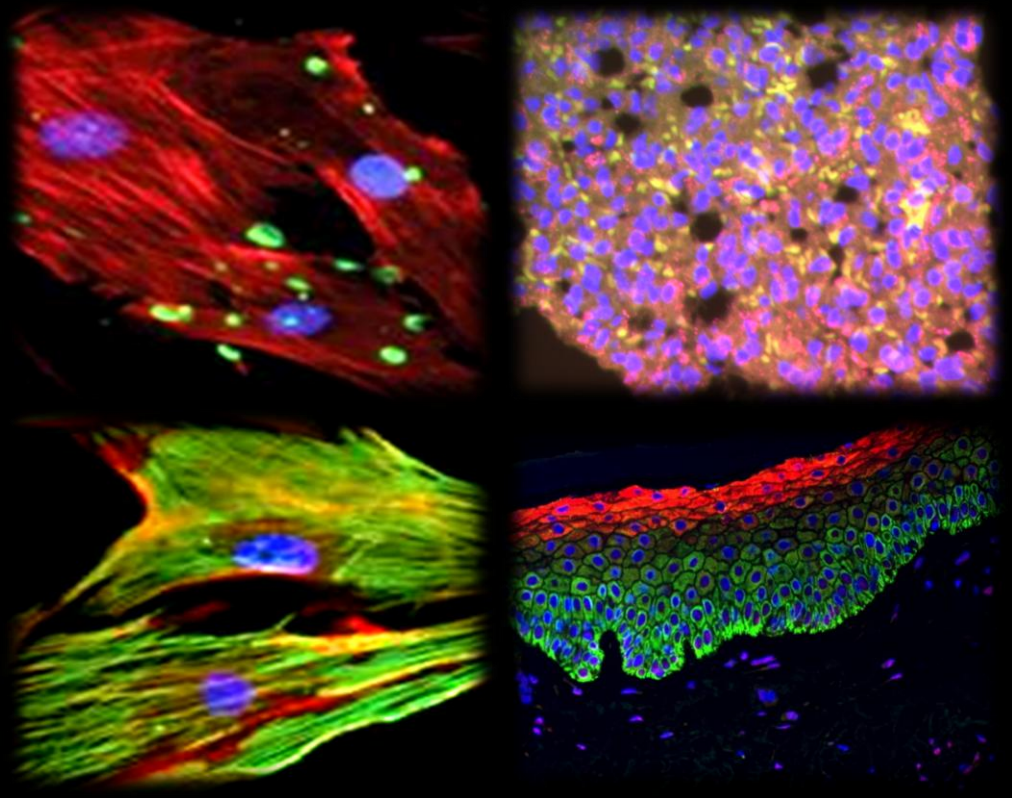
Scleroderma Research

The 12th International Workshop



23-27 July 2011 • Trinity College, Cambridge UK

Understanding the pathogenesis of scleroderma, utilising current therapies and identifying new targets and treatment strategies, leading to better management for patients with scleroderma

Advancing Scleroderma Research and Translational Medicine

The Scientific Steering Committee

Would like to thank the following for their generous support of the Workshop:

Celebrating 10 Years of Service 1998-2008
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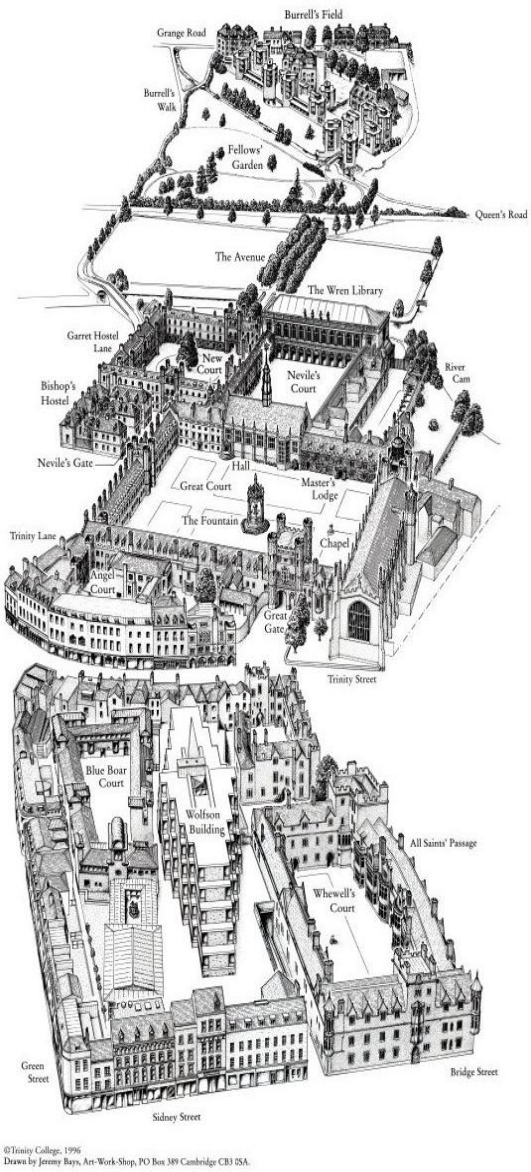


