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Anti-U11/U12 RNP antibodies in systemic sclerosis (SSc): A new serologic marker associated with pulmonary fibrosis

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Abstract

Objective—Our goal was to characterize a new serum autoantibody in patients with systemic sclerosis (SSc) directed against U11/U12 ribonucleoprotein (RNP) and to identify the clinical features associated with this autoantibody.

Methods—We identified autoantibodies directed against the U11/U12 RNP complex in sera of patients with SSc and confirmed antibody specificity by immunoprecipitation, RT-PCR and southern blotting. We determined the prevalence of these antibodies in SSc and their specificity for SSc. We compared anti-U11/U12 RNP positive and negative SSc patients on demographic, disease classification, clinical variables, and survival.

Results—We identified 33 patients with anti-U11/U12 RNP antibodies. In two consecutive series of SSc patients first seen at 10-year intervals (1994–1995 and 2004–2005), the prevalence of anti-U11/U12 RNP antibody positive patients was 15/462 (3.2%). Seventeen (52%) of these 33 patients had limited cutaneous involvement. All had Raynaud phenomenon and 82% had gastrointestinal involvement. None had “intrinsic” pulmonary arterial hypertension. The most significant clinical difference between anti-U11/U12 antibody positive and negative cohorts was the prevalence of lung fibrosis, which occurred in 79% of the anti-U11/U12 RNP antibody positive patients versus 37% of the anti-U11/U12 RNP antibody negative patients ($p < 0.0001$). Gastrointestinal involvement was also significantly increased in the anti-U11/U12 RNP antibody positive group. Patients with anti-U11/U12 RNP antibodies and pulmonary fibrosis had a 2.25 greater risk of death than anti-U11/U12 RNP negative patients with pulmonary fibrosis.

Conclusion—Anti-U11/U12 RNP antibodies are present in sera of approximately 3% of patients with SSc and are a marker for lung fibrosis which is often severe.

Keywords

autoantibodies; scleroderma; SSc; ribonucleoprotein; lung fibrosis

INTRODUCTION

Systemic sclerosis (SSc) is a connective tissue disease of unknown etiology which commonly involves the skin and internal organs. A number of serum autoantibodies have been identified in SSc patients and they serve as biomarkers of clinical features. For

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example, anti-topoisomerase I (anti-Scl 70) (1), and anti-Th/To (2) autoantibodies both are associated with an increased risk of interstitial lung disease. Anti-RNA polymerase III autoantibodies are associated with "scleroderma renal crisis" and are infrequently present in patients with significant lung disease (3,4). Anti-centromere, anti-U3 ribonucleoprotein (RNP), and anti-Th/To antibodies are more frequently detected in sera of patients with "intrinsic" pulmonary arterial hypertension (2). Anti-U3RNP antibody also is associated with scleroderma heart disease (5).

Serum autoantibodies to small nuclear RNPs have been found in patients with SSc and other connective tissue diseases. Most of these antibodies are directed against the protein component of the complex. Some antibodies recognize individual RNPs such as anti-U1 RNP or anti-U3 RNP, while others are directed against a complex of RNPs, such as anti-Sm autoantibodies, which target the uridine (U) rich complexes of U1, U2, U5, and U4/U6 RNP (6). Of the anti-RNP antibodies, anti-U1 and anti-U3 are the most frequent in SSc patients, while anti-U5 and anti-U4/U6 are rare. Anti-U4/U6 autoantibodies were initially reported in the serum of a patient with SSc (7) and subsequently in a Japanese patient with primary Sjögren syndrome (8). U4 and U6 RNAs have been shown to co-exist in a single small ribonucleoprotein particle (9), which explains their co-immunoprecipitation with antisera from patients with SSc (7). Anti-U5 RNP antibodies were identified in the serum of one Pittsburgh patient with SSc and polymyositis in overlap (10) and later in a Japanese patient with a similar overlap syndrome and large cell carcinoma of the lung (11).

U11/U12 RNPs are found in low abundance in eukaryotic cells, are components of the spliceosome, and catalyze pre-messenger RNA (mRNA) splicing of nuclear pre-mRNA introns (12). Gilliam and Steitz previously reported the presence of anti-U11/U12 RNP antibodies in one patient with diffuse cutaneous SSc (13), but this antibody may not have been specific to U11/U12 RNP since it also recognized the 5' 2,2,7-trimethyl guanosine (TMG) cap of small nuclear (sn)RNAs. Except for U6 RNA, all other U series RNAs have a unique 5' TMG cap which targets them to the nucleus (13,14). Antibodies to the TMG cap have also been reported in patients with SSc (15). However, clinical features associated with anti-U11/U12 RNP antibodies have not been examined to date.

