PAHの治療薬は，経口薬としてベラプロスト，ボセンタン，アンブリセンタン，シルデナフィル，タダラフィルであり，それぞれ膠原病に合併したPAHに対する有効性が報告されている．静注薬としては，エポプロステノールが我が国でも10年以上前から使用可能となっている．

5) 腎病変

腎障害の発症は，日本人SSc患者の10％以下に認められる低頻度の合併症であるが，生命予後に関与するとされている重要な症状である．1952年に，MooreとSheehanが高血圧を伴って，病理学的には，免疫複合体の沈着および好中球浸潤に伴う血管炎の所見は認められず，血管内皮細胞，血管平滑筋細胞，線維芽細胞の増殖を伴う内膜の肥厚が認められる腎障害を報告した．また，高レニン，アンジオテンシンII血症を呈していることが報告されており，アンジオテンシンIIの細胞増殖作用および血管収縮作用が生じ，血管内腔の狭小化が進行すると考えられている．治療としては，アンジオテンシン変換酵素阻害薬(ACEI)の投与が行われるようになり，著明な予後の改善がみられるようになった．

6) 消化管病変

SSc患者の90％以上に消化管病変を有していると考えられている．病変の部位としては，90％以上が食道である．口腔内乾燥症状を呈し，舌小帯の短縮/肥厚が生じる．食道病変の病因としては，食道下部の平滑筋層に生じる線維化に伴い，逆流性食道炎(gastroesophageal reflux disease: GERD)が惹起されることによる．診断には，バリウムによる食道造影が行われる．小腸の蠕動運動低下に伴う吐き気，腹部膨満感の症状を呈することは約半数の患者にて認められる．また，腸内細菌の異常増殖が生じ，吸収不良症候群を呈する患者も認められる．まれな症状として，腸内ガスの腸壁内または腹腔内への流入が引き起こされ，腸壁嚢胞状気腫(pneumatosis cystoides intestinalis: PCI)を引き起こす．

治療としては，GERDに対し，胃酸の逆流を防ぐ目的で，H2ブロッカー，プロトンポンプインヒビター，シサプリドの投与を行う．難治性と考えられていたが，近年，プロトンポンプインヒビターの投与により予後が著明に改善している．

7) 神経病変

SScにおいて，神経障害は頻度の低い合併症と考えていた．1970年代までの臨床研究では，SScに末梢神経障害も中枢神経障害もほとんどみられないという報告であった．しかしながら，現在では，種々の報告があり，脳神経障害，中枢神経障害，末梢神経障害，交感神経障害がそれぞれ合併することが知られている．

脳神経障害で最も頻度が高いのは，三叉神経障害である．ピッツバーグ大学からの報告では，3–10％程度にみられ，筋炎の合併が多いとされた．これらの三叉神経障害には，顔面神経などの運動神経障害は合併しない．一方，頻度は更に低いが，単独で顔面神経麻痺を合併するSScが報告されている．原因は不明であり，副腎皮質ステロイド薬の有効性に乏しいとされている[14]．

中枢神経障害は，三叉神経障害より更に頻度は少ない．痙攣，脳梗塞，脳出血などの症状が合併することはあるが，高血圧症や高脂血症がある症例，高齢者であることが多く，SScの病態と中枢神経障害が関連しているとする報告はほとんどみられない[14]．

末梢神経障害は，手指あるいは下腿の感覚低下および神経伝導速度の低下の所見が多くみられる．しかしながら，これらの頻度は，SScの10％以下であり，高頻度ではない．線維化に起因する腱鞘の肥厚は，高頻度にみられる．その結果として，手根管症候群がみられる．diffuse cutaneous SScでは，高頻度に手関節部の腱摩擦音が認められるが，そのような症例では，しばしば手根管症候群がみられる[15]．

交感神経障害は，SScの病態に高頻度にかかわっている可能性がある．SScの90％以上に認められるレイノー現象は，異常な血管の収縮と拡張により生じる．これらの血管収縮異常に交感神経が関連している可能性はある．また，腸管の蠕動運動の低下は，食道から大腸，肛門までに認められる．これらの腸管蠕動運動にも交

感神経の関与は否定できない．また，不整脈は10–20％のSScで認められる．これらの病態にも交感神経障害が関与している．

レイノー現象あるいは腸管病変の項で前述したように，SScでは，線維化と血管傷害が一元的に生じていて，それらの病態に交感神経障害がどの程度関与しているかは，いまだに不明である．種々の研究報告では，頻度は，14.3–79％と様々である[14]．

神経障害は，SScにおいて，病態形成を考えるうえで，最初の異常ではないと考えている．線維化と血管傷害が生じてくる段階で，三叉神経障害，手根管症候群，交感神経・副交感神経障害が惹起されるのだと考えている．

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トピックス

Ⅳ．関節リウマチ以外の膠原病，話題の疾患

6．混合性結合組織病

川口　鎮司

要　旨

混合性結合組織病（mixed connective tissue disease：MCTD）は，1972年に米国のGC Sharpらが提唱した疾患である．高力価を示す抗核抗体（speckle型）および抗U1-RNP抗体陽性で，全身性エリテマトーデス（systemic lupus erythematosus：SLE），全身性強皮症（systemic sclerosis，scleroderma：SSc），多発性筋炎（polymyositis：PM）の3疾患のうち，少なくとも2疾患の臨床症状を呈する疾患をMCTDと定義した．一方，2つ以上の膠原病を合併する重複症候群という概念があり，MCTDは抗U1-RNP抗体陽性の重複症候群と考えることもできる．

〔日内会誌　103：2501～2506，2014〕

Key words　抗U1-RNP抗体，MCTD，SLE，強皮症，多発性筋炎

はじめに

混合性結合組織病（mixed connective tissue disease：MCTD）は，全身性エリテマトーデス（systemic lupus erythematosus：SLE），全身性強皮症（systemic sclerosis，scleroderma：SSc），多発性筋炎（polymyositis：PM）の3疾患が混合した臨床像を呈し，血清学的に抗U1-RNP抗体を高力価で発現する疾患である．MCTDが，Sharpらにより提唱されて[1]10年後の1982年に厚生省の指導のもとMCTD研究班が発足した．興味深いことにSharpの米国よりも，本邦では膠原病の中で抗U1-RNP抗体陽性の頻度が高いことがわかってきた．これは，白人と我々日本人の遺伝子の差による可能性がある．そのため，米国よりも本邦においてより重要な疾患と考えられるようになった．1988年に粕川により，MCTD診断の手引きと治療指針が発表された[2]．1996年に東條により診断の改訂がなされ，その後，2004年に近藤により，診断基準が改定され発表された．この基準の画期的な内容は，共通所見として肺高血圧症（pulmonary hypertension：PH）が組み入れられたことである．MCTDでは，10～15％程度でPHを合併する．PHは種々の膠原病に合併することが知られているが，稀な合併症と考えられている．その中で，MCTDが最も高頻度にPHを合併することがわかってきた．PHは生命予後に関わる重要な病態であることより，MCTDでは自覚症状のないときからPHのス

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トピックス

表 1. 混合性結合組織病（mixed connective tissue disease：MCTD）診断基準（2004 年改訂版）

混合性結合組織病の概念：
全身性エリテマトーデス，強皮症，多発性筋炎などにみられる症状や所見が混在し，血清中に抗U1-RNP抗体がみられる疾患である

Ⅰ．共通所見
1．Raynaud現象
2．指ないし手背の腫脹
3．肺高血圧症

Ⅱ．免疫学的所見
抗U1-RNP抗体陽性

Ⅲ．混合所見
A．全身性エリテマトーデス様所見
1．多発関節炎
2．リンパ節腫脹
3．顔面紅斑
4．心膜炎または胸膜炎
5．白血球減少または血小板減少
B．強皮症様所見
1．手指に限局した皮膚硬化
2．肺線維症，肺拘束性換気障害または拡散能低下
3．食道低下または拡張
C．多発性筋炎様所見
1．筋力低下
2．筋原性酵素上昇
3．筋電図における筋原性異常所見

診断：
1．Ⅰの 1 所見以上が陽性
2．Ⅱの所見が陽性
3．ⅢのA，B，C項のうち，2 項目以上につき，それぞれ 1 所見以上が陽性以上
上記の 3 項目を満たす場合を混合性結合組織病と診断する

クリーニングを行っていく必要がある．

MCTDは稀な疾患と考えられているが，2012 年の厚生労働省から発表された患者統計では 10,146 名の症例が登録されていた．つまり，本邦でのMCTDの頻度は，10 万人に 8.1 人である．SLEは，約 6 万人，SSc，PMではそれぞれ約 2 万人が登録されており，これらの疾患と比較して，MCTDは希少疾患といえる．性差があり，男女比は，本邦での 1992 年の調査では，1：13.4 と圧倒的に女性に多い疾患となっている．

1．診断基準

現在，本邦では 2004 年に改訂された厚生労働省の研究班が提唱した診断基準を用いている（表 1）．この基準では，Raynaud現象，あるいは指または手背の腫脹，またはPHのうち 1 項目以上が存在することが必須条件である．血清学的指標としての抗U1-RNP抗体陽性がやはり必須項目である．以前は，MCTDでは，抗U1-RNP抗体以外の疾患特異的自己抗体は全て陰性であることが

