CLINICAL AND MOLECULAR GENETIC FEATURES OF PULMONARY HYPERTENSION IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

RICHARD C. TREMBATH, F.R.C.P., JENNIFER R. THOMSON, M.R.C.P., RAJIV D. MACHADO, B.Sc., NEIL V. MORGAN, B.Sc., CARL ATKINSON, B.Sc., INGRID WINSHIP, M.D., GERALD SIMONNEAU, M.D., NAZZARENO GALIE, M.D., JAMES E. LOYD, M.D., MARC HUMBERT, M.D., WILLIAM C. NICHOLS, PH.D., AND NICHOLAS W. MORRELL, M.D.

ABSTRACT

Background Most patients with familial primary pulmonary hypertension have defects in the gene for bone morphogenetic protein receptor II (BMPR2), a member of the transforming growth factor β (TGF-β) superfamily of receptors. Because patients with hereditary hemorrhagic telangiectasia may have lung disease that is indistinguishable from primary pulmonary hypertension, we investigated the genetic basis of lung disease in these patients.

Methods We evaluated members of five kindreds plus one individual patient with hereditary hemorrhagic telangiectasia and identified 10 cases of pulmonary hypertension. In the two largest families, we used microsatellite markers to test for linkage to genes encoding TGF-β–receptor proteins, including endoglin and activin-receptor–like kinase 1 (ALK1), and BMPR2. In subjects with hereditary hemorrhagic telangiectasia and pulmonary hypertension, we also scanned ALK1 and BMPR2 for mutations.

Results We identified suggestive linkage of pulmonary hypertension with hereditary hemorrhagic telangiectasia on chromosome 12q13, a region that includes ALK1. We identified amino acid changes in activin-receptor–like kinase 1 that were inherited in subjects who had a disorder with clinical and histologic features indistinguishable from those of primary pulmonary hypertension. Immunohistochemical analysis in four subjects and one control showed pulmonary vascular endothelial expression of activin-receptor–like kinase 1 in normal and diseased pulmonary arteries.

Conclusions Pulmonary hypertension in association with hereditary hemorrhagic telangiectasia can involve mutations in ALK1. These mutations are associated with diverse effects, including the vascular dilatation characteristic of hereditary hemorrhagic telangiectasia and the occlusion of small pulmonary arteries that is typical of primary pulmonary hypertension.

From the Division of Medical Genetics, Departments of Medicine and Genetics, University of Leicester, Leicester, United Kingdom (R.C.T., J.R.T., R.D.M., N.W.M.); the Department of Medicine, University of Cambridge School of Clinical Medicine, Addenbrooke’s and Papworth Hospitals, Cambridge, United Kingdom (C.A., N.W.M.); the Department of Molecular Medicine, School of Medicine, University of Auckland, Auckland, New Zealand (I.W.); the Service de Pneumologie, Hôpital Antoine Béclère, Assistance Publique–Hôpitaux de Paris, Université Paris-Sud, Clamart, France (G.S., M.H.); the Institute of Cardiology, University of Bologna, Bologna, Italy (N.G.); Vanderbilt University Medical Center, Nashville (J.E.L.); and the Division of Human Genetics, Children’s Hospital Medical Center, Cincinnati (W.C.N.). Address reprint requests to Dr. Trembath at the Division of Medical Genetics, Departments of Medicine and Genetics, Adrian Bldg., University of Leicester, Leicester LE1 7RH, United Kingdom, or rtrembat@hgmp.mrc.ac.uk.

Other authors were Jonathan Berg, M.D., Department of Clinical Genetics, Guy’s Hospital Campus, King’s College, London; Alessandra Manes, M.D., Institute of Cardiology, University of Bologna, Bologna, Italy; Julie McCaughran, M.D., Department of Molecular Medicine, School of Medicine, University of Auckland, Auckland, New Zealand; Michael Pauulo, B.Sc., Division of Human Genetics, Children’s Hospital Medical Center, Cincinnati; and Lisa Wheeler, B.Sc., Vanderbilt University Medical Center, Nashville.