We have identified and characterized anti-U11/U12 RNP autoantibodies in 33 patients with systemic sclerosis and have described their clinical features and disease course in comparison with SSc patients without these antibodies.

MATERIALS and METHODS

Patient samples

Serum samples were obtained with informed consent from patients seen by physicians in the Division of Rheumatology and Clinical Immunology at the University of Pittsburgh School of Medicine and stored at −80°C. All patients had a physician-confirmed diagnosis of SSc between 1982 and 2005. To determine the prevalence of anti-U11/U12 RNP antibodies, consecutive patients first evaluated during 1994–1995 and 2004–2005 (a total of 4 calendar years) with serum samples available were tested for U11/U12 RNP antibodies. For demographic, clinical features and survival comparisons, the 1982–2004 cohort of anti-U11/U12 RNP positive patients was compared with the 1994–1995 patients who had no detectable U11/U12 RNP antibodies. This earlier cohort was chosen because follow-up was available for a longer period of time (mean of 5.3 years after first visit). The rationale for using 4 years of consecutive patients as a comparison group is that these patients had all 8 other SSc-associated serum autoantibodies determined.

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Clinical information

Clinical and laboratory information obtained on first and follow-up visits on all SSc patients was prospectively collected using standardized data collection forms. The definitions for organ system involvement attributable to SSc used in this study have been published previously (2). Organ system involvement was considered present if it occurred at any time during the illness, including the follow-up period after first evaluation. Diffuse cutaneous involvement was defined as skin thickening proximal to the elbows or knees (upper arms, thighs, chest or abdomen) at any time during the illness (16). Pulmonary fibrosis was defined as interstitial fibrosis or ground glass changes on chest radiograph or HRCT scan of the lungs, respectively. Pulmonary arterial hypertension (PAH) was defined as “intrinsic” pulmonary arterial hypertension, not secondary to lung fibrosis or heart disease and required a mean PA pressure >25 mm Hg (right heart catheterization) or estimated PA systolic pressure >40 mm Hg (echocardiogram) (17). Gastrointestinal involvement was defined as one of: distal esophageal hypomotility by cine esophagram or manometry, esophageal stricture by endoscopy, colon wide-mouth succulation on x-ray, duodenal dysmotility or hypomotility or small bowel abnormality on x-ray, malabsorption syndrome, administration of antibiotics for small bowel bacterial overgrowth, or gastrointestinal scleroderma as the cause of death.

Reagents

K562 and HeLa cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin and streptomycin (Invitrogen, Carlsbad, CA). Mouse monoclonal anti-5’ 2,2,7-trimethyl guanosine cap (TMG) antibodies were purchased from Oncogene Science (Manhasset, NY).

Immunoprecipitation of proteins

Protein immunoprecipitation was done as previously described (10,18). Briefly, K562 or HeLa cells were harvested, washed with 1x PBS, and resuspended in lysis buffer (500 mM NaCl, 10 mM Tris-HCl, 0.1% Nonidet P-40, pH 8.0). Cells were sonicated on ice and cellular debris pelleted following centrifugation at 12,000 rpm for 20 min. Supernatants were used as antigen source. Serum (10 μl) was incubated with 50 μl protein A agarose (Invitrogen, Carlsbad, CA) for 16 hrs at 4°C. The antibody-bound beads were washed three times in lysis buffer and incubated with cellular extracts for 2 hrs at 4°C. Agarose-bound complexes were washed three times, resuspended in Laemmli buffer, and fractionated by SDS-PAGE.

Immunoprecipitation of ribonucleoprotein complexes

For immunoprecipitation of ribonucleoprotein complexes, unlabelled K562 or HeLa cells were harvested and sonicated in NET-2 buffer (140 mM NaCl, 50 mM Tris-HCl, 0.05% Nonidet P-40, pH 7.4) as previously described (15). Cellular debris was removed following centrifugation and cellular lysates incubated with antibody-bound protein A agarose as described above. Ribonucleoprotein complexes were extracted with phenol:chloroform:isoamyl alcohol (PCA) (50:50:1), precipitated with ethanol and separated by electrophoresis on 7M urea polyacrylamide gels. The snRNAs were visualized following silver staining.