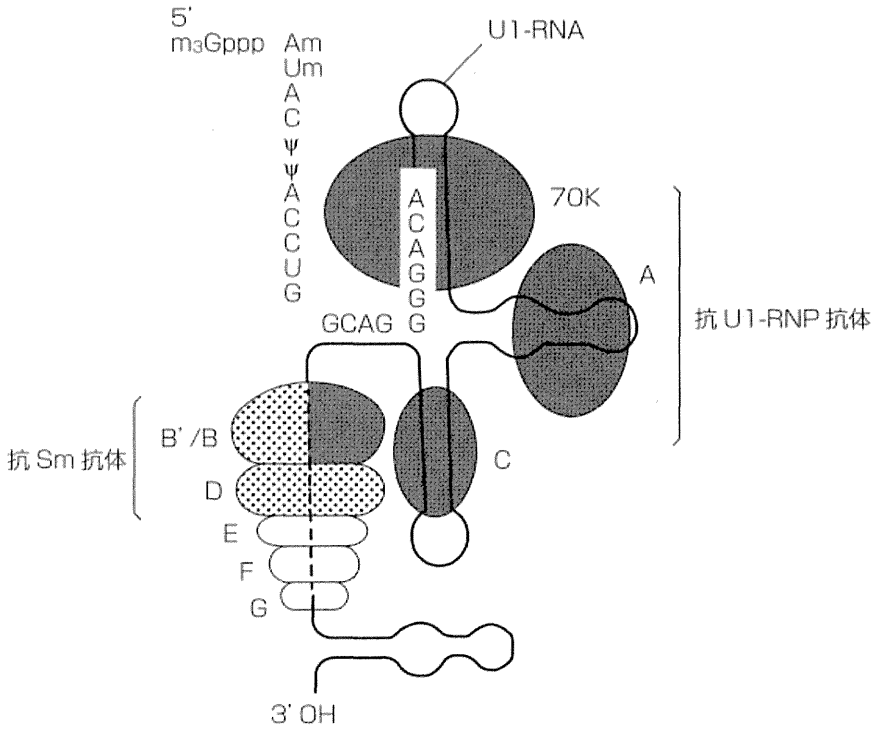

図1. U1 RNP抗原とSm抗原の構成成分
U1 RNPは，U1 RNAと9つの蛋白質で構成される．
(70K，A，B/B'，C，D，E，F，G)

基準となっていた．つまり，抗2本鎖DNA抗体，抗Sm抗体というSLEの特異抗体や抗Scl-70抗体，抗セントロメア抗体というSScの特異抗体，抗Jo-1抗体というPMの特異抗体が陽性の場合は，MCTDとは診断せず，SLEとSScあるいはSLEとPMの重複症候群と診断することが一般的であった．2004年の基準では，抗U1-RNP抗体が陽性であれば，SLE，SScもしくはPMの分類基準に合致する症例においても，その2疾患以上の臨床症状を合併していれば，MCTDと診断できる．しかしながら，診断時には，SLE，SScもしくはPMのそれぞれの分類基準は満たさず重複症候群とは診断できない症例で，抗U1-RNP抗体陽性例をMCTDと診断するのが筆者は妥当だと考える．表1に示す診断基準の混合所見において，それぞれの項目で1項目以上が陽性であればMCTDと診断するとしているのは，SLE，SSc，PMのそれぞれの分類基準を満たさない症例こそがMCTDであると考えているからである．一方，診断時にはそれぞれの分類基準は満たさなくても，経過を追っていくうちにSLE，SScもしくはPMの分類基準を満たす症例に時々遭遇する．自然経過を追った研究があり，MCTDと診断後，5～10年の経過を観察できた症例においては，6割程度がSLE，SScもしくはSLEとSScの重複症候群と診断できる症例があり，MCTDとしか診断できないまま経過する症例は3割程度であった[3]．

2. 抗U1-RNP抗体の臨床との関連

MCTDは，前述したように3種類の膠原病の重複症状を呈することが臨床症状としての特徴であるが，自己抗体の発現から考えれば，抗U1-RNP抗体陽性膠原病である．抗U1-RNP抗体陽性の膠原病の特徴は，Raynaud現象，多関節炎，白

表2. 臨床症状の頻度（1992年の全国調査の結果，n=850）

臨床症状	陽性症例（%）
共通項目	
Raynaud現象	97.4
指，手背腫脹	91.8
SLE所見	
多発関節炎	81.2
リンパ節腫脹	30.0
顔面紅斑	37.3
心膜炎	13.9
胸膜炎	13.5
白血球減少	44.4
血小板減少	14.9
強皮症所見	
手指硬化	58.4
肺線維症	32.1
肺拘束障害	30.1
肺拡散障害	37.2
食道蠕動低下	22.0
多発性筋炎所見	
筋力低下	41.7
筋原性酵素上昇	34.8
筋電図異常	30.7

血球減少，無菌性髄膜炎，それにPHである．MCTDと診断された症例ばかりでなく，MCTD，SLE，SSc，PMのどれにも分類できない未分類結合組織病（undifferentiated connective tissue disease：UCTD）においても同様の臨床症状はよくみられる．つまり，抗U1-RNP抗体を産生する自己免疫異常は，同時に，特徴的な臨床症状に関連すると考えている．この抗体はHLA（human leukocyte antigen）に起因しており，それが，白人に比較して我々日本人では2倍以上の高頻度で検出される理由である．本邦では，HLADQB1*0302に関連していることが三森らにより報告された[4]．抗体産生機序としては，自己抗原と他の抗原との分子相同性による交差反応性が関与している可能性が示されている．抗U1-RNP抗体の対応抗原の構成成分を図1に示した．U1RNPの70K蛋白とレトロウイルス$p30^{gag}$やインフルエンザウイルス，C蛋白とヘルペスウイルス，70KやB蛋白とサイトメガロウイルスおよびEB（Epstein-Barr）ウイルスとの間に分子相同性が認められ，それらのウイルス感染と自己抗体産生との関与が示唆されている．しかし，MCTDの病因とウイルス感染との関連はいまだに明らかにはなっていない．

3. 臨床症状

初発症状で最も多いのはRaynaud現象である．次に手指の浮腫，関節炎が続く．1992年に厚生省の研究班で全国のMCTD患者850例に対しての調査が行われた．当時の10%以上の患者の臨床情報である．表2にそのまとめを示す．やはり，ほとんどの症例でRaynaud現象があり，関節炎が80%以上の症例で認められた．手指，手背の腫脹と手指の皮膚硬化が次に高頻度にみられた．SScの診断には至らないが，強皮症用の症状が多くの症例で認められるのがMCTDの臨床症状の特徴である．また，関節リウマチ（rheumatoid arthritis：RA）と鑑別が困難な多発関節炎を高頻度で呈する．SLE症状としては，この関節炎症状が最も多く，次にしばしば白血球減少がみられる．一方，SLEとしては重要な臨床症状である腎障害（糸球体障害）は少ない．また，神経障害もSLEと比較して少ない．しかしながら，MCTDに特徴的な神経障害として三叉神経痛と無菌性髄膜炎がある．正確な頻度は不明であるが，それぞれMCTDの5%，10%程度に見られる．

生命予後に関わる重要な臨床症状はPHである．5%以下の一部の症例ではあるが，間質性肺炎（interstitial pneumonia：IP）が進行して肺線維症を呈する症例やSLEと同様に腎障害，中枢神経障害，血小板減少を呈する症例がある．

本邦のMCTDの特徴であり，臨床症状において留意すべきなのは，PHの合併である．肺高血圧症は近年，多くの治療方法が確立され，この

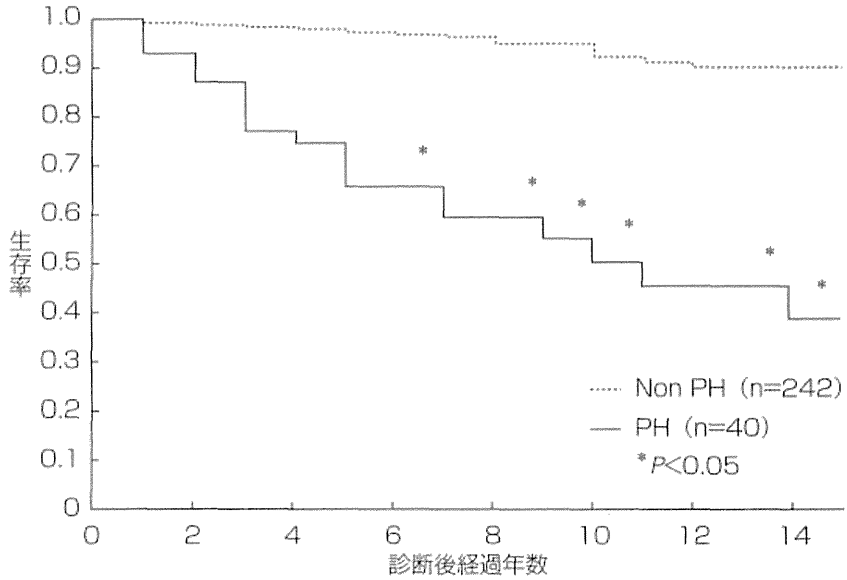

図2. MCTDの生命予後　肺高血圧症合併例（pulmonary hypertension：PH）と非合併例（non pulmonary hypertension：PH）

表3. 肺高血圧症の臨床分類（2013年）

Group 1	肺動脈性肺高血圧症（PAH）
	1.1 特発性PAH
	1.2 遺伝性
	1.3 薬物および毒物誘発性
	1.4 各種疾患に伴うPAH
	1.4.1 結合組織病（SLE，SScやMCTDなど）
Group 2	左心疾患による肺高血圧症
Group 3	肺疾患および/または低酸素血症による肺高血圧症
Group 4	慢性血栓塞栓症性肺高血圧症
Group 5	明確ではない他因子性の機序を伴う肺高血圧症

