Pulmonary Hypertension in Scleroderma Spectrum of Disease: Lack of Bone Morphogenetic Protein Receptor 2 Mutations

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ABSTRACT. ***Objective***. To determine whether mutations in the bone morphogenetic protein receptor 2 gene (BMPR2), initially reported in primary pulmonary hypertension, were present in patients with pulmonary arterial hypertension and scleroderma spectrum of disease.

Methods. BMPR2 gene mutations were determined using nucleic acid sequencing in 24 patients with pulmonary arterial hypertension and scleroderma spectrum of disease and in 2 control groups, 96 healthy North American individuals and 100 Israeli Ashkenazi Jews. The patients also had antinuclear antibody determinations and underwent right heart catheterization.

Results. One BMPR2 guanine to adenine (G to A) mutation in exon 13 was found in a 59-year-old Ashkenazi Jewish woman with the limited cutaneous variant, a normal chest radiograph, and positive anticentromere and rheumatoid factor autoantibodies. However, this mutation is thought to be a polymorphism because the same mutation was also found in an ethnically matched healthy Ashkenazi Jew.

Conclusion. Pulmonary arterial hypertension in scleroderma spectrum of disease was not associated with heterogeneous germline mutations of BMPR2. (J Rheumatol 2002;29:2379–81)

Key Indexing Terms:
SYSTEMIC SCLEROSIS PULMONARY ARTERIAL HYPERTENSION
BONE MORPHOGENETIC PROTEIN RECEPTOR 2

Pulmonary arterial hypertension (PAH) is a recognized clinical component of scleroderma spectrum of disease[1-3]. This spectrum includes systemic sclerosis (SSc), with diffuse cutaneous (dSSc) and limited cutaneous (lSSc) variants, mixed connective tissue disease (MCTD), and overlap syndrome. The frequency of PAH varies from 33% in dSSc[1] to 10–50% in the CREST syndrome, a lSSc variant with calcinosis cutis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia[2,3]. PAH in CREST syndrome is more likely to have direct involvement of the pulmonary vasculature, whereas dSSc is more likely to have interstitial lung disease[3]. The pathology, similar to that of primary pulmonary hypertension (PPH), consists of medial hypertrophy, intimal proliferation, and fibrosis, as well as plexiform lesions of the small and medium size pulmonary arterioles[4,5]. However, the endothelial proliferation has been found to be polyclonal in SSc and monoclonal in PPH[6,7].

Heterozygous germline mutations in bone morphogenetic protein receptor II gene (BMPR2), a member of the TGF-B superfamily, have recently been reported in both familial and sporadic PPH[8-11]. The missense, nonsense, and frameshift mutations in BMPR2, private to each family, were predicted to result in a dysfunctional receptor unable to inhibit vascular proliferation. To determine if these mutations could be implicated in the pathogenesis of PAH, we compared BMPR2 mutations in 24 patients with PAH and scleroderma spectrum of disease to 2 healthy control groups: 96 North Americans and 100 Israeli Ashkenazi Jews.

MATERIALS AND METHODS

Patients. The study group (approved by our institutional review board) consisted of 24 patients: 15 women and 8 men with scleroderma spectrum of disease referred to the New York Presbyterian Pulmonary Hypertension Center for evaluation of PAH between 1992 and 2000. An additional blood sample was obtained at a Pulmonary Hypertension Association meeting from a woman with SSc and PAH receiving epoprostenol. The unavailability of blood excluded another 8 patients. PAH was confirmed during right heart catheterization. The mean pulmonary artery pressure was 48 ± 13 mm Hg, mean right atrial pressure 10 ± 7 mm Hg, cardiac index 2.1 ± 0.8 l/min/m^2, and pulmonary vascular resistance index 24 ± 16 Wood units × m^2.

Table 1 illustrates the type of scleroderma spectrum of disease, lung involvement [chest radiograph or computerized tomographic (CT) scan],

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Supported by NIH-HBLI 60056.

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Submitted January 7, 2002; revision accepted May 28, 2002.

Table 1. Clinical and serological variables in 24 patients with pulmonary arterial hypertension and scleroderma spectrum disease.