PULMONARY hypertension, defined as the sustained elevation of mean pulmonary arterial pressure above 25 mm Hg at rest and 30 mm Hg during exercise, is a major cause of progressive right-sided heart failure leading to premature death.1 Pulmonary hypertension often results from chronic lung disease leading to hypoxemia, recurrent thromboembolism, or left-sided heart disease, but the pathogenesis is poorly understood. Less commonly, a primary defect of the pulmonary arterial vasculature — characterized by increased medial thickness, intimal fibrosis, and plexiform lesions of capillary-like channels — causes occlusion of small pulmonary arteries, a condition known as primary pulmonary hypertension.2,3

Primary pulmonary hypertension has an incidence of 1 to 2 cases per million people per year.4 The disease may occur at any age but has a peak onset in the third decade of life. Untreated patients with this progressive condition have a median survival of less than three years after diagnosis.5 At least 6 percent of patients with primary pulmonary hypertension have a family history of the condition.6 Kindreds demonstrate autosomal dominant inheritance with markedly reduced penetrance of the gene associated with primary pulmonary hypertension; a proportion of those who inherit the gene have no symptoms.7 The gene for primary pulmonary hypertension, which encodes bone morphogenetic protein receptor II (BMPR2) and is located on human chromosome 2,7,9 is a member of the transforming growth factor β (TGF-β) superfamily of receptors (Fig. 1).10,11

Pulmonary hypertension that is clinically and histologically indistinguishable from primary pulmonary hypertension may occur in persons with hereditary hemorrhagic telangiectasia, an autosomal dominant vascular dysplasia.12,13 Abnormalities in endothelial cells in patients with hereditary hemorrhagic telangiectasia are associated with mucocutaneous telangiectases.
These lead to recurrent epistaxis and gastrointestinal blood loss as well as arteriovenous malformations, particularly in the pulmonary, hepatic, and cerebral circulations.\(^9\) Pulmonary arteriovenous malformations create clinically significant right-to-left shunts, causing hypoxemia, paradoxical embolism, stroke, and cerebral abscesses.\(^9\) Mutations in two genes encoding additional TGF-\(\beta\) receptors—namely, endoglin and activin-receptor–like kinase 1 (\(\text{ALK1}\))—which are located on chromosomes 9 and 12, respectively, underlie hereditary hemorrhagic telangiectasia (Fig. 1).\(^12,17,18\)

We investigated the genetic basis of pulmonary hypertension in subjects with hereditary hemorrhagic telangiectasia. We identified kindreds affected by hereditary hemorrhagic telangiectasia and pulmonary hypertension, determined the clinical and pathological features of the cohort, and then searched for molecular genetic defects within the TGF-\(\beta\)–receptor pathway. The analysis was undertaken by the University of Leicester, Leicester, United Kingdom, which holds the data.

METHODS

Clinical Evaluation

Written informed consent was obtained from all participants in accordance with the requirements of the ethics committees of the Leicestershire Health Authority, United Kingdom, and the Ministry of Health, Auckland, New Zealand, and the institutional review board of the Vanderbilt University Medical Center. Between April and September 2000, all family members underwent pedigree analysis and clinical assessment. Hereditary hemorrhagic telangiectasia was diagnosed with the use of current international consensus criteria. These require the presence of any three of the following: an affected first-degree relative, recurrent spontaneous epistaxis, mucocutaneous telangiectasia, and documented visceral manifestations.\(^9\) The diagnosis of pulmonary hypertension was determined through clinical evaluation, chest radiography, electrocardiography, Doppler echocardiography, right-heart catheterization, and histologic examination of tissue obtained by excision of a lung or at autopsy. Other known causes of elevated pulmonary arterial pressure (for example, chronic lung disease associated with hypoxemia, thromboembolism, intracardiac shunting, and left ventricular failure) were excluded. The diagnosis was established without knowledge of genotypic status. To determine the prevalence of mutations in \(\text{ALK1}\) in subjects with primary pulmonary hypertension who had no personal or family history of hereditary hemorrhagic telangiectasia, we also assessed 11 subjects with familial primary pulmonary hypertension and 24 subjects with sporadic primary pulmonary hypertension, in whom the results of analysis for mutations in \(\text{BMPR2}\) were negative; the clinical features of these subjects have previously been described.\(^20,21\)