Antibody crosslinking and western blot analysis

Human serum immunoglobulins were crosslinked to protein A agarose beads as previously described (18). Agarose-bound immunoglobulins were incubated with sonicated K562 or HeLa cell extracts for 2 hours at 4°C. The complex-bound beads were washed and samples were resolved by SDS-PAGE. Membranes were incubated with control or patient sera at a

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dilution of 1:250 followed by a secondary horseradish peroxidase-conjugated anti-human IgG antibody (Pierce Biotechnology, Rockford, IL). Signals were detected following chemiluminescence and autoradiography.

Identification of U11 and U12 snRNA

RT-PCR—For reverse transcription of U11 and U12 snRNA, K562 proteins were immunoprecipitated with patient sera as described above. The RNA was reverse transcribed using Superscript II (Invitrogen, Carlsbad, CA) and the following primers: 5'- aagggcgccgggaccaa –3' for U11 or 5'- ggcagatcgcaactcccagg –3' for U12 snRNA. cDNAs were amplified using the primers used for reverse transcription in combination with 5'- ggcttctgtcgtgagtgg –3' for U11 or 5'- taacgattcggggtgacgcc –3' for U12. Amplification conditions were 20 cycles of 95°C for 1 min, 68°C for 1 min and 72°C for 1 min. DNA products were separated by electrophoresis on 12% non-denaturing acrylamide gels. The expected cDNA products were 127 bp and 116 bp for U11 and U12, respectively. The DNA products were cloned into the pGEM-T vector (Promega, Madison, WI) and sequences of cloned cDNAs were confirmed using an ABI Prism 377 automated sequencer (Perkin-Elmer, Norwalk, CT). These cDNAs were used as probes in Southern blot analysis described below.

Southern blot analysis—cDNA obtained above by RT-PCR was resolved on a 1.5% agarose gel. The DNA was denatured and transferred to a supported nitrocellulose membrane. The membrane was hybridized to [^{32}P]-labeled U11 or U12 cDNA. Signals were visualized following autoradiography.

Statistical analysis

Baseline characteristics were compared by Student t-test for continuous data, and chi-square, or Fisher's exact test where appropriate, for discrete data. All statistical analyses were two-tailed and results were considered significant if p values were less than 0.05. Survival was evaluated with the Kaplan-Meier method. Mortality risks in patients with pulmonary fibrosis were estimated using Cox proportional hazards modeling techniques.

RESULTS

Identification of anti-U11/U12 RNP autoantibodies in a patient with SSc

Immunoprecipitation of ribonucleoprotein complexes in the course of an ongoing analysis of autoantibodies in SSc revealed the presence of a snRNA of comparable electrophoretic mobility to U11 snRNA in one patient (Figure 1A).

It has been shown previously that U11 and U12 snRNAs have a TMG cap, similar to other TMG-capped snRNAs. To confirm the presence of the TMG cap and identify proteins complexed with TMG-capped snRNAs, cellular proteins were immunoprecipitated using resin-crosslinked monoclonal anti-TMG antibodies. Proteins were eluted from the resin, separated on SDS-PAGE and analyzed by western blot analysis using normal serum, anti-Sm, anti-U1 RNP, anti-U11/U12 RNP, or anti-TMG positive sera. Anti-U11/U12 RNP autoantibodies recognized a 65–68 kD protein band precipitated by anti-TMG antibody (Figure 1B). A protein of similar size had been previously shown to be associated with U11 snRNA in eukaryotic cells (12,13).

Confirmation of U11/U12 immunoprecipitation

Sera from a healthy donor, patients with anti-U11/U12 RNP, anti-Sm, anti-TMG, and anti-U3 RNP antibodies, and a purified monoclonal anti-TMG antibody were used to

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immunoprecipitate their corresponding antigens. Immunoprecipitated small nuclear RNAs were reverse transcribed using a U11-specific 3' primer or a U12-specific 3' primer followed by amplification using U11 or U12 forward and reverse primers, respectively. Amplified cDNAs were resolved on a 12% acrylamide gel. Figure 2A shows a cDNA corresponding to the expected size of U11 (127 bp) or U12 (116 bp) amplified from RNP precipitated with sera positive for anti-U11/U12 RNP, anti-Sm, or anti-TMG antibodies and with monoclonal anti-TMG antibody but not sera from a healthy ANA negative donor and a patient positive for anti-U3 RNP antibodies.