Patient	Diagnosis	Chest Radiograph (CR) or CT	ANA	Autoantibodies ENA	Scl 70	Centromere	Other
1 49 F	dSSc	Basilar fibrosis	1:2560 S	U1RNP, Sm,Ro	–	–	
2 44 F	dSSc	Normal CR	1:2560 N	La	–	–	
3 61 F	1SSc	Pericardial effusion	1:1280 S	–	–	+	
4 58 F	lSSc	Normal CR	1:640 N	–	–	–	RF,DNA
5 30 F	MCTD	Normal CR	1:640 S	U1RNP, Ro,	–	–	DNA
6 59 F	lSSc	Normal CR	1:1280 H, S	Ro	–	+	
7 57 M	lSSc	Moderate emphysema	1:1280 S, N	–	–	–	Hepatitis C
8 25 F	dSSc	Normal CR	1:2560 N	La	–	–	
9 55 F	lSSc	Interstitial	1:640 N	–	–	–	
10 60 F	lSSc	Abnormal V/Q scan	1:1260 S, N	–	–	–	LAC
11 52 F	dSSc	Unknown	NT		NT		
12 56 F	lSSc	Hyperinflation	1:1280 S	–	–	+	RF
13 76 F	dSSc	Periph nodules	1:1280 S	–	–	+	IgG aCL
14 67 M	lSSc	Normal CR	1:1280 S	–	–	+	RF
15 54 F	lSSc	Emphysema	1:2580 S	U1RNP	–	NT	RF
16 73 M	lSSc	Old granuloma	1:640 S	NT	NT	+	
17 61 M	lSSc	Reticular, nodular	+, no titer	–	–	–	
18 72 F	dSSc	Interstitial fibrosis	1:1260 S, N	–	–	–	
19 63 M	lSSc	Treated tuberculosis	1:640 S	–	–	+	
20 45 M	lSSc	Interstitial fibrosis	1:2560 S	–	–	–	
21 63 M	lSSc	Interstitial fibrosis		+	+	–	
22 44 F	lSSc	Normal CR	1:640 S	–	–	+	
23 59 F	lSSc	Normal CR	1:2560 S	–	–	+	RF
24 50 M	lSSc	Interstitial fibrosis	1:640 S	–	–	–	

Patient 6 had Crohn's disease; Patients 9 and 22 had hyper- or hypothyroidism; Patient 16 had cirrhosis of the liver. S: speckled, N: nucleolar, H: homogeneous, ENA: extractable nucleoprotein with Sm, Ro, La and U1ribonucleoprotein antigens. RF: rheumatoid factor, LAC: lupus anticoagulant, IgG aCL: IgG anticardiolipin antibody, NT: not tested. dSSc: diffuse cutaneous SSc. lSSc: limited cutaneous SSc.

and antinuclear antibody (ANA)/autoantibodies in the 24 patients. Their ages ranged from 25 to 73 years, the majority older than 50 years. Twenty-three had SSc defined by the criteria of the American Rheumatism Association[12]. Seventeen had lSSc (CREST syndrome variant)[13], 6 dSSc[13], and one MCTD[14]. Six patients with lSSc, one MCTD, and 2 dSSc had normal lung fields on chest radiograph, whereas the remaining 15 patients had abnormal lung findings on radiograph or CT. Two patients with lSSc had thyroid disease, one Crohn's ileitis, and one hepatitis C antibody without cirrhosis. One patient, with a positive lupus anticoagulant antibody, had chronic thromboembolic disease. None used anorexigens.

ANA were positive in all except for the patient whose blood was obtained off-site and not tested. When determined, the ANA pattern was speckled or nucleolar except for one homogeneous pattern. Eight of the 17 patients with lSSc had anticentromere antibodies and another lSSc patient with severe pulmonary fibrosis had anti-Scl70. Three dSSc patients with one or more antibodies to DNA, La, U1RNP, or Sm antigens could have been classified as MCTD or "overlap syndrome." Five had positive rheumatoid factor and one a positive anticardiolipin IgG antibody.

Healthy controls. None of the 96 North American controls or the 100 Israeli Ashkenazi Jews had PAH.

BMPR2 mutations. BMPR2 gene mutations were determined in the 24 patients, 96 North American controls, and in 100 Israeli Ashkenazi Jews as another control group, as described[8].

RESULTS

The single BMPR2 mutation found in the one patient with lSSc was considered a polymorphism because it was found in only one of 100 Ashkenazi Jews and in none of the 96 North American controls; furthermore this mutation has not been reported previously[8-11]. The patient was a 59-year-old Ashkenazi Jewish woman with lSSc, normal chest radiograph, and positive anticentromere and rheumatoid factor autoantibodies (Table 1, Patient 23). She had a guanine to adenine (G2948A) mutation in exon 13 predicted to result in an arginine to glutamine (R983Q) substitution. Exon 13 is in the carboxy terminal end of the intracellular part of BMPR2 and could be predicted to result in a dysfunctional receptor.