Genetic Studies

Linkage Analysis

DNA was isolated from peripheral-blood lymphocytes, paraffin-embedded lung tissue, or dried blood spots from family members as indicated in Figures 2 and 3.\(^12,22,23\) Genetic-linkage analysis was performed in the largest families, Family 1 and Family 2, with the use of the following polymorphic microsatellite markers: D9S560, D9S315, and D9S61 for endoglin; D12S347, D12S368, and D12S325 for \(\text{ALK1}\); and D2S2289, D2S346, and D2S3009 for \(\text{BMPR2}\), as previously described.\(^24\) Two-point lod scores were calculated with the use of the MLINK program.\(^25\)

Identification of Mutations in \(\text{ALK1}\) and \(\text{BMPR2}\)

The protein-coding sequence of \(\text{ALK1}\) (exons 2 through 10) was amplified from genomic DNA with the use of primers complementary to the intronic sequences near the intron–exon boundaries, as previously described.\(^26\)

The fragments were amplified by the polymerase chain reaction (PCR) and excess primer was removed from the amplified fragments with the use of a purification kit (QiAquick, Qiagen, Crawley, West Sussex, United Kingdom) and sequenced with a dye-terminator cycle-sequencing system (ABI PRISM 377, Perkin–Elmer Applied Biosystems, Foster City, Calif.). Analysis of \(\text{BMPR2}\) sequence (exons 1 through 13) and gene dosage (exons 1 and 12) was performed in each family member with pulmonary hypertension as previously described\(^20,21\) (information on primer sequences is available as Supplementary Appendix 1 with the full text of this article at http://www.nejm.org).

Confirmation of Mutations and Segregation of Genotypes

Identified variants of \(\text{ALK1}\) were confirmed by resequencing of independent samples or, for the substitution of thymine for cytosine at position 1450 (Family 1) and the deletion of cytosine at position 37 (Family 5), by restriction-enzyme digestion with \(\text{Fnu4HI}\). For the latter, the required exons were amplified, digested with restriction enzyme, and size-fractionated on a 3 percent agarose gel. The presence or absence of sequence variants in DNA samples from family members and from 75 normal controls was also ascertained by sequence analysis or restriction-enzyme digestion.

Histologic and Immunohistochemical Analysis

Normal lung tissue was obtained from unused material excised from donors for heart–lung transplantation and diseased lung from four subjects in Families 1 and 3 (Table 1). For immunohistochemical analysis, sections were incubated at a dilution of 1:80 with either a polyclonal antibody against activin-receptor–like kinase 1 (provided by Dr. D. Marchuk) or the monoclonal anti–endothelial-cell marker anti-CD31 (JC/70A, from Dako, Ely, Cambridgeshire, United Kingdom) for 60 minutes at room temperature and washed before incubation with biotinylated secondary antibody. Antigen was visualized with the use of the avidin–biotin–peroxidase technique (Vectastain ABC, Vector Laboratories, Peterborough, United Kingdom) with diaminobenzidine substrate. The specificity of immunostaining was demonstrated by the absence of signal in sections incubated with control mouse IgG (Sigma, St. Louis) or in sections processed after omission of the primary antibody.