To further confirm the identity of the bands, amplified cDNAs were cloned and sequenced. The nucleotide sequences of the cDNAs completely matched the U11 and U12 cDNA sequences in GenBank. Sequenced cDNAs were further used as radiolabeled probes in a southern blot assay to confirm the presence of U11 and U12 cDNA in the RT-PCR reactions. As shown in Figure 2B, southern blot analysis using a U11 or U12 radiolabeled cDNA probe confirmed the presence of U11 and U12 cDNA in samples precipitated by anti-U11/U12 RNP, anti-Sm, and anti-TMG, and amplified by PCR, whereas no U11 or U12 cDNA was detected in samples precipitated with healthy donor serum or anti-U3 RNP positive serum.

Sera from patients with SSc immunoprecipitate U11/U12 RNP complex

Comparison of sera from other SSc patients to the index serum revealed that 32 additional SSc patients were positive for anti-U11/U12 RNP autoantibodies. Figure 3 shows immunoprecipitation results from representative patients whose sera were positive for anti-U11/U12 RNP antibodies. U11/U12 snRNAs precipitated by SSc patient sera were compared to those precipitated by standard sera corresponding to U1, U2, U3, U4/U6, and U5 RNP, Sm, and TMG. Antibody reactivity to U11/U12 RNP complexes was also detected in serial serum samples from several patients (patients 1–3).

Prevalence and specificity of anti-U11/U12 RNP in SSc

The prevalence of U11/U12 RNP antibodies was calculated in two consecutive two-year periods. During 1994–1995, 4 of 244 consecutive new SSc patients (1.6%) with serum available for testing had anti-U11/U12 RNP antibodies and no other SSc-associated serum autoantibody. During 2004–2005, 11 of 218 consecutive new SSc patients (5.0%) had anti-U11/U12 RNP antibodies. Thus the overall prevalence of anti-U11/U12 RNP antibodies in these two time periods combined was 3.2%. The presence of anti-U11/U12 antibodies was not associated with the presence of any of the other SSc-associated antibodies. Although low levels of anti-U1 and U2 RNP antibodies were detected in some anti-U11/U12 RNP positive sera by immunoprecipitation (Figure 3), less sensitive assays such as double immunodiffusion failed to detect the presence of these antibodies. Anti-U11/U12 RNP antibodies were specific to SSc patients and were not detected in sera of 272 patients with polymyositis, dermatoymyositis, rheumatoid arthritis, systemic lupus erythematosus, or connective tissue diseases in overlap. Anti-U11/U12 RNP antibodies were also not detected in 24 patients with idiopathic pulmonary fibrosis.

Characteristics of anti-U11/U12 RNP positive patients

The 15 anti-U11/U12 RNP positive patients in the above 4 years were combined with 18 anti-U11/U12 RNP positive patients identified from other years during 1982–2004. Table 1 shows the demographic and clinical characteristics of the 33 anti-U11/U12 RNP antibody positive SSc patients compared with the 240 consecutive U11/U12 RNP negative SSc patients first evaluated in 1994–1995. There were no age or sex differences between the groups. A somewhat lower proportion of anti-U11/U12 RNP positive patients were Caucasian (79% vs. 90%, NS). Twenty-eight (85%) of the anti-U11/U12 RNP antibody

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positive patients and 206 (86%) of the anti-U11/U12 RNP antibody negative patients fulfilled the American College of Rheumatology (formerly American Rheumatism Association) preliminary criteria for classification as definite SSc (16). Half of the anti-U11 RNP positive patients had limited cutaneous involvement (52%). The mean maximum total skin thickness score (TSS) using the modified Rodnan skin scoring method (mRSS) (19) in anti-U11/U12 RNP patients was 4.6 for patients with limited cutaneous disease (lcSSc), and 30.2 for patients with diffuse cutaneous SSc (dcSSc). The comparison group had similar mean maximum skin thickness scores.

Clinical features of anti-U11/U12 RNP antibody positive patients

All 33 anti-U11/U12 RNP positive patients had Raynaud phenomenon. Gastrointestinal (GI) tract involvement was equally frequent in both groups. Two of the anti-U11/U12 RNP positive patients had skeletal muscle involvement, two had renal crisis, and none had "intrinsic" (i.e. without associated pulmonary fibrosis) pulmonary arterial hypertension. All anti-U11/U12 RNP antibody positive patients tested had gastrointestinal involvement compared with 75% of anti-U11/U12 RNP negative patients ($p = 0.0110$).

