The TFIID complex is a new autoantibody target in systemic sclerosis

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Systemic sclerosis (SSc) is characterized by systemic fibrosis, vascular dysfunction, and the presence of antinuclear autoantibodies. The major SSc-associated antinuclear antibodies include anti-centromere, anti-topoisomerase I, anti-RNA-polymerase III and anti-fibrillarin autoantibodies [1]. These autoantibodies are generally mutually exclusive and define clinically relevant subgroups.

In up to 10% of persons with SSc, the target antigens of the antinuclear autoantibodies are unknown. Using an unbiased immunoprecipitation-mass spectrometry (IP-MS) approach we recently identified novel and rare autoantigens in SSc, including telomere- and telomerase-associated proteins and components of the spliceosome [2,3]. Using IP-MS, we here describe another new target antigen in SSc.

We examined a database of IP-MS identifications of 106 persons with SSc from two Belgian academic hospitals [Ghent University Hospital (n=48) and University Hospitals Leuven (n=58)] without autoantibody specificity as tested by commercial assays, previously described in reference [2], for potential novel autoantibody targets associated with transcription. TATA-binding protein (TBP)-associated factors (TAFs) were precipitated by sera of four persons (two from Ghent, two from Leuven) (confirmed in a second experiment, supplemental table 1). Together with TBP, TAFs form the three-lobed transcription factor IID complex (TFIID) (figure 1B). The TFIID complex recognizes core promoter sequences on DNA. The binding of TFIID is the first step of the assembly of the preinitiation complex, which is needed to position RNA polymerase II onto the correct DNA sequence to start transcription [4].

In order to identify additional cases, we tested samples from individuals with SSc with unidentified autoantibodies from 3 extra hospitals [Cliniques Universitaires Saint-Luc (Belgium) (n=45), Chris Hani Baragwanath Hospital (South Africa) (n=28) and Centre

Hospitalier Universitaire d'Angers (France) (n=13)] by IP-MS. The analysis was done by QTOF MS (method described in supplemental data). Two additional cases precipitating TFIID subunits were identified (supplemental table 2). Figure 1A and supplemental table 3 summarize the results from a confirmatory experiment including all 6 anti-TFIID-positive cases and 6 controls (3 healthy controls and 3 topoisomerase-I antibody-positive disease controls). In the 6 index cases, different subunits of the TFIID complex were identified in the immunoprecipitate: TAF2, TAF4, TAF4B, TAF5, TAF6, TAF8, TAF9, TAF9B, TAF10, TAF11, TAF12 and TAF13. TBP was identified in 2/6 of the cases. In controls, only TAF9 and TAF9B were precipitated. In order to confirm the specificity of anti-TIFIID antibodies for SSc, IP-MS was performed in healthy individuals (n=34) and in patients with inflammatory myopathy (IIM, n=12), Sjögren's disease (SJD, n=12), systemic lupus erythematosus (SLE, n=36) and anti-topoisomerase-I-, anti-centromere- or anti-RNA polymerase III-positive SSc (n=28). TAF9, TAF9B and TAF10 were often precipitated in these control samples (supplemental table 4). The HEp-2 indirect immunofluorescence patterns of the anti-TFIID antibody-positive patients were nuclear speckled (n=4), nuclear speckled/homogeneous and nuclear speckled/nucleolar. Clinical features are shown in figure 1A and supplemental table 5. Five of 6 patients had interstitial lung disease and 4/6 patients had limited cutaneous SSc. None of the anti-TFIID antibody-positive patients had scleroderma renal crisis or pulmonary arterial hypertension.

Autoantibodies against TBP have been described in up to 24% of persons with systemic sclerosis, overlap syndromes and systemic lupus erythematosus [5]. However, in this study purified recombinant TBP, without the other subunits of the TFIID complex, was used as protein source [5]. Notably, we found TBP in the immunoprecipitate of some anti-TFIID-positive samples. As we did not encounter

similar IP-MS results with sera of persons with SLE, IIM and other controls, the anti-TFIID autoantibodies identified here are most likely distinct from the non-specific reactivity identified in the study of Chauhan et al. [5]. Similarly, we observed nonspecific precipitation of TAF9, TAF9B and TAF10 subunits, but precipitation of multiple (other) subunits was restricted to the six index patients. The exact epitope of the anti-TFIID autoantibodies in persons with SSc is currently unclear, but it is possible that it is a conformational autoepitope that depends on higher-order protein interactions in the protein complex which are not easily captured by recombinant protein-based assays.

In conclusion, we describe TFIID as a new target of autoantibodies in SSc, thereby expanding the spectrum of SSc-associated autoantigens related to transcription. In the future, international multicentric IP-MS studies are needed to characterize and further delineate potential clinical associations of anti-TFIID autoantibodies, in parallel to other new and rare autoantibodies in SSc.

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Ethics statements

The study was approved by the local Ethical Committees: S60347/B32220071204, S65989 (University Hospitals Leuven); B67020084358 (Ghent University Hospital). MTA was in place between University Hospitals Leuven and Cliniques Universitaires Saint-Luc, Centre Hospitalier Universitaire d'Angers and University of Bath.