RESULTS

We identified five kindreds (Families 1, 2, 3, 4, and 6) and one individual patient (in Family 5) (Fig. 2 and 3) affected by hereditary hemorrhagic telangiectasia, among whom there were 10 cases of pulmonary hypertension (Table 1). Stigmata of hereditary hemorrhagic telangiectasia were not observed in five of these subjects, each of whom was less than 30 years of age (Table 1). Family 1 was noteworthy for the early age of onset of pulmonary hypertension in three members (Table 1 and Fig. 2). Pulmonary arteriovenous malformations were detected in Subject II-1 from Family 3 and Subject II-1 from Family 5; in the latter subject, the malformation was embolized after the onset of pulmonary hypertension. In the remaining eight subjects with pulmonary hypertension, pulmonary arteriovenous malformations were ruled out through a combination of chest radiography, high-resolution helical computed tomography of the thorax, studies of
Figure 1. Signaling Pathway of the Transforming Growth Factor β (TGF-β) Superfamily.

In the extracellular space, ligands to the TGF-β superfamily of receptors bind either to an accessory protein, which presents the ligand to the type II receptor, or directly to the type II receptor on the cell membrane. The binding of the ligands to the type II receptor then leads to binding of the type I receptor to form a heteromeric receptor complex at the cell surface. This results in phosphorylation and activation of the kinase domain of the type I receptor, which initiates phosphorylation of cytoplasmic signaling proteins termed receptor Smads (R-Smads). Phosphorylated R-Smad binds to a collaborating Smad (Co-Smad), and the resulting complex moves from the cytoplasm into the nucleus. The Smad complex associates with a DNA-binding partner in the cell nucleus and interacts with various other transcription factors in a cell-specific manner to regulate gene transcription and to mediate the effects of signaling by the TGF-β superfamily of receptors at the cellular level. Germ-line mutations of the gene encoding the type II receptor, bone morphogenetic protein receptor II, underlie primary pulmonary hypertension. Defects of the type I receptor, activin-receptor–like kinase 1, and the accessory protein, endoglin, cause hereditary hemorrhagic telangiectasia. In addition, mutations in the ALK1 gene also predispose subjects to the development of pulmonary hypertension. The specific extracellular ligands, cell-surface receptors, cytoplasmic Smad proteins, and nuclear transcription factors that are involved with signaling of bone morphogenetic protein II and activin-receptor–like kinase 1 in the pulmonary circulation have not been identified.
arterial oxygen saturation, and pulmonary angiography and hemodynamic studies or were excluded on examination of excised lung tissue or at autopsy.

Analysis of three polymorphic markers on chromosome 12 supported linkage of the disease to the \textit{ALK1} locus in two families (likelihood of independent coinheritance, 1 in 100 and 1 in 125 in Families 1 and 2, respectively). Because mutations in \textit{ALK1} are known to cause hereditary hemorrhagic telangiectasia, the nine exons encoding the protein product were amplified and sequenced in probands with pulmonary hypertension from five families (Families 1, 2, 3, 4, and 6) and one subject from Family 5. Novel heterozygous sequence variants of \textit{ALK1} were identified in the genomes of probands in four of five kindreds (Families 1, 2, 3, and 4) and one subject from Family 5 who had been adopted and had no available family history (Table 1 and Fig. 4). A deletion of a single nucleotide (cytosine) at position 37 in exon 2 in Subject II-1 from Family 5 and a substitution (thymine for cytosine) at position 1468 in exon 10 in Family 4 are predicted to lead to premature truncation of the mature protein, by changing the reading frame and by introducing a stop codon, respectively.

Three sequence variants lead to alterations in 1 of the 503 amino acids of the activin-receptor–like kinase 1: a deletion of an aspartic acid at position 254 encoded by exon 6 (Family 3), a substitution of tryptophan for arginine at position 411 encoded by exon 8 (Family 2), and a substitution of tryptophan for arginine at position 484 encoded by exon 10 (Family 1) (Table 1). No mutations in \textit{ALK1} were detected in 11 subjects with familial cases and 24 subjects with sporadic cases of isolated primary pulmonary hypertension, who were known to be negative for mutations in \textit{BMPR2}.