Most patients in the anti-U11/U12 RNP antibody positive group (29/33) and the 1994–1995 comparison group (202/240) were evaluated for pulmonary fibrosis by radiography or high resolution CT scanning. The vast majority (>80%) of patients in both groups had only routine chest radiographs performed as HRCT was more commonly available only after 1995. Pulmonary fibrosis was significantly more frequent in anti-U11/U12 RNP positive vs. negative patients (23/29 or 79% versus 74/202 or 37%, $p < 0.0001$). The proportion of U11/U12 RNP antibody positive patients with PF who developed dyspnea related to PF during the first four years of illness (83%) was significantly greater that the proportion of U11/U12 RNP negative patients (55%) ($p = 0.0205$). Pulmonary fibrosis was equally distributed among patients with diffuse (90%) and limited cutaneous (81%) involvement. There was no difference in the proportion of anti-U11/U12 RNP positive and anti-U11/U12 RNP negative patients with pulmonary fibrosis whose dyspnea on exertion began prior to the first physician diagnosis of SSc (52% and 41%, respectively). There was also no significant difference in the lowest FVC% predicted between the two groups. Neither was there any difference in the proportion of patients in the two groups who were treated with corticosteroids and/or immunosuppressive drugs for their SSc or lung disease.

Table 2 shows the frequency of radiographic pulmonary fibrosis at any time during the course of the disease in all patients with SSc followed at the University of Pittsburgh from 1982 –2004, according to the presence of different SSc-associated serum autoantibodies. Patients with anti-U11/U12 RNP autoantibodies have the highest frequency of pulmonary fibrosis among all patient groups.

Survival in anti-U11/U12 RNP antibody positive and negative patients

Ten-year cumulative survival rate was assessed from first symptom attributable to scleroderma in all anti-U11/U12 RNP antibody patients compared to 240 consecutive anti-U11/U12 RNP negative patients seen between 1994–1995. There was no significant difference in 10-year survival rate between the U11/U12 RNP-positive patients (66%) and the U11/U12 RNP-negative (73%) patients (log-rank $p = 0.69$).

Mortality in anti-U11/U12 RNP antibody positive patients

We next compared the proportion of patients with pulmonary fibrosis who died of this disease complication using as denominator all patients with known causes of death. The highest mortality from pulmonary fibrosis was in anti-U11/U12 RNP positive patients. Eleven (38%) of the anti-U11/U12 RNP antibody patients died during follow-up (mean

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follow-up 6.9 years). All 11 deaths were attributed to SSc lung disease (9 patients) or its complications (1 lung cancer, 1 pneumonia). Mortality from pulmonary fibrosis or its complications in the anti-U11/U12 RNP positive patients (11/23 or 48%) was significantly higher than that in patients with all other autoantibodies combined (68/415, or 16%; $p < 0.0001$). Death due to pulmonary fibrosis in the anti-U11/U12 RNP positive patients also was significantly more frequent when compared to the other two major lung fibrosis-associated autoantibodies, anti-topoisomerase I and anti-Th/To, combined ($p = 0.0002$). Among the causes of death in the U11/U12 negative comparison group, “primary” PAH was the most frequent, but other causes were represented including renal, cardiac and gastrointestinal scleroderma, and non-scleroderma causes such as cancer and infections.

Survival of patients with pulmonary fibrosis

We then examined survival in anti-U11/U12 RNP antibody positive patients with pulmonary fibrosis (n=23) and all other anti-U11/U12 RNP antibody negative SSc patients in our series with pulmonary fibrosis (n=74). Survival was 39% among anti-U11/12 RNP positive patients with a median follow-up of 6.9 years, and 34% among anti-U11/U12 RNP negative patients with a longer median follow-up of 9.7 years ($p = 0.47$). However, after adjusting for age at symptom onset, gender and tobacco use, the presence of anti-U11/12 RNP antibodies among SSc patients with pulmonary fibrosis was associated with a 2.25 greater risk of death or lung transplant compared to anti-U11/U12 RNP antibody negative individuals at any point in time (Table 3).

DISCUSSION

We have identified and characterized anti-U11/U12 RNP autoantibodies in a group of patients with systemic sclerosis. To determine the prevalence of this antibody in SSc, we evaluated two series of consecutive SSc patients first evaluated during 1994–1995 and 2004–2005 (4 total years). Fifteen (3.2%) of these 462 SSc patients had anti-U11/U12 autoantibodies and no other SSc-associated autoantibodies. As a comparison, the prevalence of these antibodies in a pediatric cohort of patients with systemic sclerosis was 7% (unpublished observations). These antibodies are specific to SSc.