DISCUSSION

This is the first survey of the frequency and type of BMPR2 gene mutations in patients with PAH associated with the scleroderma spectrum of disease. The clinical and serological findings in the 24 patients with PAH and scleroderma spectrum of disease were heterogeneous and were similar to previous reports[1-3]. This study lacked lung biopsies, which would have helped to determine whether the PAH was due to pulmonary vasculopathy or was secondary to parenchymal lung disease, or both. However, both clinical subsets were represented, since at least 7 patients had normal chest findings and another 7 had findings compatible with pulmonary fibrosis.

The single polymorphism found here, in the ethnically similar single patient and control, contrasts with the 50% frequency found in familial PPH[8,9,11] and the 26% frequency

reported in sporadic PPH[10]. The method used to detect mutations, nucleic acid sequencing, could have missed large heterozygous deletions in BMPR2. Also, the BMPR2 promotor and introns were not rigorously evaluated, although all of the intron/exon boundaries of BMPR2 were screened. We did not screen the 125,000 base pairs (3,000,000 for all 24 patients) because the probability of finding disease predisposing mutations is low, while the chances of finding false positive differences is very high given the rate of DNA sequence variation in humans. Despite these technical limitations and the small sample size, it appears that other genes and/or mechanisms remain to be characterized in PAH associated with the scleroderma spectrum of disease. In addition, BMPR2 mutations need to be determined in larger numbers of patients and controls of Ashkenazi ethnicity to determine whether this polymorphism represents a risk factor for PAH.

ACKNOWLEDGMENT

The authors are indebted to Deborah Elstein, MD, Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel, for providing DNA control samples from the Ashkenazi Jews.

REFERENCES

1. Ungerer RG, Tashkin DP, Furst D, et al. Prevalence and clinical correlates of pulmonary arterial hypertension in progressive systemic sclerosis. Am J Med 1983;75:65-74.
2. Stupi AM, Steen VD, Owens GR, Barnes EL, Rodnan GP, Medsger TA. Pulmonary hypertension in the CREST syndrome variant of systemic sclerosis. Arthritis Rheum 1986;29:515-24.
3. Salneri R, Rodman GP, Leon DR, Shaver JA. Pulmonary hypertension in the CREST syndrome variant of progressive systemic sclerosis (scleroderma). Ann Intern Med 1977;86:394-9.
4. Young RH, Mark GJ. Pulmonary vascular changes in scleroderma. Am J Med 1978;64:998-1004.
5. Al-Sabbagh MR, Steen VD, Zee BC, et al. Pulmonary arterial histology and morphometry in systemic sclerosis: a case-control autopsy study. J Rheumatol 1989;16:1038-42.
6. Lee S-D, Shroyer KR, Markham NE, Cool CD, Voelkel NF, Tuder RM. Monoclonal endothelial cell proliferation is present in primary but not secondary pulmonary hypertension. J Clin Invest 1998;101:927-34.
7. Cool CD, Kennedy D, Voelkel NF, Tuder RM. Pathogenesis and evolution of plexiform lesions in pulmonary hypertension associated with scleroderma and human immunodeficiency virus. Hum Pathol 1997;28:434-42.
8. Deng Z, Morse JH, Slager SL, et al. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor II-gene. Am J Hum Genet 2000;67:737-44.
9. Lane KB, Machado RD, Pauciulo MW, et al. Heterozygous germline mutations in BMPR2, encoding a TGF-B receptor, cause familial primary pulmonary hypertension. The International PPH Consortium. Nat Genet 2000;26:81-4.
10. Thompson JR, Machado RD, Pauciulo MW, et al. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-B family. J Med Genet 2000;37:741-5.
11. Machado RD, Pauciulo MW, Thompson JR, et al. BMPR2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. Am J Hum Genet 2001; 68:92-102.
12. Masi AT, Rodnan GP, Medsger TA, et al. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee: Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581-90.
13. LeRoy EC, Black C, Fleischmajer R, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 1988;15:202-5.
14. Alarcon-Segovia D, Villareal M. Classification and diagnostic criteria for mixed connective tissue disease and anti-nuclear antibodies. Amsterdam: Excerpta Medica; 1987:33-40.