In Family 6, a previously reported \textit{ALK1} sequence variant was detected in Subject III-2, who had hereditary hemorrhagic telangiectasia (Fig. 3). However, segregation analysis demonstrated that this \textit{ALK1} variant was not inherited by Subjects IV-3, V-1, and V-2 (Fig. 3). To investigate the genetic basis of the pulmonary hypertension in Subject V-1 further, we performed mutational analysis of \textit{BMPR2}. A partial deletion in \textit{BMPR2} that included exon 12 was identified and shown to be maternally inherited (Table 1). Subject IV-4, who was from an unrelated family, reported no personal or family history of pulmonary hypertension, in keeping with the low penetrance of mutant \textit{BMPR2} alleles. Thus, the presence of both \textit{ALK1}-associated hereditary hemorrhagic telangiectasia and \textit{BMPR2}-related pulmonary hypertension in Family 6 may be due to chance alone. No mutations in \textit{BMPR2} were identified in probands with pulmonary hypertension from Families 1, 2, 3, and 4 or in Subject II-1 from Family 5.

To support the likelihood that these novel variants of \textit{ALK1} cause disease, we first confirmed that each

---

**Figure 2. Segregation of the Cytosine-to-Thymine Mutation at Position 1450 in ALK1 with Pulmonary Hypertension and Hereditary Hemorrhagic Telangiectasia in Family 1.**

The DNA-sequence chromatograms for the subjects tested are shown below the pedigree. Unaffected family members (Subjects II-3, I-2, and II-5) have two normal alleles (cytosine in blue) at position 1450 of \textit{ALK1}. Family members with pulmonary hypertension, hereditary hemorrhagic telangiectasia, or both (Subjects II-2, III-1, III-2, III-3, and II-4) have one normal allele (cytosine in blue) and one mutant allele (thymine in red) at position 1450, indicated by the letter Y. Squares denote male family members, circles female family members, symbols with a slash deceased family members, black symbols members with both pulmonary hypertension and hereditary hemorrhagic telangiectasia, gray symbols members with hereditary hemorrhagic telangiectasia only, hatched symbols members with pulmonary hypertension only, and open symbols members unaffected by either condition.
variant cosegregated with the disease in available affected family members, as shown for Family 1 (Fig. 2). None of the five defects in ALK1 were found in 150 normal chromosomes; therefore, they are unlikely to represent silent polymorphisms. Each of the amino acid alterations has been conserved through evolution and across related type I receptors of the TGF-β superfamily (Fig. 4).

Histologic assessment of the pulmonary vasculature was available for four patients from Families 1 and 3. Each had characteristic features of end-stage plexogenic pulmonary hypertension similar to those seen in patients with primary pulmonary hypertension (Fig. 5). Immunohistochemical analysis demonstrated expression of activin-receptor-like kinase 1 to be predominantly in the vascular endothelium of normal and diseased lungs (Fig. 5); activin-receptor-like kinase 1 colocalized with the CD31 endothelial-cell marker in serial sections. Expression of activin-receptor-like kinase 1 was also observed in the endothelium of occlusive and plexiform lesions (Fig. 5).

In our study the clinical, hemodynamic, and histologic features of pulmonary hypertension associated with mutations in ALK1 were indistinguishable from the features of pulmonary hypertension due to occlusive vascular lesions associated with defects in BMPR2 (Fig. 5).10,11,20,21

DISCUSSION
The histologic and pathophysiological features of hereditary hemorrhagic telangiectasia and primary pulmonary hypertension might seem to be distinct. Pul-
TABLE 1. CLINICAL FEATURES AND MUTATIONS IN SUBJECTS WITH PULMONARY HYPERTENSION AND A PERSONAL OR FAMILY HISTORY OF HEREDITARY HEMORRHAGIC TELANGIECTASIA.*