Contribution

J-BV, EDL and XB designed the study. J-BV, XB, DD, BM and TDH analyzed the data. XB and J-BV drafted the manuscript. J-BV, DD, BM and TDH performed the IP-MS experiments. J-BV, VS, YP, PDH, JL, DB, CB EDL and XB collected patient data and revised the draft manuscript. All authors revised the draft manuscript.

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Competing interests

All authors have completed the ICMJE uniform disclosure form at https://www.icmje.org/disclosure-of-interest/ and declare: no support from any organization for the submitted work; VS has received research grants, consulting fees and support from Boehringer-Ingelheim, Janssen-Cilag, Argenx BV, WebMD Global

LLC and BKC Moving Media Makers BV (outside the scope from the submitted work); NM was member of the data safety monitoring board for NIHIR funded Astute Trial (outside the scope from the submitted work); XB was part of a scientific advisory committee for Inova Diagnostics and Thermo Fisher (outside the scope from the submitted work).

Patient and public involvement

Patients were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Figure 1 Anti-TFIID autoantibodies

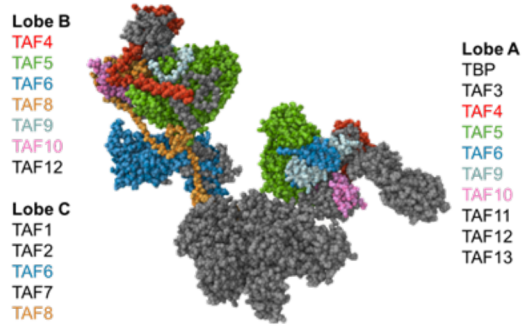
A

Identified TFIID proteins	Abbr.	#1	#2	#3	#4	#5	#6	DC1	DC2	DC3	HC1	HC2	HC3
TFIID subunit 1	TAF1	0	0	0	0	0	0	0	0	0	0	0	0
TFIID subunit 2	TAF2	0	0	61250	0	43086	0	0	0	0	0	0	0
TFIID subunit 3	TAF3	0	0	0	0	0	0	0	0	0	0	0	0
TFIID subunit 4	TAF4	94303	163797	192601	98662	157561	171092	0	0	0	0	0	0
TFIID subunit 4B	TAF4B	0	5153	0	0	0	0	0	0	0	0	0	0
TFIID subunit 5	TAF5	58593	119503	129468	64821	140278	155704	0	0	0	0	0	0
TFIID subunit 6	TAF6	74148	152870	154420	74191	154218	150588	0	0	0	0	0	0
TFIID subunit 7	TAF7	0	0	0	0	0	0	0	0	0	0	0	0
TFIID subunit 8	TAF8	34354	71481	54896	0	82083	80606	0	0	0	0	0	0
TFIID subunit 9	TAF9	132676	315343	220972	146620	202760	192164	41912	49050	53309	58472	49227	70107
TFIID subunit 9B	TAF9B	57704	109858	44499	47549	63538	88554	14154	9997	5405	15176	13411	14068
TFIID subunit 10	TAF10	96158	169525	122595	62200	128845	124886	0	0	0	0	0	0
TFIID subunit 11	TAF11	0	34449	0	0	0	32076	0	0	0	0	0	0
TFIID subunit 12	TAF12	0	0	19998	0	0	0	0	0	0	0	0	0
TFIID subunit 13	TAF13	0	59166	45804	17361	0	0	0	0	0	0	0	0
TATA-box-binding protein	TBP	0	37326	48964	0	0	0	0	0	0	0	0	0
DNA topoisomerase I	SCL70	0	0	0	0	0	0	2077867	2677551	869261	0	0	0

B

Clinical features						
Limited cutaneous involvement						
Diffuse cutaneous involvement						
Interstitial lung disease						
Elevated creatine kinase						
Malignancy						

C



A Hi-3 values of precipitated proteins by sera from 6 anti-TFIID-positive index patients (#1-#6), 3 anti-topoisomerase I-positive SSc disease controls (DC1, DC2, DC3) and 3 healthy controls (HC1, HC2, HC3). Hi-3 values represent per protein the sum of the intensity of the top three most intense peptides and are an estimate of the relative abundance of the protein in the immunoprecipitate. Confidence color scores of the protein identifications are shown (green, yellow; see supplemental methods). Samples #1 and #2 are from Ghent University Hospital, samples #3 and #4 are from University Hospitals Leuven, sample #5 is from Cliniques Universitaires Saint-Luc and sample #6 is from Centre Hospitalier Universitaire d'Angers.

B Clinical features of 6 anti-TFIID positive individuals with SSc

C Structure of TFIID (https://doi.org/10.2210/pdb6MZL/pdb), with color matching to subunits from panel A. Made with Mol* Viewer [6].

Abbr. abbreviation, DC disease control, HC healthy control, TFIID transcription factor IID, TAF TATA-box-binding protein (TBP)-associated factor