10年で生命予後がめざましく改善された．2013年には，ニースで第5回国際肺高血圧症会議が開催され，臨床分類や診断基準，治療方針が検討された．そこでは，PHをその病態により5つのカテゴリーに分類してそれぞれの治療方法を考案している（表3）[5]．MCTDに伴うPHは，肺動脈性肺高血圧症（pulmonary arterial hypertension：PAH）というGroup 1に分類される．肺動脈の前毛細血管に病変があり，肺血管の内膜，中膜の肥厚により肺動脈圧が上昇し，右心不全を呈することが推測される．PAHを合併するMCTD症例の生命予後は，2002年の本邦での報告では，図2に示すように5年生存率は60%であった．PAHを合併していない症例では，10年生存率が90%以上であることから，PAH合併は生命予後を悪化させることがわかる[6]．

4. 治療方針

MCTDの特異的な治療方法はない．炎症が強い症例では，副腎皮質ステロイド薬の治療を行う．SLEに伴う症状がみられれば，その重症度に

トピックス

あわせて副腎皮質ステロイド薬の投与量を検討する．また，筋症状に関しても副腎皮質ステロイド薬の適応となる．一方，皮膚の硬化やRaynaud現象というSSc症状に関しては，副腎皮質ステロイド薬の有効性は乏しく，血管拡張薬での対症療法となる．シクロホスファミド（cyclophosphamide），アザチオプリン（azathioprine），カルシニューリン阻害薬という免疫抑制薬の併用は，ステロイド抵抗性の腎障害や中枢神経障害，IPに対して用いられる．関節破壊を伴う多関節炎を呈するMCTDでは，RAに準じてメトトレキサート（methotrexate）などの抗リウマチ薬併用する．多関節炎に対して非ステロイド性抗炎症薬（non-steroidal anti-inflammatory drugs：NSAIDs）が用いられることもあるが，無菌性髄膜炎を誘発するという報告があり，留意する必要がある．

生命予後に重要なPAHを合併したときの治療は近年急速に進歩している．PAHに特異的な治療としての薬剤が開発されたからである[7~9]．本邦では，プロスタサイクリン製剤としてベラプロスト徐放薬，ホスホジエステラーゼ5（phosphodiesterase 5：PDE5）阻害薬，エンドセリン受容体拮抗薬（ERA）の3系統の経口薬剤が使用可能である．PDE5阻害薬は，一酸化窒素により誘導されるcGMP(cyclic guanosine monophosphate)を増強することにより血管を拡張させる．また，最も強力な血管収縮因子として知られているエンドセリンの作用を抑制する薬剤として開発されたのがERAである．このPDE5阻害薬とERAは，肺動脈に特異性が高いことよりPAHの治療薬として開発され，有用性が報告された．MCTDでは，副腎皮質ステロイド薬や免疫抑制薬の有効性が本邦を中心に報告されており[10,11]，PAH特異的な治療薬と同時に用いられる．実際の治療としては，PAHの合併が認められれば，シクロホスファミド間欠的静注療法を行う．0.5~0.6 g/体表面積（m^2）の量を4週間に1度のペースで3~6回行う．同時にプレドニゾロン（prednisolone：PSL）を体重あたり0.6~0.8 mgの量を内服する．この免疫抑制療法に反応性がない場合には，早々にPAH特異治療を併用する．

著者のCOI (conflicts of interest) 開示：川口鎮司：講演料（アクテリオンファーマシューティカルズジャパン，グラクソ・スミスクライン，ファイザー）

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Arthritis Care & Research
Vol. 66, No. 4, April 2014, pp 575–584
DOI 10.1002/acr.22163

ORIGINAL ARTICLE

Autoantibodies to RuvBL1 and RuvBL2: A Novel Systemic Sclerosis–Related Antibody Associated With Diffuse Cutaneous and Skeletal Muscle Involvement

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Objective. To identify and characterize a novel systemic sclerosis (SSc)–related autoantibody directed against a complex consisting of RuvBL1 and RuvBL2 (RuvBL1/2) and to assess its clinical correlations.

Methods. We first analyzed 316 consecutive patients with SSc who were evaluated at Kanazawa University Hospital. Controls included 290 patients with other connective tissue diseases, interstitial lung disease alone, or autoimmune hepatitis, and 50 healthy subjects. Autoantibody specificities were analyzed using RNA and protein immunoprecipitation assays. Autoimmune targets were affinity purified using patients' sera and subjected to liquid chromatography mass spectrometry. SSc patients in another institution in Japan and the University of Pittsburgh cohort were also included in analysis for evaluating clinical correlations.

Results. By protein immunoprecipitation assay, 6 SSc sera (1.9%) reacted with doublets with molecular weights of ~50 kd. Liquid chromatography mass spectrometry of the partially purified autoantigen and additional immunoblot-based analyses revealed that this antibody specificity recognized RuvBL1/2. Anti-RuvBL1/2 antibody was exclusively detected in SSc patients. SSc patients with anti-RuvBL1/2 in both the Japanese and Pittsburgh cohorts consistently had higher frequencies of SSc in overlap with myositis and diffuse skin thickening than those without anti-RuvBL1/2. Compared with other autoantibodies related to SSc/myositis overlap (anti–PM-Scl and anti-Ku), anti-RuvBL1/2 was distinctive in terms of its associations with older age at SSc onset, male sex, and a high frequency of diffuse cutaneous involvement.

Conclusion. Anti-RuvBL1/2 antibody is a novel SSc-related autoantibody associated with a unique combination of clinical features, including myositis overlap and diffuse cutaneous involvement.

INTRODUCTION

Systemic sclerosis (SSc; scleroderma) is a connective tissue disease characterized by excessive fibrosis of skin and internal organs, immune system activation, and microvascular damage. There are variable clinical presentations ranging from limited to diffuse cutaneous involvement.

Supported by a research grant on intractable diseases from the Japanese Ministry of Health, Labour and Welfare; the Scleroderma Research Fund (University of Pittsburgh Division of Rheumatology and Clinical Immunology); the Taub Scleroderma Laboratory Fund (Chicago, Illinois); the Arthritis Foundation Western PA Chapter (Shoemaker Fund); and the Zale Foundation (Dallas, Texas).

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Dr. Takehara has received consulting fees (less than $10,000) from Japan Benessis.

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Submitted for publication February 20, 2013; accepted in revised form September 3, 2013.

Significance & Innovations

- We have identified a novel systemic sclerosis (SSc)–related autoantibody reactive with a complex consisting of RuvBL1 and RuvBL2 (RuvBL1/2) that is involved in many important cellular processes, such as transcription and DNA repair.
- Anti-RuvBL1/2 antibody is highly specific to SSc, but its prevalence is very low, ranging from 1–2%.
- Anti-RuvBL1/2 antibody is associated with a unique combination of clinical features, including older age at SSc onset, a higher proportion of men, and higher frequencies of myositis overlap and diffuse skin thickening.
- Detection of anti-RuvBL1/2 antibody is useful for the diagnosis and clinical subgrouping of SSc patients.

While the etiology of SSc remains unclear, autoimmunity is considered to be involved in the pathophysiology. In particular, serum antinuclear antibodies (ANAs) are detected in more than 95% of patients and are a hallmark of the disease (1,2).

A variety of ANAs have been reported to be specific for SSc, including anti–topoisomerase I (anti–topo I), anticentromere, anti–RNA polymerase III (anti–RNAP III), anti-Th/To, and anti–U3 RNP antibodies. They are present at diagnosis and are almost mutually exclusive to each other. Importantly, ANAs are closely associated with distinct clinical subsets. For example, anti–topo I, anti–RNAP III, and anti–U3 RNP are associated with diffuse cutaneous SSc (dcSSc), while anticentromere and anti-Th/To are detected mainly in patients with limited cutaneous SSc (lcSSc) (1–4). These SSc-related antibodies have close associations with distinctive internal organ involvement, such as interstitial lung disease (ILD), scleroderma renal crisis, and pulmonary arterial hypertension. In addition, anti–U1 RNP, anti-Ku, and anti–PM-Scl are frequently found in SSc patients who have features of another connective tissue disease (overlap syndrome). Therefore, detection of SSc-related antibodies is useful not only in diagnosis, but also in prediction of subsequent organ involvement and prognosis. These SSc-related autoantibodies are identified in ~80% of the entire SSc population, suggesting the possibility that other autoantibodies remain undiscovered. Indeed, a recent study by some of the authors has identified a new SSc-related ANA reactive with U11/U12 RNP that correlates with severe ILD (5).

We routinely examined ANAs using immunoprecipitation (IP) assays in sera with various autoimmune diseases. During this screening process, we found several SSc sera that commonly reacted with doublets with molecular weights of ~50 kd. Using a series of biochemical and molecular analyses, autoantigens targeted by this antibody specificity were identified as a complex consisting of RuvBL1 and RuvBL2 (RuvBL1/2). Further clinical evaluations have revealed that the anti-RuvBL1/2 antibody is a new SSc-related autoantibody associated with diffuse skin thickening and skeletal muscle involvement.