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>Subject (Family)</th>
<th>Subject (Family)</th>
<th>Subject (Family)</th>
<th>Subject (Family)</th>
<th>Subject (Family)</th>
<th>Subject (Family)</th>
<th>Subject (Family)</th>
<th>Subject (Family)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>III-1</td>
<td>III-2</td>
<td>III-3</td>
<td>III-6</td>
<td>II-1</td>
<td>II-4</td>
<td>V-1</td>
<td>II-1†</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(1)</td>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>(3)</td>
<td>(4)</td>
<td>(5)</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Age at death (yr)‡</td>
<td>7</td>
<td>2</td>
<td>9</td>
<td>46</td>
<td>50</td>
<td>34</td>
<td>54</td>
<td>39</td>
</tr>
<tr>
<td>Known features of HHT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of HHT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NK</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Telangiectasia</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pulmonary AVM</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Hepatic AVM</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Other AVM</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Estrogen therapy</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Known features of PH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at onset (yr)†</td>
<td>6.0</td>
<td>1.5</td>
<td>1.5</td>
<td>29.0</td>
<td>45.0</td>
<td>31.0</td>
<td>27.0</td>
<td>33.0</td>
</tr>
<tr>
<td>Family history of PH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>NK</td>
</tr>
<tr>
<td>Symptoms at onset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breathlessness</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fatigue</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Syncope</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Chest pain</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Right-sided heart catheterization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean PAP (mm Hg)</td>
<td>–</td>
<td>55</td>
<td>120</td>
<td>74</td>
<td>45</td>
<td>55</td>
<td>70</td>
<td>54</td>
</tr>
<tr>
<td>CO (liters/min)</td>
<td>–</td>
<td>0.86</td>
<td>–</td>
<td>4.82</td>
<td>4.81</td>
<td>6.90</td>
<td>2.90</td>
<td>2.97</td>
</tr>
<tr>
<td>CI (liters/min/m²)</td>
<td>–</td>
<td>1.56</td>
<td>–</td>
<td>2.50</td>
<td>4.40</td>
<td>1.43</td>
<td>1.75</td>
<td>1.75</td>
</tr>
<tr>
<td>PAWP (mm Hg)</td>
<td>–</td>
<td>9</td>
<td>–</td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>PVR (resistance units)</td>
<td>–</td>
<td>53.5</td>
<td>–</td>
<td>13.9</td>
<td>8.3</td>
<td>7.0</td>
<td>20.7</td>
<td>15.8</td>
</tr>
<tr>
<td>Response to vasodilators</td>
<td>NK</td>
<td>NK</td>
<td>No</td>
<td>NK</td>
<td>No</td>
<td>NK</td>
<td>NK</td>
<td>No</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vasodilators</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Transplantation</td>
<td>–</td>
<td>–</td>
<td>HLT</td>
<td>–</td>
<td>DLT</td>
<td>SLT</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Histology of the lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Explant</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Autopsy</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>ALK1 mutation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon§</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Amino acid position</td>
<td>R484W</td>
<td>R484W</td>
<td>R484W</td>
<td>R411W</td>
<td>254delD</td>
<td>254delD</td>
<td>Q490X</td>
<td>L136s(+1aa)†</td>
</tr>
<tr>
<td>Type of mutation</td>
<td>Missense</td>
<td>Missense</td>
<td>Missense</td>
<td>Missense</td>
<td>Deletion</td>
<td>Deletion</td>
<td>Nonsense</td>
<td>Frame shift</td>
</tr>
<tr>
<td><strong>BMPR2 mutation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
|**†**No family history was available for this patient, who was adopted.

‡The current ages of the surviving subjects, Subject V-1 from Family 4 and Subject V-1 from Family 6, are 28 and 20 years, respectively.

§A substitution of glutamic acid for arginine at position 67 in exon 3 of ALK1 was detected in Subject III-2 in Family 6 that was not inherited by Subjects IV-2, V-1, and V-2.