Pulmonary fibrosis (PF) is currently a leading cause of death in patients with SSc. In over 2000 of our Pittsburgh systemic sclerosis patients, PF accounted for 44% of the SSc-associated deaths (4). The presence of anti-U11/U12 RNP antibodies was highly associated with pulmonary fibrosis (79%). In our experience, two other SSc-associated antibodies are predictive of pulmonary fibrosis. PF was present in 48% of 87 SSc patients with anti-Th/To antibodies and limited cutaneous involvement and was found in 48% of anti-topoisomerase I antibody positive SSc patients with diffuse cutaneous involvement (1,2). Furthermore, we have shown that SSc patients positive for anti-U11/U12 RNP antibodies are at a higher risk of death from pulmonary fibrosis-related causes than patients with any of the other eight SSc-associated autoantibodies. Thus, screening for anti-U11/U12 RNP antibodies can serve as a marker for severe pulmonary fibrosis in SSc.

In published series of SSc patients, up to 90% have one of 8 serum autoantibodies, including anti-RNA polymerase III, anti-topoisomerase I, anti-centromere, anti-Th/To, anti-PM-Scl, anti-U1 RNP, anti-U3 RNP, and anti-Ku (21). Anti-U11/U12 RNP antibody is detected in approximately 3% of SSc patients and is thus a ninth SSc-associated serum autoantibody, which raises the proportion of all SSc patients with one of these nine autoantibodies to nearly 95%.

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In conclusion, we have identified and characterized a novel autoantibody targeted against U11/U12 RNP in SSc patients with a high frequency of pulmonary fibrosis, which is often severe and fatal.

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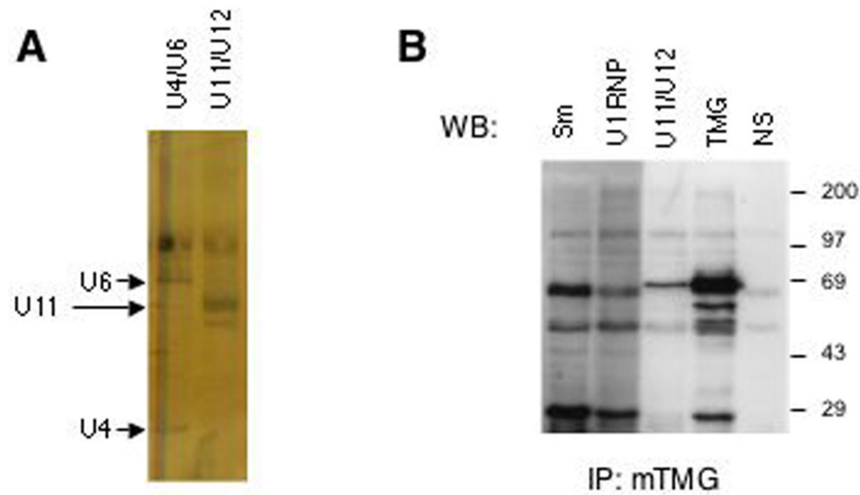

Figure 1.

Immunoprecipitation of U11/U12 snRNP complex. (A) K562 cells extracts were used for immunoprecipitation assays using sera of SSc patients. (B) Western blot analysis of proteins precipitated by a monoclonal anti-TMG antibody and probed with human serum. A monoclonal anti-TMG antibody (mTMG) was used to precipitate ribonucleoprotein complexes containing TMG-capped U RNAs. Precipitated samples were analyzed by western blot using sera from a healthy individual (NS) or from patients positive for anti-Sm (Sm), anti-U1 RNP, anti-U11/U12 RNP, or anti-TMG antibodies. A protein of approximately 65 kD is shared by anti-U11/U12 RNP and anti-TMG complexes.