PATIENTS AND METHODS

Patients and controls. We enrolled 316 consecutive Japanese patients with physician-confirmed SSc at Kanazawa University Hospital between 1995 and 2009. Two hundred ninety-one patients (92%) fulfilled the 1980 American College of Rheumatology (ACR) preliminary criteria for SSc (6). Disease controls were randomly selected from among all patients who visited Kanazawa University Hospital during the same period and were evaluated for ANA specificity, including 60 with systemic lupus erythematosus (SLE), 20 with polymyositis (PM), 80 with dermatomyositis (DM), 30 with rheumatoid arthritis (RA), 80 with ILD without features suggestive of connective tissue disease, and 20 with autoimmune hepatitis (AIH). All patients with SLE and RA fulfilled the respective ACR criteria (7,8), while those with PM or DM satisfied definite or probable PM or DM according to the Bohan and Peter criteria (9). The diagnosis of AIH was based on the criteria proposed by the International Autoimmune Hepatitis Group (10). Fifty healthy individuals were used as controls. We used another SSc cohort from Keio University Hospital that included 272 consecutive Japanese patients with SSc first evaluated between 1995 and 2006. Of these, 240 (88%) fulfilled the ACR preliminary criteria for SSc (6).

Additional SSc patients were obtained from the Scleroderma Databank at the University of Pittsburgh. All patients had physician-confirmed SSc between 1981 and 2011 (5). To determine the prevalence of anti-RuvBL1/2 antibody, 463 consecutive SSc patients first evaluated during 1994–1995 and 2004–2005 (a total of 4 calendar years) with serum samples available were tested for anti-RuvBL1/2 antibody. Ninety-six percent of these patients fulfilled the ACR preliminary criteria for SSc (6). For analysis of clinical correlations, anti-RuvBL1/2–positive patients identified in the 1982–2005 cohorts were used for comparisons to increase the number of patients. We further included SSc patients with anti–PM-Scl or anti-Ku (without concomitant SSc-related antibodies) first evaluated between 1980 and 2004 with a diagnosis of SSc (11). All sera were obtained with written informed consent, as approved by the individual institutional review boards.

Clinical assessments. Clinical and laboratory information obtained on all SSc patients at the first and followup visits was prospectively collected using standardized data collection forms. SSc patients were classified as having dcSSc, lcSSc, or SSc in overlap (12). The maximum modified Rodnan total skin thickness score (MRSS) during the disease course was recorded. The definitions for organ system involvement attributable to SSc (5,11,13) are listed in Supplementary Table 1 (available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22163/abstract).

ANA assay. ANA was detected by indirect immunofluorescence performed on HEp-2 cell slides (MBL) as the substrate, combined with fluorescein isothiocyanate–conjugated anti-human IgG. In some instances, mouse anti-RuvBL1 (clone 3G4-1F8, Abnova) or anti-RuvBL2

monoclonal antibody (mAb; clone 42, BD Biosciences) diluted at 1:100 was used in combination with fluorescein isothiocyanate–conjugated anti-mouse IgG.

IP assays. An IP assay was performed using extracts of the leukemia cell line K562, as previously described (14). Briefly, 10 μl of patients' sera, or 1 μl of anti-RuvBL1 or anti-RuvBL2 mAb, was mixed with protein A–Sepharose CL-4B (Pharmacia Biotech). For protein analysis, antibody-coated Sepharose beads were incubated with ^{35}S-methionine–labeled K562 cellular extracts, and immunoprecipitated materials were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Radiolabeled polypeptide components were analyzed by autoradiography. For RNA analysis, antibody-bound Sepharose beads were incubated with unlabeled K562 cellular extracts. RNA components were extracted from the immunoprecipitated materials and were applied to urea-PAGE, followed by staining with silver.

Identification of autoantibodies. Autoantibodies in all sera from the Japanese and Pittsburgh cohorts were screened using indirect immunofluorescence and RNA and protein IP assays. Antibodies except anticentromere were identified by IP assays based on precipitating patterns of RNAs or proteins, which were identical to those produced by reference sera commonly used in Kanazawa, Keio, and Pittsburgh. Autoantibody specificities recorded included those to centromere, topo I, RNAP III, Th/To, U1 RNP, U2 RNP, U3 RNP, U5 RNP, U4/U6 RNP, U11/U12 RNP, Ku, PM-Scl, aminoacyl–transfer RNA synthetases, SRP, Mi-2, melanoma differentiation–associated gene 5 protein, transcription intermediary factor 1γ, NXP-2, SAE, SSA, SSB, Sm, and ribosomal. Antibodies to topo I, RNAP III, centromere (MBL), and SSA (Ro 60 and Ro 52; ThermoFisher Scientific) were evaluated using commercially available kits.

Purification and identification of autoantigens. Autoantigens recognized by patients' sera were isolated by affinity purification as described previously (15), with some modifications. Briefly, a mixture of sera from 2 SSc patients containing autoantibodies of interest was incubated with protein A–Sepharose CL-4B overnight at 4°C. The antibody–protein A complex was cross-linked by treatment with dimethyl pimelimidate (Pierce) for 1 hour, and subsequently incubated with K562 cellular extracts. Proteins bound were eluted by treatment with a buffer containing 3 *M* $MgCl_2$ and 1 m*M* dithiothreitol, concentrated, and fractionated on SDS–polyacrylamide gel. After both edges of the gel were stained with silver, a portion of the gel corresponding to the molecular weight of the protein of interest was cut out from the gel, and protein components were subjected to amino-terminal amino acid sequencing by nanoscale high-performance liquid chromatography on a C18 column (MAGIC 2002, Michrom BioResources) coupled to a tandem mass spectrometer (Q-Tof2, Waters Micromass) (16). Sequence data were used to search a compiled protein database that was composed of protein database NCBInr, which is publicly available (http://www.ncbi.nlm.nih.gov/) as of November 7, 2007.

Immunoblots. Antibody reactivities to RuvBL1 and RuvBL2 were further evaluated using 2 different immunoblot-based assays (17). First, protein components immunoprecipitated from K562 extracts by patients' sera were subjected to immunoblots. The K562 cellular lysates were used as a positive control. The immunoprecipitates and the K562 lysates were fractionated on SDS–polyacrylamide gels and transferred onto nitrocellulose membranes. After blocking with 5% nonfat milk, the membranes were incubated with a 1:250 dilution of mouse anti-RuvBL1 or anti-RuvBL2 mAb. After incubation with horseradish peroxidase–conjugated goat anti-mouse IgG antibodies (eBioscience), antibody binding was detected using an enhanced chemiluminescence kit (Thermo Scientific).

We also used recombinant RuvBL1 and RuvBL2 proteins expressed in *Escherichia coli* (rRuvBL1 and rRuvBL2, respectively; Abnova), which encompassed human full-length amino acid sequences of these proteins fused with glutathione S-transferase (GST), as antigens in immunoblots. These proteins were fractionated and transferred onto nitrocellulose membranes. An edge of the membrane was stained by amide black, and the remaining portion was incubated with a 1:100 dilution of serum samples, or 1:1,000 dilution of mouse anti-RuvBL1 or anti-RuvBL2 mAb. After incubation with alkaline phosphatase–conjugated goat anti-mouse or anti-human IgG antibodies (Cappel), immunoreactive bands were visualized by development with 4-nitro-blue tetrazolium chloride/BCIP (Sigma-Aldrich).

Enzyme-linked immunosorbent assay (ELISA). rRuvBL1 and rRuvBL2 were used as an antigen in ELISA as described previously (18). Briefly, polyvinyl 96-well plates were coated with purified recombinant proteins (0.5 μg/ml) and incubated with a 1:100 dilution of serum samples. Samples were tested in duplicate, and the results were expressed as the optical density at 405 nm.

Depletion of antibodies reactive with rRuvBL1 or rRuvBL2 in serum. Patients' sera were preincubated with an excess amount (1 μg) of rRuvBL1, rRuvBL2, or the mixture of these proteins for 2 hours at 4°C, and were subjected to IP assay or immunoblots.

Statistical analysis. All continuous data are shown as the mean ± SD. The chi-square test or Fisher's exact test was employed for comparison of frequencies, when appropriate. Continuous variables were compared using the Mann-Whitney test.

RESULTS

Detection of a novel SSc-related autoantibody reactive with 50-kd doublets. During our routine assessment of autoantibody profiles using protein IP assay, we noticed

that several SSc sera precipitated strong doublet protein bands at a molecular weight of ~50 kd (Figure 1). Since unlabeled immunoglobulins fractionated on the gels interfered with the electrophoretic motility of these bands, the sizes of the doublet bands varied noticeably and it was difficult to determine the precise molecular weights of these proteins. This pattern of doublet protein IP was not consistent with any reported SSc-related autoantibodies, and was detected in 6 (1.9%) of 316 consecutive SSc patients in the Kanazawa cohort. An identical IP pattern was also found in 4 (1.5%) of 272 consecutive SSc patients in the Keio cohort. In contrast, this antibody specificity was not detected in any sera obtained from a total of 290 patients with SLE, PM, DM, RA, ILD alone, or AIH, or 50 healthy controls, indicating that the anti–50-kd doublet is a novel SSc-specific autoantibody.

Identification of RuvBL1/2 as autoantigens. All 10 sera that immunoprecipitated 50-kd doublets commonly produced a speckled nuclear pattern on indirect immunofluorescence at a titer ranging from 1:160 to 1:1,280 (Figure 2A). This pattern was characterized by condensation of staining during prophase, followed by attenuation of staining in the chromosomal area of the metaphase mitotic cells (Figure 2B). Four (40%) of the positive sera also produced fine granular cytoplasmic staining, but there was no protein commonly immunoprecipitated by those sera. On the other hand, none of the 10 sera with antibodies to 50-kd doublets precipitated small nuclear or cytoplasmic RNA by IP.