PORTERS LODGE

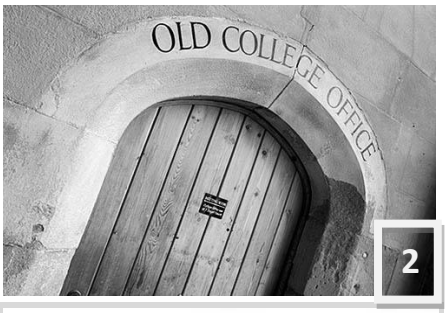
1

OLD COLLEGE OFFICE

Conference Office 01223 766 278

Opening times:

Saturday	1-7PM
Sun-Tues	8AM–6PM
Wednesday	8AM-4PM



2

THE HALL

Meals

Breakfast served

Sunday	7.45AM
Mon-Weds	8.00AM

Banquet

Sunday	7.30PM


5

FRAZER ROOM

Opening times:

Saturday	5-7PM
Sunday	3-4PM
Mon-Weds (Conference Office)	

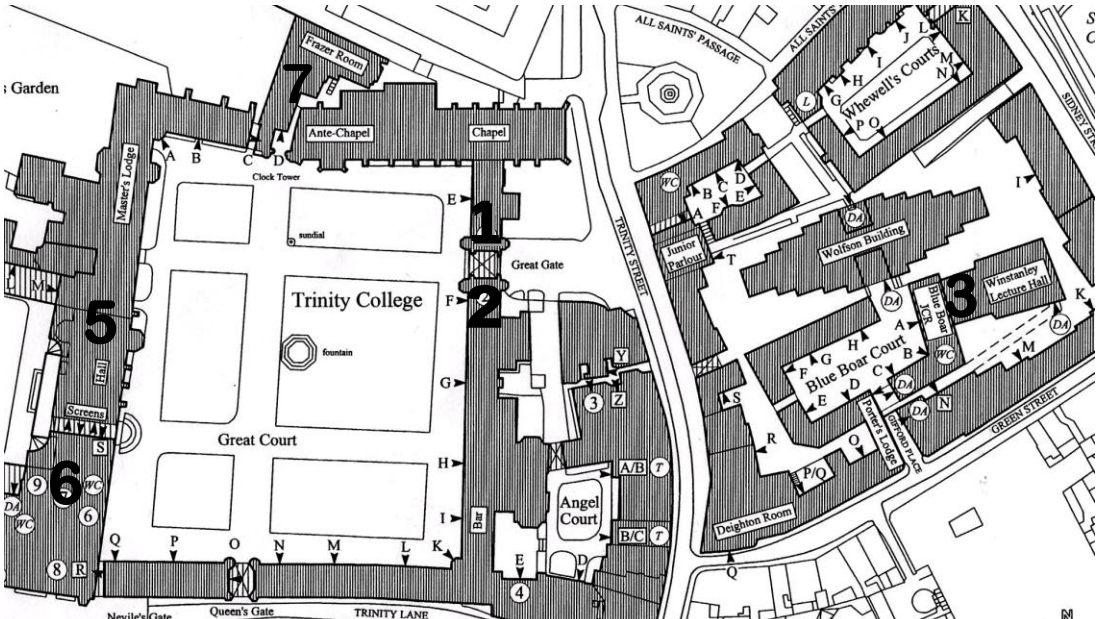

7

WINSTANLEY LECTURE THEATRE 3

Opening Lecture

OLD COMBINATION ROOM 6

Drinks Reception