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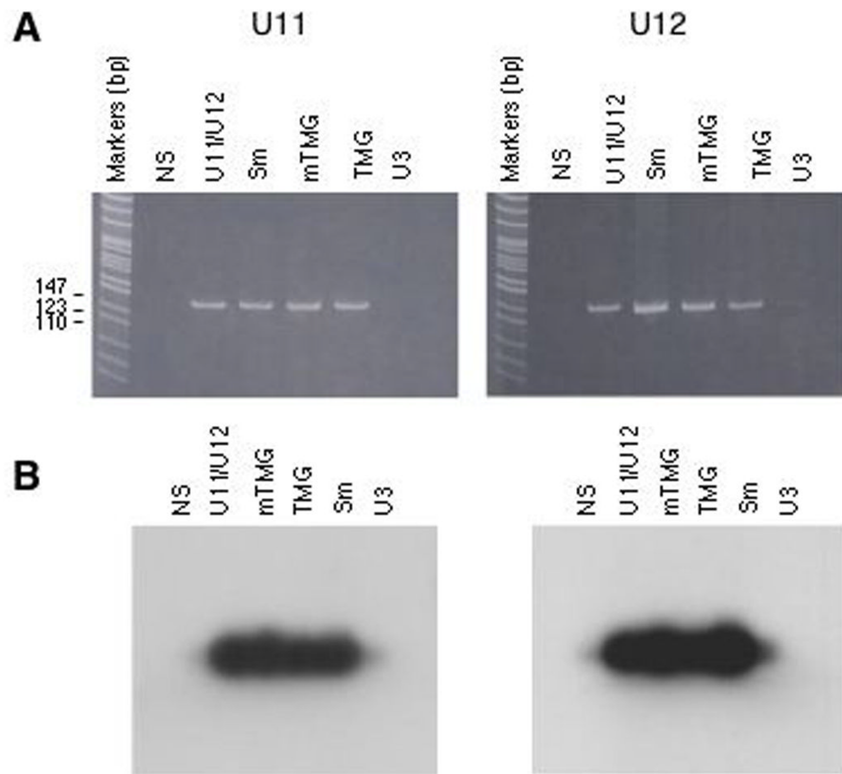

Figure 2.

Molecular confirmation of U11 and U12 snRNA. (A) Sera from a healthy donor (NS) or patients positive for anti-U11/U12 RNP (U11/U12), anti-Sm (Sm), anti-TMG (TMG), anti-U3 RNP (U3) antibodies, or a monoclonal anti-TMG antibody (mTMG) were used in immunoprecipitation reactions. Immunoprecipitated complexes were used in RT-PCR with U11 (left panel) or U12 (right panel) specific primers. Amplified products were visualized by electrophoresis on polyacrylamide gels and ethidium product staining. (B) An aliquot of the amplified DNA was electrophoresed on a 2% agarose gel and used in southern blotting with a radiolabeled cDNA probe corresponding to U11 (left panel) or U12 (right panel). Anti-U11/U12 patient sera precipitate the U11/U12 RNP complex containing the corresponding U RNA. U11 and U12 RNAs can be reverse transcribed and amplified using the designed primers.

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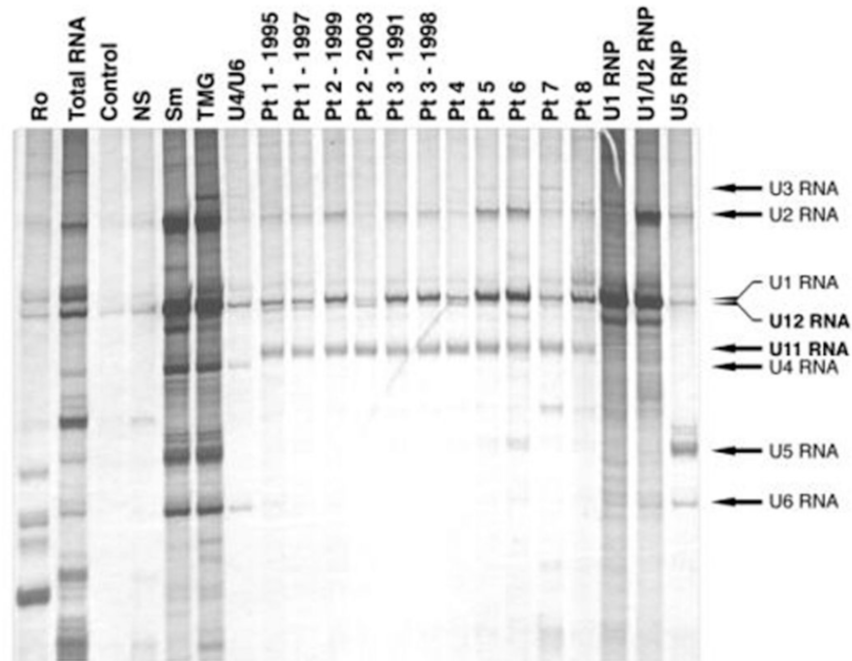

Figure 3.