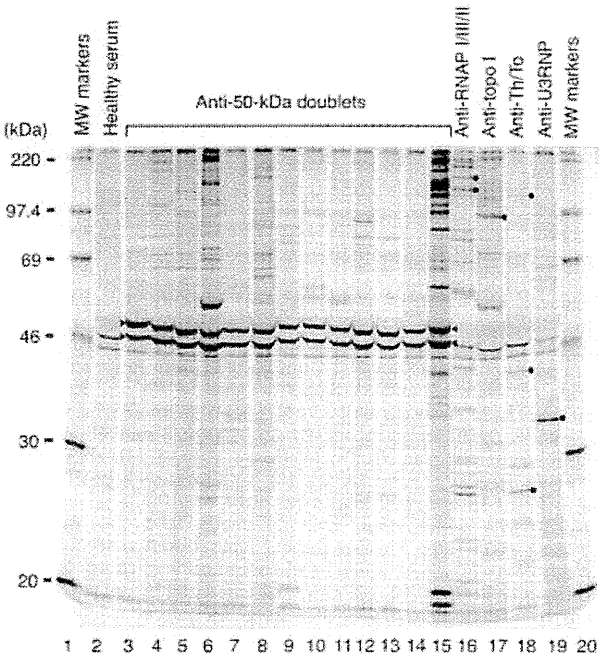

Figure 1. Detection of autoantibodies reactive with the 50-kd doublets by a protein immunoprecipitation assay. Immunoprecipitates from ^{35}S-methionine–labeled K562 cellular extracts were subjected to 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by autoradiography. Lanes 1 and 20 = molecular weight (MW) markers; lane 2 = healthy serum; lanes 3–15 = sera from systemic sclerosis (SSc) patients positive for the anti–50-kd doublets (RuvBL1/2) from the Kanazawa cohort (lanes 3–8) and Pittsburgh cohort (lanes 9–15); lane 16 = SSc serum with anti–RNA polymerase I/III/II (anti–RNAP I/III/II); lane 17 = SSc serum with anti–topoisomerase I (anti-topo I); lane 18 = SSc serum with anti-Th/To; lane 19 = SSc serum with anti–U3 RNP. **Arrowheads** show the 50-kd doublets. **Dots** in sera positive for anti–topo I, anti–RNAP III, anti-Th/To, and anti–U3 RNP show the main target antigens.

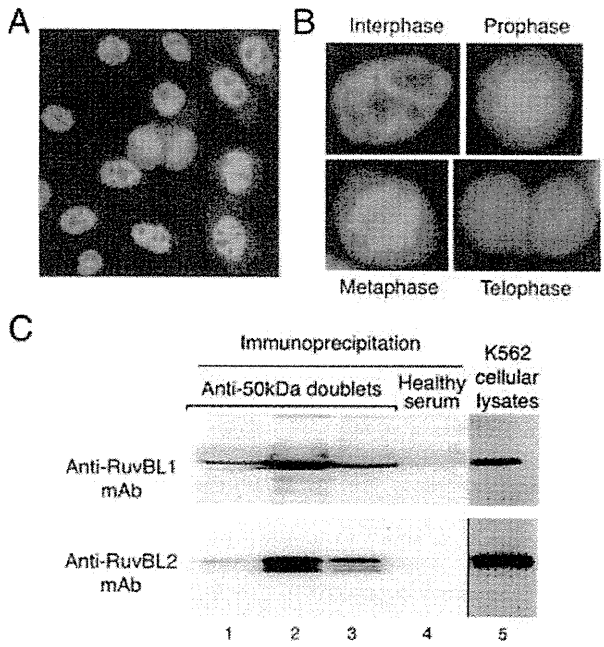

Figure 2. Identification of RuvBL1/2 as the 50-kd doublets. **A,** An indirect immunofluorescent staining pattern on HEp-2 cell slides produced by a systemic sclerosis (SSc) serum positive for anti–50-kd doublets (corresponding to lane 4 in Figure 1). Original magnification × 200. **B,** High-magnification views of HEp-2 cells in various cell cycle phases stained by an SSc serum positive for anti–50-kd doublets. Original magnification × 400. **C,** Detection of RuvBL1 and RuvBL2 in immunoprecipitates using immunoblots. Protein components immunoprecipitated from K562 cellular extracts by 3 representative SSc sera positive for the anti–50-kd doublets (lanes 1–3; corresponding to lanes 3, 4, and 7 in Figure 1) and healthy serum (lane 4) were subjected to immunoblots probed with anti-RuvBL1 (**upper**) or anti-RuvBL2 (**lower**) monoclonal antibodies (mAb). Untreated K562 cellular lysates, instead of immunoprecipitates, were used as controls (lane 5).

To identify autoantigens recognized by antibodies to 50-kd doublets, we partially purified the 50-kd doublets from K562 extracts using the 2 SSc sera samples that showed an ANA titer of ≥1:640 (corresponding to lanes 4 and 7 in Figure 1). When amino acid sequences obtained by liquid chromatography mass spectrometry were subjected to the protein database NCBInr search, the majority of them were derived from human immunoglobulins. However, 8% of 456 sequences completely matched amino acid sequences of the amino-terminal portion of RuvBL1, which is an ATP-binding nuclear protein that belongs to the family of ATPase associated with diverse cellular activities (19). RuvBL1 is present in the nucleus as a complex with RuvBL2, which is a structurally related protein (19). Interestingly, RuvBL1 and RuvBL2 form a double hexamer and interact through their ATPase insert domain (20). The

molecular weights of RuvBL1 and RuvBL2 are reported to be 49 kd and 48 kd, respectively (19), raising the hypothesis that the 50-kd doublets correspond to RuvBL1 and RuvBL2. To test this hypothesis, we examined whether proteins immunoprecipitated by anti–50-kd doublets contained RuvBL1 and RuvBL2 (Figure 2C). The immunoprecipitates of 3 representative SSc sera samples positive for the anti–50-kd doublets contained both RuvBL1 and RuvBL2, but those of healthy serum did not. In total, immunoprecipitates of all 10 SSc sera with anti–50-kd doublets contained both RuvBL1 and RuvBL2. In contrast, all of the 20 control sera from SSc patients negative for the anti–50-kd doublets failed to precipitate any of these proteins. Therefore, the 50-kd doublets targeted by the novel SSc-related autoantibody were confirmed to be RuvBL1 and RuvBL2.

Autoantibodies to the RuvBL1/2 complex are specific to SSc. It has been reported that anti-RuvBL1 antibody is detected in a small proportion of patients with SLE, PM/DM, RA, or AIH by ELISA using a recombinant RuvBL1 fragment expressed in *E coli* (21). This is inconsistent with our findings obtained from IP assay: detection of anti-RuvBL1/2 was exclusive to SSc patients. To examine reasons for this discrepancy, we carried out antibody detection assays using rRuvBL1 and rRuvBL2 individually as antigens. In immunoblots, we occasionally found antibodies reactive with rRuvBL1 or rRuvBL2 in the sera from patients with SSc, PM, DM, SLE, RA, or AIH (Figure 3A). None of those sera reacted with GST alone (data not shown). We used ELISA to further screen a larger number of sera (Figures 3B and C). As a result, antibodies to rRuvBL1 and rRuvBL2 were detected in sera from patients with various diseases, irrespective of the presence or absence of reactivity to RuvBL1/2 by IP. When cutoff values were set at 2 SDs above the mean of healthy controls, anti-rRuvBL1 was detected in 6 (86%) of 7 SSc sera with anti-RuvBL1/2 antibody by IP, but in 8 (5%) of 159 sera without anti-RuvBL1/2 ($P < 0.00001$); anti-rRuvBL2 was detected in 5 with anti-RuvBL1/2 (71%), but in 41 without anti-RuvBL1/2 (26%; $P = 0.03$). These findings suggest that antibodies reactive with RuvBL1/2 detected by IP assay and those reactive with individual rRuvBL1 and rRuvBL2 are distinct repertoires, although anti-RuvBL1/2 antibodies frequently coexisted with antibodies to rRuvBL1 or rRuvBL2.

One potential explanation for different specificities between antibodies detected by IP assay and recombinant protein-based assays is their recognition of distinct epitopes. The IP detects antibodies recognizing epitopes present on the native RuvBL1/2 complex, whereas assays using rRuvBL1 or rRuvBL2 may detect antibodies reactive with epitopes not present on the native complex. To examine this possibility, we used commercially available anti-RuvBL1 and anti-RuvBL2 mAb, which were generated by immunization of mice with synthetic peptides encoding amino acid sequences unique to each protein. These mAb failed to precipitate RuvBL1/2 by IP assay (see Supplementary Figure 1A, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22163/abstract) and failed to stain the nucleus of HEp-2 cells by indirect immunofluorescence. These characteristics were consistent with patients' sera that reacted with rRuvBL1 and/or rRuvBL2 by immunoblots and ELISA. In addition, SSc sera that immunoprecipitated the RuvBL1/2 complex retained their reactivity even after antibodies reactive with rRuvBL1, rRuvBL2, or both were depleted