All workshop sessions will be held in

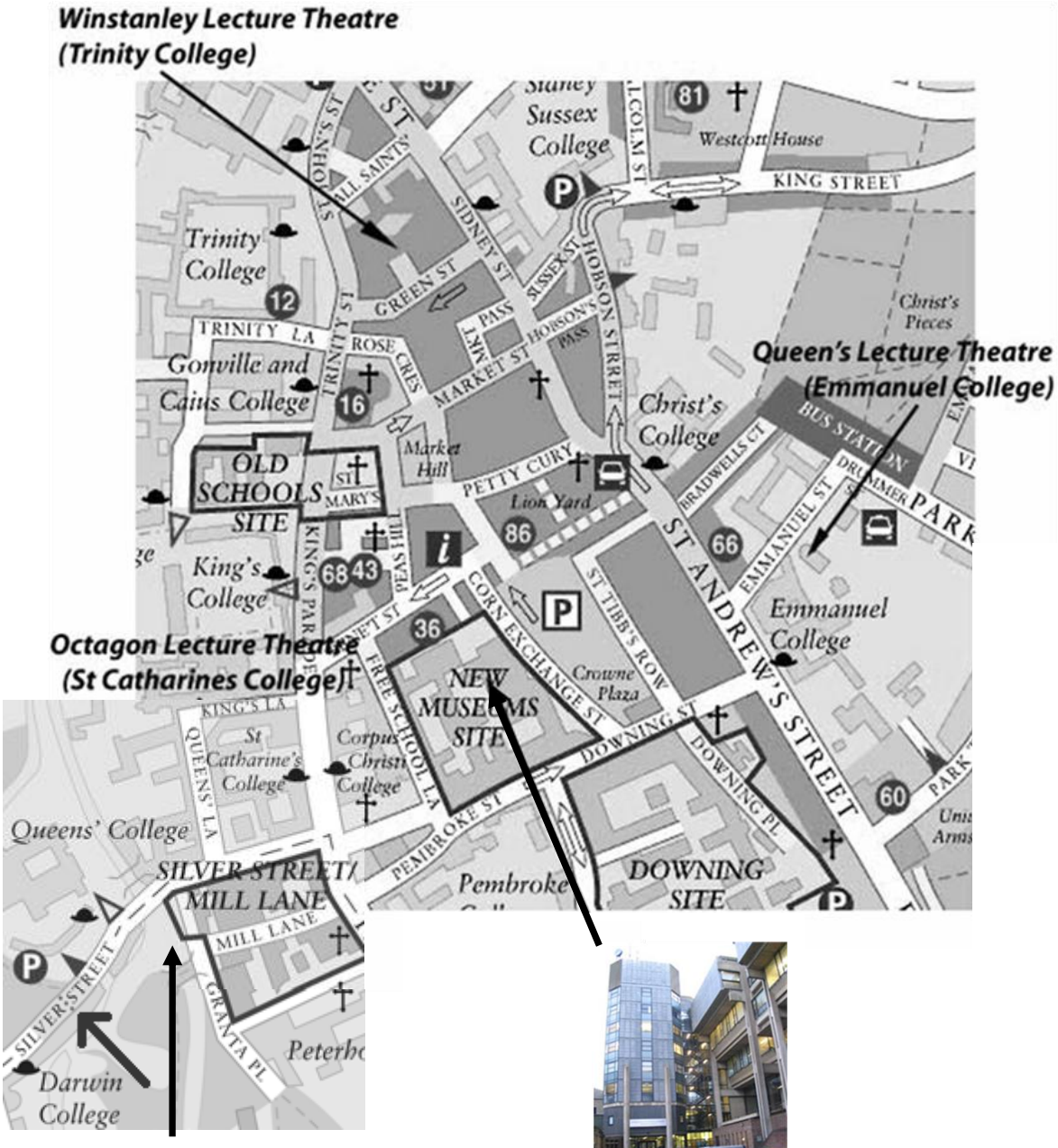
The Babbage Lecture Theatre

New Museums Site

Pembroke Street

Cambridge

CB2 3QH



(LUNCH) University Centre

BABBAGE LECTURE THEATRE

SATURDAY 23 JULY — *Winstanley Lecture Theatre*

1-PM-6PM	REGISTRATION	Conference Office
5-7PM	POSTER SET UP	Frazer Room
6:30-7:30	**OPENING LECTURE** Integrin-mediated adhesion and signalling in health and disease	**Reinhard Fässler** ***Chairs~Thomas Krieg and John Varga***
7:30	Drinks Reception	