Identification of anti-U11/U12 RNP antibodies in additional SSc patients. Ribonucleoprotein complexes were immunoprecipitated from K562 cell extracts. Sera from a healthy donor (normal serum; NS), or patient sera positive for anti-Ro, anti-Sm (Sm), anti-TMG (TMG), anti-U4/U6 RNP (U4/U6), anti-U11/U12 RNP (Pt 1–8), anti-U1 RNP, anti-U1 and U2 RNP (U1/U2), or anti-U5 RNP antibodies are shown. Serial serum samples from patients 1–3 were obtained at the indicated years. A marker of all U RNAs is shown (Total RNA). The negative control (Control) represents protein agarose beads incubated with extracts in the absence of human serum. Immunoglobulins in sera of patients 1–8 precipitate U RNAs of similar size to U11 and U12 RNAs.

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TABLE 1

Characteristics of 33 systemic sclerosis (SSc) patients with anti-U11/U12 RNP antibody and 240 consecutive SSc patients without this antibody who were first evaluated during 1994–1995.

	Anti-U11/U12 RNP antibody positive patients (n = 33)	**Anti-U11/U12 RNP antibody negative patients (n = 240)**	**P value**
Demographic Features			
Age at SSc onset (mean years)	41.1 ± 16.9	43.2 ± 15.8	p = 0.4760
Caucasian	26 (79%)	215 (90%)	p = 0.1288
Female	24 (73%)	186 (78%)	p = 0.6967
Disease Classification			
Diffuse cutaneous (dcSSc)	16 (48%)	121 (50%)	p = 0.9821
Limited cutaneous (lcSSc)	17 (52%)	117 (49%)	p = 0.9106
Organ System Involvement			
Peripheral Vascular	33 (100%)	234 (98%)	p = 0.7754
Skin: mean maximum TSS	17.0 ± 15.2	15.5 ± 13.7	p = 0.5660
dcSSc	30.2 ± 10.7	26.0 ± 12.0	p = 0.1830
lcSSc	4.6 ± 3.9	4.9 ± 3.1	p = 0.6940
Joints/Tendons	26 (79%)	177 (74%)	p = 0.6827
Skeletal Muscle	2 (6%)	37 (15%)	p = 0.2401
Gastrointestinal Tract	26/26* (100%)	120/161 (75%)	p = 0.0110
Pulmonary Fibrosis	23/29 (79%)	74/202 (37%)	p = 0.0001
Pulmonary Hypertension	0 (0%)	27 (11%)	p = 0.0946
Heart	6/25 (24%)	48/175 (27%)	p = 0.7180
Kidney (renal crisis)	2 (6%)	27 (11%)	p = 0.5446

*denominator = number of patients studied

TSS = Total Skin Score (20)

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TABLE 2

Frequency of pulmonary fibrosis (PF) in systemic sclerosis (SSc) patients with different SSc-associated autoantibodies, from the University of Pittsburgh Scleroderma Databank (1982–2004)*, compared with SSc patients in this study.

SSc-associated autoantibody	Frequency of PF	Significance vs. U11/U12
U11/U12 RNP	23/33† (70%)	
Centromere	59/436 (14%)	$p < 0.0001$
Ku	6/14 (43%)	NS
PM-Scl	27/65 (42%)	$p < 0.009$
RNA-Polymerase III	63/343 (18%)	$p < 0.0001$
Th/To	47/121 (39%)	$p < 0.002$
Topoisomerase I	204/425 (48%)	$p < 0.02$
U1 RNP	36/123 (29%)	$p < 0.0001$
U3 RNP	16/84 (19%)	$p < 0.0001$

*Overlap patients excluded; no patient had more than one SSc-associated autoantibody

†Denominator = total number of antibody positive patients

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TABLE 3

Effect of anti-U11/U12 RNP positive status on mortality, after adjustment of common risk factors.

	Variable Estimate ± Standard Error	Hazard Ratio	95% CI	p-value
Age at Symptom Onset	0.06 ± 0.01	1.06	1.04–1.09	<.0001
Male	0.26 ± 0.29	1.30	0.73–2.31	0.36
Tobacco Use	−0.63 ± 0.48	0.53	0.21–1.36	0.18
Anti-U11/U12 Positive	0.81 ± 0.32	2.25	1.20–4.24	0.01