterial hypertension in BMPR2 and ALK1 mutation carriers. Am J Cardiol. 2012;110(4):586–93.

29. Zhang R, Dai LZ, Xie WP, Yu ZX, Wu BX, Pan L, Yuan P, Jiang X, He J, Humbert M, Jing ZC. Survival of Chinese patients with pulmonary arterial hypertension in the modern treatment era. Chest. 2011;140(2):301–9.

30. Majka S, Hagen M, Blackwell T, Harral J, Johnson JA, Gendron R, Paradis H, Crona D, Loyd JE, Nozik-Grayck E, Stenmark KR, West J. Physiologic and molecular consequences of endothelial Bmpr2 mutation. Respir Res. 2011;12(1):84.

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Zhang X, Zhang C, Li Q, Gu H. TGF-β receptor mutations and clinical prognosis in Chinese pediatric patients with idiopathic/hereditary pulmonary arterial hypertension. Pulm Circ. 2022;12:e12076. https://doi.org/10.1002/pul2.12076