¶L136s(+1aa)† indicates a premature truncation one amino acid after the mutation in the leucine residue at codon 13.

‖Del3’ indicates a partial deletion of the 3’ end of BMPR2, including exon 12, detected in DNA available from Subjects V-1 and IV-4 in Family 6.
GENETIC FEATURES OF PULMONARY HYPERTENSION IN HEREDITARY HEMORRHAGIC TELANGIECTASIA

Figure 4. ALK1 and Identified Mutations.

ALK1 encodes a serine–threonine kinase; the hatched area denotes the sequence encoding the signal peptide, the black area the sequence encoding the transmembrane region, and the gray area the sequence encoding the kinase domain. Previously reported mutations in ALK1 are shown above the ALK1 complementary DNA (cDNA). The mutations in ALK1 detected in the families in this study and their nucleotide positions are shown below the ALK1 cDNA. Circles denote missense mutations (amino acid substitutions), squares nonsense mutations (an amino acid replaced by a stop codon), triangles deletions of a nucleotide, crosses insertions of a nucleotide, and the diamond both the insertion and the deletion of nucleotides. The gray circle denotes the missense mutation in ALK1 detected in Family 6. Beneath these symbols, the homology results show the similarity of human activin-receptor–like kinase 1 to mouse activin-receptor–like kinase 1 and other human type I receptors in the TGF-β family (i.e., activin-receptor–like kinases 2, 3, 4, 5, and 6). Amino acid positions are shown in bold (254, 411, and 484), with the deletion of aspartic acid (D) and the substitutions of arginine (R) for tryptophan (W).

Pulmonary arteriovenous dilatation is the hallmark of lung involvement in hereditary hemorrhagic telangiectasia, leading to decreased pulmonary vascular resistance and increased cardiac output, with normal to low pulmonary arterial pressure. In contrast, primary pulmonary hypertension is characterized by obliteration of small pulmonary arteries, leading to increased pulmonary vascular resistance, marked elevation of pulmonary arterial pressure, and ultimately, a reduction in cardiac output.

We identified a total of 10 subjects with pulmonary hypertension from 5 families with hereditary hemorrhagic telangiectasia and 1 subject with no available family history. In each of these subjects, we excluded left-ventricular high-output failure secondary to systemic arteriovenous malformations and thromboembolic disease after estrogen therapy for telangiectasias as potential causes of elevated pulmonary arterial pressure. Of the four subjects from whom lung tissue was available (Table 1), pulmonary arterial lesions similar to those reported among subjects harboring mutations in BMPR2 were observed (Fig. 5). Since the clinical and histologic features of these subjects resembled those of subjects with primary pulmonary hypertension, we considered the possibility that the association with hereditary hemorrhagic telangiectasia reflected basic defects underlying the inherited predisposition to pulmonary hypertension.

Molecular analysis of these kindreds demonstrated that mutations in ALK1 may lead to occlusion of the pulmonary arteries together with vascular dilatation manifested as telangiectasia and arteriovenous malformations. This apparent dichotomy may be explained in part by current knowledge of the TGF-β signaling pathway (Fig. 1). After ligand binding, a type II receptor (for example, bone morphogenetic protein receptor II) forms a heteromeric complex with a type I receptor (for example, activin-receptor–like kinase 1) and may associate with an accessory receptor such as endoglin. This results in activation of the kinase domain of the type I receptor, initiating phosphorylation of cytoplasmic proteins and subsequent gene transcription.

Immunohistochemical analysis demonstrated the presence of activin-receptor–like kinase 1 in diseased pulmonary vascular endothelium, suggesting that this cell type is critical to the pathogenesis of both hereditary hemorrhagic telangiectasia and pulmonary hyper-
Figure 5. Photomicrographs of Paraffin-Embedded Lung Sections from a Control Subject (Panel A) and Subjects with Plexogenic Pulmonary Hypertension and Mutations in ALK1 (Panels B, C, D, E, and F).