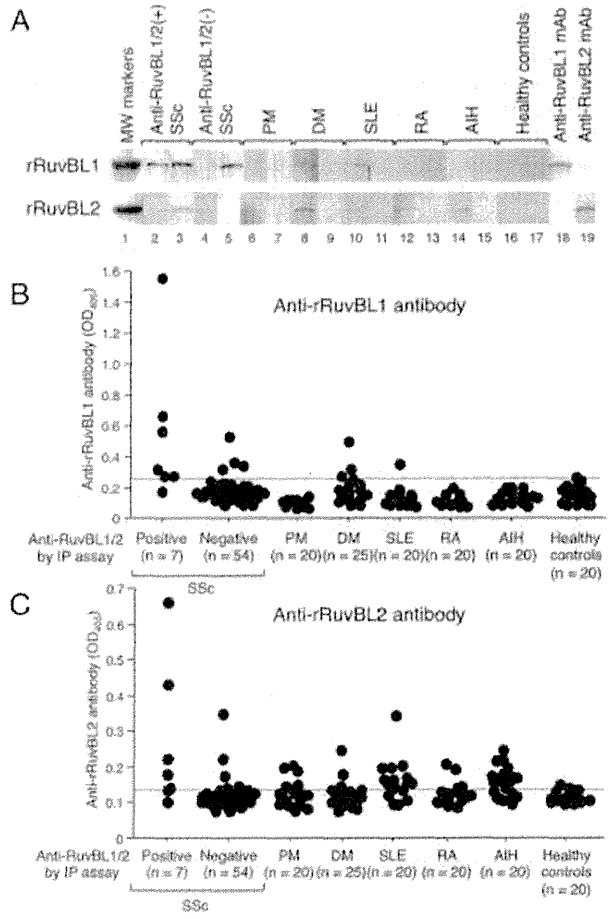

Figure 3. Detection of antibodies to recombinant RuvBL1 and RuvBL2 (rRuvBL2 and rRuvBL2). **A,** Immunoblots using rRuvBL1 (**upper**) and rRuvBL2 (**lower**) as antigens. Systemic sclerosis (SSc) sera positive for anti-RuvBL1/2 antibody by immunoprecipitation (IP) assay (lanes 2 and 3), SSc sera negative for anti-RuvBL1/2 antibody by IP assay (lanes 4 and 5), polymyositis (PM) sera (lanes 6 and 7), dermatomyositis (DM) sera (lanes 8 and 9), systemic lupus erythematosus (SLE) sera (lanes 10 and 11), rheumatoid arthritis (RA) sera (lanes 12 and 13), autoimmune hepatitis (AIH) sera (lanes 14 and 15), healthy control sera (lanes 16 and 17), anti-RuvBL1 monoclonal antibody (mAb; lane 18), and anti-RuvBL2 mAb (lane 19) are shown. Lane 1 indicates molecular weight (MW) markers, and a band corresponds to 75 kd. **B** and **C,** Antibodies to rRuvBL1 and rRuvBL2 measured by enzyme-linked immunosorbent assay in 61 sera from SSc patients, 20 from PM patients, 25 from DM patients, 20 from SLE patients, 20 from RA patients, 20 from AIH patients, and 20 from healthy controls. SSc patients were divided into 2 groups based on the presence or absence of anti-RuvBL1/2 antibodies detected by IP assay. Broken lines show cutoff levels for positivity, which were set at 2 SDs above the mean of healthy controls (0.25 for anti-rRuvBL1 antibody and 0.14 for anti-rRuvBL2 antibody). OD_{405} = optical density at 405 nm.

Table 1. Clinical profiles in SSc patients with and without anti-RuvBL1/2 antibody in 2 independent Japanese cohorts*

Demographic features and organ involvement	Kanazawa cohort		Keio cohort		2 cohorts combined		
	Anti-RuvBL1/2 negative (n = 310)	Anti-RuvBL1/2 positive (n = 6)	Anti-RuvBL1/2 negative (n = 268)	Anti-RuvBL1/2 positive (n = 4)	Anti-RuvBL1/2 negative (n = 578)	Anti-RuvBL1/2 positive (n = 10)	P†
Age at SSc onset, mean ± SD years	47.2 ± 14.6	58.0 ± 14.5	42.2 ± 13.6	58.3 ± 7.9	44.9 ± 14.1	58.1 ± 12.1	0.008
Male sex	62 (20)	3 (50)	29 (11)	2 (50)	91 (16)	5 (50)	0.01
Disease classification							< 0.00001‡
dcSSc alone	95 (31)	2 (33)	78 (29)	0	173 (30)	2 (20)	
lcSSc alone	206 (66)	0	142 (53)	2 (50)	348 (60)	2 (20)	
SSc in overlap	9 (3)	4 (67)	48 (18)	2 (50)	57 (10)	6 (60)	
Cutaneous involvement							
Diffuse	102 (33)	5 (83)	93 (35)	2 (50)	195 (34)	7 (70)	0.04
Limited	208 (67)	1 (17)	175 (65)	2 (50)	383 (66)	3 (30)	
Diffuse within overlap, no./total (%)	2/9 (22)	3/4 (75)	15/48 (31)	2/2 (100)	17/57 (30)	5/6 (83)	0.01
Maximum MRSS, mean ± SD							
Diffuse cutaneous involvement	18.4 ± 8.5	20.6 ± 10.3	21.9 ± 6.1	21.0 ± 1.4	20.0 ± 7.4	20.7 ± 7.5	0.8
Limited cutaneous involvement	3.9 ± 3.6	8§	5.0 ± 2.9	5.0 ± 1.4	4.4 ± 3.3	6.0 ± 3.9	0.2
Organ involvement							
Peripheral vasculature	275 (89)	6 (100)	259 (97)	4 (100)	534 (92)	10 (100)	0.8
Skeletal muscle	31 (10)	4 (67)	35 (13)	2 (50)	66 (11)	6 (60)	0.00003
Gastrointestinal tract	143 (46)	3 (50)	172 (64)	3 (75)	315 (54)	6 (60)	0.7
Interstitial lung disease	137 (44)	5 (83)	147 (55)	2 (50)	284 (49)	7 (70)	0.3
PAH	25 (8)	1 (17)	17 (6)	0	42 (7)	1 (10)	0.8
Heart	34 (11)	4 (67)	23 (9)	1 (25)	57 (10)	5 (50)	0.0003
Kidney (renal crisis)	6 (2)	0	11 (4)	0	17 (3)	0	0.7

* Values are the number (percentage) unless indicated otherwise. SSc = systemic sclerosis; dcSSc = diffuse cutaneous SSc; lcSSc = limited cutaneous SSc; MRSS = modified Rodnan total skin thickness score; PAH = pulmonary arterial hypertension.
† *P* values were calculated after the 2 cohorts were combined.
‡ $P = 0.03$ and $P < 0.00001$ in frequencies of lcSSc alone and SSc in overlap between anti-RuvBL1/2–positive and –negative patients, respectively.
§ Only 1 patient had limited cutaneous involvement.

completely (see Supplementary Figure 1B, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22163/abstract). These findings together suggest that anti-RuvBL1/2 detected by IP exclusively in SSc patients recognizes the native complex.

Clinical features associated with anti-RuvBL1/2 antibody in the Japanese cohorts. We first divided SSc patients in the Kanazawa and Keio cohorts into 2 groups according to the presence or absence of anti-RuvBL1/2 and compared clinical characteristics between the groups (Table 1). Since the number of SSc patients with anti-RuvBL1/2 antibody was small in individual cohorts, statistical analysis was conducted by combining the 2 cohorts into one. SSc patients with anti-RuvBL1/2 were older at onset and were more frequently men than those without anti-RuvBL1/2 ($P = 0.008$ and $P = 0.01$, respectively). Of note, 60% of patients with anti-RuvBL1/2 were classified as having SSc in overlap, which was significantly higher than the frequency of SSc in overlap in those without this antibody ($P < 0.00001$). Diffuse cutaneous involvement was more common in patients with anti-RuvBL1/2 than in those without ($P = 0.04$). Interestingly, the majority of patients with SSc in overlap also had diffuse skin thickening. In terms of organ involvement, skeletal muscle disease was significantly more frequent in anti-RuvBL1/2–positive patients than in anti-RuvBL1/2–negative patients ($P = 0.00003$). Heart involvement was also more commonly found in patients with anti-RuvBL1/2 ($P = 0.0003$).

Although some anti-RuvBL1/2–positive sera precipitated additional proteins by IP assay, no known autoantibodies, including SSc- and myositis-related antibodies, were detected, while one was positive for anti-SSA antibody by a commercial kit. No patient received medications that could potentially induce autoantibody production, including statins, antihypertensive drugs, and biologic agents. None of the patients had malignancy diagnosed concurrently with or within 3 years before or after the diagnosis of SSc.

Clinical features associated with anti-RuvBL1/2 antibody in the Pittsburgh cohort. To further examine clinical correlations with anti-RuvBL1/2 antibody, we analyzed SSc patients in the Pittsburgh cohort. By routine screening of SSc patients' sera with IP assay during 1982–2005, 27 SSc sera were found to have anti-RuvBL1/2 antibody. Four positive patients had other SSc-related antibodies (1 with both anti–RNAP III and anti-Ku and 3 with one of each of

Table 2. Clinical profiles in SSc patients with and without anti-RuvBL1/2 antibody in the Pittsburgh cohort*

Demographic features and organ involvement	Anti-RuvBL1/2 negative (n = 458)	Anti-RuvBL1/2 positive (n = 27)	*P*
Age at SSc onset, mean ± SD years	44.0 ± 15.5	46.0 ± 15.1	0.5
Male sex	104 (23)	10 (37)	0.1
White race	414 (90)	25 (93)	1.0
Disease classification			< 0.00001†
dcSSc alone	215 (47)	8 (30)	
lcSSc alone	207 (45)	3 (11)	
SSc in overlap	36 (8)	16 (59)	
Cutaneous involvement			
Diffuse	226 (49)	18 (67)	0.08
Limited	232 (51)	9 (33)	
Diffuse within overlap, no./total (%)	11/36 (31)	10/16 (63)	0.04
Maximum MRSS, mean ± SD			
Diffuse cutaneous involvement	26.5 ± 12.0	20.0 ± 8.4	0.02
Limited cutaneous involvement	4.5 ± 3.8	7.6 ± 8.7	0.03
Organ involvement			
Peripheral vasculature	449 (98)	22 (81)	< 0.00001
Skeletal muscle	64 (14)	16 (59)	< 0.00001
Gastrointestinal tract, no./total (%)	239/310 (77)	17/18 (94)	0.08
Interstitial lung disease, no./total (%)	157/374 (42)	11/22 (50)	0.3
PAH, no./total (%)	56/285 (20)	2/15 (13)	0.8
Heart, no./total (%)	70/353 (20)	5/23 (22)	0.8
Kidney (renal crisis)	50 (11)	1 (4)	0.4