Histologic analysis of normal lung shows thin-walled peripheral pulmonary arteries (open arrow in Panel A) and normal alveolar capillaries (solid arrows in Panel A). In contrast, regions of the alveolar capillary bed in subjects with pulmonary hypertension show capillary dilatation (arrows in Panel B), the presence of plexiform lesions composed of thin-walled capillary channels (arrows in Panel C), and thick-walled peripheral pulmonary arteries occluded by a cellular intimal proliferation (arrows in Panel D). Immunohistochemical analysis performed with a polyclonal antibody against activin-receptor–like kinase 1 demonstrated cellular localization of activin-receptor–like kinase 1 to the pulmonary vascular endothelium in normal arteries and in arteries of patients with pulmonary hypertension (arrows in Panel E), including the capillary channels containing occlusive and plexiform lesions (arrows in Panel F). The sections in Panels A, B, C, and D were stained with hematoxylin and eosin. The sections in Panels E and F were counterstained with hematoxylin. (Panels A, B, C, D, and F, approximately ×200; Panel E, ×800.)
tension. TGF-β signaling affects both vascular differentiation and proliferation, and overexpression of the TGF-β1 ligand promotes intimal growth and apoptosis simultaneously in vascular endothelium. The pleiotropic nature of TGF-β as a growth factor offers a potential explanation for the variable complications of hereditary hemorrhagic telangiectasia. The net effect of dysfunction of activin-receptor-like kinase 1 may depend on local vascular interactions and other environmental or genetic factors.

The majority of defects in BMPR2 in patients with primary pulmonary hypertension are predicted to generate either a substantially truncated protein or a reduction in the functional activity of the mature protein, a mechanism known as haploinsufficiency. The deletion of the cytosine residue at position 37 in ALK1 is predicted to result in a markedly abbreviated transcript, suggesting that a similar mechanism perturbs the function of activin-receptor-like kinase 1 in inherited pulmonary hypertension associated with hereditary hemorrhagic telangiectasia. No mutations in ALK1 were identified in 35 subjects with isolated primary pulmonary hypertension.

The management of pulmonary hypertension in patients with mutations in ALK1 should include a careful assessment for recognized complications of hereditary hemorrhagic telangiectasia. Pulmonary arteriovenous malformations are considered to be more common among patients with defects in endoglin, yet we identified such lesions in two subjects with mutations in ALK1. The possibility of pulmonary as well as systemic vascular malformations should be considered in all patients with hereditary hemorrhagic telangiectasia, irrespective of genotype. Similarly, the possibility of hereditary hemorrhagic telangiectasia should be considered in any patient who presents with unexplained pulmonary hypertension.

Our findings suggest that the TGF-β signaling pathway is an important mechanism for the pathogenesis of pulmonary hypertension. Defects in activin-receptor-like kinase 1 may trigger divergent signaling pathways that remodel the pulmonary artery, resulting in dilated and occlusive vascular phenotypes. Genes that encode other components of the TGF-β pathway, or those that are transcriptionally regulated by the pathway, might also be associated with pulmonary hypertension.

**REFERENCES**

27. Klaus DJ, Gallione CJ, Anthony K, et al. Novel missense and frame-

We are indebted to the patients and their families for their participation; to Drs. R. Radley-Smith and J. Neuse for their assistance in identifying families; to Drs. B. Merrick, S. Stewart, and M. Burke for histologic analysis; and to Professor L. Young for critical reading of the manuscript.

Supported by grants from the British Heart Foundation (RG/2000/012, to Drs. Trembath and Morrell) and the National Institutes of Health (HL61997, to Drs. Nihols and Loyd, and HL48164, to Dr. Loyd). Dr. Thomson is the recipient of a Clinical Training Fellowship from the Medical Research Council of the United Kingdom.


Copyright © 2001 Massachusetts Medical Society.