* Values are the number (percentage) unless indicated otherwise. Denominators are the number of patients with objective testing for organ involvement. SSc = systemic sclerosis; dcSSc = diffuse cutaneous SSc; lcSSc = limited cutaneous SSc; MRSS = modified Rodnan total skin thickness score; PAH = pulmonary arterial hypertension.
† $P = 0.001$ and $P < 0.00001$ in frequencies of lcSSc alone and SSc in overlap between anti-RuvBL1/2–positive and –negative patients, respectively.

anti–RNAP III, anti-Ku, and anti-Th/To). Myositis-related antibodies were not detected, but anti-SSA antibody was found in 2 sera. Regarding medications that could conceivably induce autoantibody production, we examined those taken prior to the serum sample that demonstrated anti-RuvBL1/2 antibodies. These included, alone or in combination, D-penicillamine (n = 7), methotrexate (n = 8), and anti–tumor necrosis factor biologic agents (n = 2). Angiotensin-converting enzyme inhibitors (n = 2) and statins (n = 0) were infrequently or not prescribed. Only 1 patient had lung cancer 10 months after anti-RuvBL1/2 was identified.

When the prevalence of anti-RuvBL1/2 antibody was calculated in 2 consecutive 2-year periods, including 1994–1995/2004–2005, 5 (1.1%) of 463 consecutive new SSc patients with serum available for testing had anti-RuvBL1/2 antibody.

For assessment of clinical correlations with anti-RuvBL1/2, demographic and clinical characteristics of the 27 anti-RuvBL1/2–positive SSc patients were compared with the 458 consecutive anti-RuvBL1/2–negative SSc patients first evaluated in 1994–1995/2004–2005 (Table 2). In contrast to the Japanese cohorts, there was no difference in age at SSc onset between the groups, but the proportion of men among the anti-RuvBL1/2–positive patients tended to be higher. The distribution of SSc subsets was different between patients with and without anti-RuvBL1/2 ($P < 0.00001$). More than half of the anti-RuvBL1/2–positive patients were classified as having SSc in overlap. Diffuse skin thickening was more common in patients with anti-RuvBL1/2 in general and in those with SSc in overlap compared with those without. In terms of organ involvement, peripheral vascular involvement was less common and skeletal muscle involvement was more common in patients with anti-RuvBL1/2 than in those without ($P < 0.00001$ for both comparisons). There were no differences in frequencies of other organ involvements, including the heart, between these 2 groups.

Comparison of clinical features in SSc patients with 3 autoantibodies associated with SSc/myositis overlap. Anti–PM-Scl and anti-Ku are representative SSc-related antibodies associated with inflammatory myopathy (1,2). To examine potential differences in clinical correlations between anti-RuvBL1/2 and these 2 other antibodies, clinical features were compared between SSc patients who were identified to have anti-RuvBL1/2, anti–PM-Scl, and anti-Ku (Table 3). In this analysis, SSc patients in the Japanese and Pittsburgh cohorts were combined. Anti–PM-Scl was not found in the Japanese cohort, which is consistent with a previous report (22). Age at SSc onset was older and the proportion of men was higher in patients with anti-RuvBL1/2 ($P = 0.0001$ and $P = 0.002$, respectively). Approximately half of the patients were classified as having SSc in overlap in all 3 groups, but diffuse skin thickening was more prevalent in patients

Table 3. Comparisons of clinical profiles in SSc patients with 3 autoantibodies associated with SSc/myositis overlap*

Demographic features and organ involvement	Anti-RuvBL1/2 positive (n = 37)†	Anti–PM-Scl positive (n = 76)	Anti-Ku positive (n = 44)	Overall *P*
Cohort, no.				
2 Japanese institutions	10	0	13	
University of Pittsburgh	27	76	31	
Age at SSc onset, mean ± SD years	59.3 ± 14.4	37.6 ± 17.7	38.4 ± 15.5	0.0001‡
Male sex	15 (41)	8 (16)	8 (18)	0.002§
White race	25 (68)	74 (97)	26 (59)	< 0.00001¶
Disease classification				0.02#
dcSSc alone	13 (35)	12 (16)	15 (34)	
lcSSc alone	5 (14)	28 (37)	10 (23)	
SSc in overlap	19 (51)	36 (47)	19 (43)	
Cutaneous involvement				
Diffuse	25 (68)	22 (29)	20 (45)	0.0004**
Limited	12 (32)	54 (71)	24 (55)	
Diffuse within overlap, no./total (%)	12/19 (63)	10/36 (28)	5/19 (26)	0.02††
Typical DM rash	4 (11)	24 (32)	6 (14)	0.01‡‡
Maximum MRSS, mean ± SD				
Diffuse cutaneous involvement	20.3 ± 8.4	11.0 ± 8.8	22.7 ± 12.4	0.01§§
Limited cutaneous involvement	7.2 ± 5.6	4.6 ± 3.5	5.6 ± 4.8	0.1
Organ involvement				
Peripheral vasculature	32 (86)	68 (91)	40 (91)	0.8
Skeletal muscle	21 (57)	39 (51)	22 (50)	0.7
Gastrointestinal tract, no./total (%)	23/28 (82)	24/46 (52)	29/40 (73)	0.04¶¶
ILD, no./total (%)	18/32 (56)	31/62 (50)	19/44 (43)	0.5
PAH, no./total (%)	3/25 (12)	3/59 (5)	4/28 (14)	0.4
Heart, no./total (%)	10/33 (30)	6/54 (11)	8/40 (20)	0.08
Kidney (renal crisis)	1 (3)	6 (8)	1 (2)	0.9

* Values are the number (percentage) unless indicated otherwise. Denominators are the number of patients with objective testing for organ involvement. SSc = systemic sclerosis; dcSSc = diffuse cutaneous SSc; lcSSc = limited cutaneous SSc; DM = dermatomyositis; MRSS = modified Rodnan total skin thickness score; ILD = interstitial lung disease; PAH = pulmonary arterial hypertension.
† Two anti-RuvBL1/2–positive patients had concomitant anti-Ku antibody.
‡ $P = 0.0001$ and $P = 0.001$ between anti-RuvBL1/2–positive and anti–PM-Scl–positive groups and between anti-RuvBL1/2–positive and anti-Ku–positive groups, respectively.
§ $P = 0.0002$ between anti-RuvBL1/2–positive and anti–PM-Scl–positive groups.
¶ $P < 0.00001$ for comparisons both between anti-RuvBL1/2–positive and anti–PM-Scl–positive groups and between anti–PM-Scl–positive and anti-Ku–positive groups.
# $P = 0.01$ between anti-RuvBL1/2–positive and anti–PM-Scl–positive groups.
** $P = 0.0001$ between anti-RuvBL1/2–positive and anti–PM-Scl–positive groups.
†† $P = 0.02$ and $P = 0.03$ between anti-RuvBL1/2–positive and anti–PM-Scl–positive or anti-Ku–positive groups, respectively.
‡‡ $P = 0.01$ and $P = 0.03$ between anti-RuvBL1/2–positive and anti–PM-Scl–positive groups and between anti–PM-Scl–positive and anti-Ku–positive groups, respectively.
§§ $P = 0.001$ between anti-RuvBL1/2–positive and anti–PM-Scl–positive groups.
¶¶ $P = 0.01$ between anti-RuvBL1/2–positive and anti–PM-Scl–positive groups.

with anti-RuvBL1/2 ($P = 0.0004$). In particular, anti-RuvBL1/2–positive patients with SSc in overlap more frequently had diffuse cutaneous involvement than did patients positive for anti–PM-Scl or anti-Ku ($P = 0.02$). DM rashes were less frequent in patients with anti-RuvBL1/2 and anti-Ku than in those with anti–PM-Scl ($P = 0.01$). The maximum MRSS in patients with diffuse cutaneous involvement was greater in those with anti-RuvBL1/2 than in those with anti–PM-Scl ($P = 0.001$). Gastrointestinal and heart involvement was less common in patients with anti–PM-Scl compared with those patients with the other antibodies ($P = 0.04$ and $P = 0.08$, respectively).

DISCUSSION

We have identified and characterized a novel autoantibody reactive with an RuvBL1/2 complex in a small group of SSc patients. Anti-RuvBL1/2 antibody was detectable by IP assay exclusively in SSc patients. In addition, anti-RuvBL1/2 seldom coexisted with other known autoantibody specificities. Based on these characteristics, anti-RuvBL1/2 should be listed as one of the SSc-related autoantibodies, although its prevalence in SSc patients is low (1–2%). RuvBL1 (also known as RVB1, TIP49, and pontin) and RuvBL2 (also known as RVB2, TIP48, and reptin) are highly conserved eukaryotic proteins and form a double hexamer in the nucleus as a scaffolding molecule. Recent studies have implicated the RuvBL1/2 complex in many cellular processes, such as transcription, DNA repair, chromatin remodeling, and small nucleolar RNP assembly (19).

We also confirmed and extended the finding by Makino et al (21). Specifically, antibodies to rRuvBL1 or rRuvBL2 were detected in a small proportion of patients with vari-

ous connective tissue diseases or AIH. However, these antibodies were distinct from anti-RuvBL1/2 detected in SSc patients, in terms of recognition of the RuvBL1/2 complex by IP assay. It is highly likely that SSc-specific autoantibodies preferentially recognize epitopes present on the native RuvBL1/2 complex, probably a double hexamer, whereas autoantibodies detected nonspecifically in patients with various connective tissue diseases may react with epitopes present on RuvBL1 and RuvBL2, but not on the native complex. In this regard, many SSc-related antibodies have been shown to preferentially recognize autoantigens with native conformations (23–26).

Clinical features associated with anti-RuvBL1/2 included a high frequency of SSc in overlap with skeletal myopathy and diffuse skin thickening. These correlations were originally detected in the Japanese cohorts and replicated in the North American cohort. Therefore, anti-RuvBL1/2 should be included in a group of autoantibodies associated with SSc/myositis overlap, including anti–PM-Scl and anti-Ku. Interestingly, among these 3 antibodies, anti-RuvBL1/2 is associated with a unique combination of clinical features, including an older age at SSc onset and higher frequencies of men and diffuse skin thickening.

Intriguingly, both RuvBL1/2 and Ku are required for the DNA damage response. Specifically, RuvBL1/2 is involved in sensing DNA damage and recruitment of repair proteins at the site of damage (19), while the Ku heterodimer plays a key role in the repair of DNA double-strand breaks by forming a complex with the DNA-dependent protein kinase catalytic subunit (27). Conversely, PM-Scl, also known as an exosome complex, plays a critical role in processing of various RNAs, including ribosomal and small nucleolar RNAs (19,28). RuvBL1/2 is also involved in assembly and trafficking of small nucleolar RNAs (19). These shared features of RuvBL1/2, Ku, and PM-Scl may provide insights into pathologic consequences in SSc and skeletal myopathy.

In summary, we have identified a novel SSc-related ANA reactive with an RuvBL1/2 complex. Anti-RuvBL1/2 antibody is a serologic marker for SSc/myositis overlap with diffuse cutaneous involvement.

ACKNOWLEDGMENTS

The authors would like to thank Ms M. Matsubara, T. Hayashi, N. Yoshifuji (Kanazawa University), and M. Ishida (Keio University) for their technical assistance.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Kuwana had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Kaji, Medsger, Takehara, Fujimoto, Kuwana.

Acquisition of data. Kaji, Fertig, Medsger, Satoh, Hoshino, Hamaguchi, Hasegawa, Lucas, Ogawa, Sato.

Analysis and interpretation of data. Kaji, Medsger, Lucas, Fujimoto, Kuwana.

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Reconciling Healthcare Professional and Patient Perspectives in the Development of Disease Activity and Response Criteria in Connective Tissue Disease–related Interstitial Lung Diseases

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ABSTRACT. Interstitial lung diseases (ILD), including those related to connective tissue disease (CTD), and idiopathic pulmonary fibrosis (IPF) carry high morbidity and mortality. Great efforts are under way to develop and investigate meaningful treatments in the context of clinical trials. However, efforts have been challenged by a lack of validated outcome measures and by inconsistent use of measures in clinical trials. Lack of consensus has fragmented effective use of strategies in CTD-ILD and IPF, with a history of resultant difficulties in obtaining agency approval of treatment interventions. Until recently, the patient perspective to determine domains and outcome measures in CTD-ILD and IPF had never been applied. Efforts described here demonstrate unequivocally the value and influence of patient involvement on core set development. Regarding CTD-ILD, this is the first OMERACT working group to directly address a manifestation/comorbidity of a rheumatic disease (ILD) as well as a disease not considered rheumatic (IPF). The OMERACT 11 proceedings of the CTD-ILD Working Group describe the forward and lateral process to include both the medical and patient perspectives in the urgently needed identification of a core set of preliminary domains and outcome measures in CTD-ILD and IPF. (First Release Feb 1 2014; J Rheumatol 2014;41:792–8; doi:10.3899/jrheum.131251)

Key Indexing Terms:
INTERSTITIAL LUNG DISEASE
OUTCOME AND PROCESS ASSESSMENT
RHEUMATOID ARTHRITIS
CONNECTIVE TISSUE DISEASE
PATIENT PERSPECTIVE
SYSTEMIC SCLEROSIS

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Background

The Connective Tissue Disease Interstitial Lung Disease (CTD-ILD) Working Group convened as a special interest group (SIG) during the Outcome Measures in Rheumatology 11 (OMERACT 11) conference to examine concurrence of results between the Delphi exercise among healthcare professionals and the focus group sessions with patient participants. The SIG included, in addition to OMERACT participants, 2 patient research partners with ILD (DL, CS), the principal investigator (LAS), a director of the patient expert investigation (SM), a director of the medical expert investigation (ELM), and the representative from the OMERACT executive (VS).

The OMERACT CTD-ILD Working Group is an international multidisciplinary effort to develop consensus on criteria to measure disease activity and therapeutic response in CTD-ILD. The group first met in November 2008 to address outcome measures in CTD-ILD by developing a multitiered Delphi process to obtain opinions from a broad array of expert pulmonologists, rheumatologists, and cardiologists, as well as a patient perspective strategy.

ILD is one of the leading causes of mortality related to underlying CTD pulmonary disease in systemic sclerosis (SSc)[1,2] and is a major cause of morbidity and mortality in CTD such as rheumatoid arthritis (RA), idiopathic inflammatory myopathy, and Sjögren syndrome. Studies suggest that while mortality rates associated with some CTD have declined, mortality rates associated with CTD-associated pulmonary disease have increased[3,4].

Many complexities of CTD-ILD exist; however, presently there is no consensus on measures to use for assessment of disease activity or treatment response in CTD-ILD. Drug development and assessment of treatment efficacy have been diminished by a relative paucity of data on validated outcome measures in CTD.

Traditional measures of disease activity in ILD are easily confounded by extrapulmonary manifestations of underlying CTD, and although instruments are imperfect, even in a disease such as IPF, whose characteristics are limited to the lung, the group embarked on addressing IPF in comparison to CTD-ILD to provide opinion simultaneously on outcome measures for both disease groups. The CTD-ILD SIG is the first working group, to our knowledge, to take an interest in a comorbid manifestation of a rheumatic disease.

During OMERACT 11, the CTD-ILD SIG presented results to date and engaged meeting participants (providers and patients) in further discussions regarding this progress.

We summarize studies conducted to develop response criteria, domains identified, and progress leading up to OMERACT 11.

Medical Expert Consensus

Rheumatology, pulmonary, cardiology, radiology, and pathology specialists with expertise in IPF and/or CTD-ILD, as well as statisticians, advised on construction of and participation in a structured 3-tiered Internet-based Delphi process to develop consensus on outcome measures reflective of disease activity and therapeutic responsiveness.

The healthcare professional (HCP) Delphi exercise was designed to identify domains and instruments perceived as important outcomes in the context of a 1-year multicenter randomized controlled trial (RCT) of a promising treatment for IPF and/or CTD-ILD. This consensus process included 254 medical experts (physicians with research and clinical expertise in ILD) from 36 countries and 6 continents. In an effort to representatively reflect the views and maintain the true voice of the expert community, the process was initiated with an unrestricted collection of domains and instruments suggested by the HCP. This method of data collection created the voting survey. Throughout the consensus process, the Delphi addressed both CTD-ILD and IPF in parallel tracts, to identify commonalities and differences among outcome measures between these 2 entities.

The Delphi process used a Web-based data collection system that featured links to original publications and subsequent articles addressing validation of all instruments that were identified by a comprehensive Medline literature review. An "Inter-Expert Educational Component" allowed participants to upload commentary, articles, and links for review, as well as challenge or defend inclusion of a domain or instrument.

Using cluster analyses, the 3-part Delphi resulted in selection of 5 domains each for IPF and CTD-ILD that were further supported by high mean and median ratings (Table 1). Surviving instruments, also analyzed by cluster analysis, are shown in Table 2, with supporting high median and mean scores. In addition, surviving instruments of "increasing or decreasing steroids and/or immunosuppressive medications" survived as a marker of disease activity in the "Medications" domain. Please see discussion under "OMERACT 11 Proceedings" below.

Patient Perspective Investigation

The patient-centered investigation was planned with the following objectives: to collect information relevant to the patient experience to determine domains important to patients in assessing disease activity and its effect, provide their perspective on currently used instruments in IPF/CTD-ILD, and to recognize aspects of these diseases relevant to patients potentially not considered by investigators. This strategy was intended to promote understanding of the disease based on the priorities, central experiences, and subtle day-to-day challenges faced by patients that investigators rarely witness.

The investigative team included 3 rheumatologists with expertise in CTD-ILD, a pulmonologist, a patient research partner, and a senior qualitative researcher. All members engaged in study design, implementation, analysis, and interpretation of the results. No preconceived themes or codification were imposed upon the data collected for deductive analysis. Rather, the team adopted an inductive methodology to preserve views expressed by the patients within their own frames of reference, whereby data collected through focus group interviews underwent iterative analyses from which a codification system emerged. Each transcript from each focus group was

Table 1. Results of cluster analysis of the 3-tiered healthcare professional Delphi process. Five domains each were identified for connective tissue disease–related interstitial lung disease (CTD-ILD) and idiopathic pulmonary fibrosis (IPF). Values are ratings on a 9-point scale.

Domain Name	CTD-ILD median/mean	IPF median/mean
Dyspnea	8.0/7.8	8.0/8.1
Health-related quality of life	8.0/7.7	8.0/7.8
Lung imaging	9.0/8.3	9.0/8.3
Lung physiology/function	9.0/8.7	9.0/8.7
Survival	8.0/8.2	9.0/8.4