SUNDAY 24 JULY — *Babbage Lecture Theatre*

8AM - 6PM	REGISTRATION / POSTER SET UP	Conference Office / Frazer Room
08:45	**WELCOME AND INTRODUCTION**	**CAROL BLACK AND ROBERT LAFYATIS**
Session 1 **9:00-10:30**	**The Clinical and Research Challenges of Scleroderma**	***Chairs~Daniel Furst and Jaap van Laar***
09:00	Translational biology and new therapies	John Varga
09:35	Epigenetic modifications in SSc	Bashar Kahaleh
10:10	*Discussion*	
10:30	*Morning Coffee*	
Session 2 **11:00-12:30**	**Immunology: Innate Immunity**	***Chairs~Robert Lafyatis and Hans Stauss***
11:00	Immune aspects of dendritic cells	Timothy Radstake
11:25	Apoptosis and the immune response	Keith Elkon
11:50	Molecular diversity of the IFN pathway	Peggy Crow
12:15	*Discussion*	
12:30	LUNCH	
Session 3 **1:30-3:00**	**Infection and Environmental Triggers**	***Chairs~Armando Gabrielli and Arnold Postlethwaite***
1:30	Infections and autoimmunity	Irun Cohen
1:55	Infectious diseases, innate immunity and fibrosis	Cory Hogaboam
2:20	Nephrogenic Systemic Fibrosis as a scleroderma model	Sergio Jimenez
2:45	*Discussion*	
3:00	*Afternoon Tea*	
Session 4 **3:30-4:30**	**Young Investigators**	***Chairs~Carol Black and Robert Lafyatis***
3:30	Introduction	
	Top Five Abstract Awards Winners (10 minute presentations)	Lara Bossini-Castillo, Jasper Broen, David Lagares Miltiadis Paliouras, Tatiana Rodriguez-Reyna
4:30	END OF SESSION	
5:30-7:00	***POSTER VIEWING***	

MONDAY 25 JULY — *Babbage Lecture Theatre*

Session 5 **09:00-10:50**	**Inflammation and Mesenchymal Stroma**	***Chairs~Kristofer Rubin and Maria Trojanowska***
09:00	Effects of matrix density on cellular phenotype (not only in carcinoma)	Valerie Weaver
09:25	Regulation of extracellular matrix production by hypoxia: implications for cancer and fibrosis	Amato Giaccia
09:50	Stromal reactions in carcinoma and its similarity and differences to tissue fibrosis	Michael Ostrowski
10:15	LOXL2 in pathologic fibrotic microenvironment	Victoria Smith
10:40	*Discussion*	
10:50	*Morning Coffee*	
Session 6 **11:20-12:45**	**What causes Scleroderma?** **General Round Table Discussion Session**	***Chairs~Bashar Kahaleh, Maureen Mayes, Neil McHugh, Arnold Postlethwaite, Timothy Radstake, Douglas Veale, Ken Welsh***
11:20	Introduction and Round Table Discussion	
12:45	LUNCH	
Session 7 **1:45-3:15**	**Genomics and Post-Genomics**	***Chairs~John Varga and Michael Whitfield***
1:45	Methylation determines fibroblast activation and fibrogenesis in the kidney	Michael Zeisberg
2:10	Transcriptional analysis of models — comparative analysis	Michael Whitfield
2:35	Genetic of complex autoimmune diseases	Javier Martin
3:00	*Discussion*	
3:15	TEA BREAK	
Session 8 **3:45-5:15**	**Novel Regulatory Pathways in Fibrosis**	***Chairs~Humphrey Gardner and Marco Matucci Cerinic***
3:45	Sphingosine 1 phosphate in health and disease	Nigel Pyne
4:10	Non canonical WNT signalling in fibrosis and angiogenesis	Francesco Del Galdo
4:35	Epithelial cells promote dermal fibroblast activation via IL-1α in systemic sclerosis	Richard Stratton
5:00	*Discussion*	
5:15	*END OF SESSION*	
7:30	***BANQUET:*** ***Speaker ~*** **SIR GREGORY WINTER**	

TUESDAY 26 JULY — *Babbage Lecture Theatre*

Session 9 **09:00-10:30**	**The Fibrillin Area and Fibrosis**	***Chairs~Thomas Krieg and Kristofer Rubin***
09:00	Fibrillins: Key extracellular determinants of tissue formation and fibrosis	Francesco Ramirez
09:25	Mechanisms of TGF–β activation	Daniel Rifkin
09:50	Fibrillin related diseases	Elizabeth Gerber
10:15	*Discussion*	
10:30	*Morning Coffee*	
Session 10 **11:00-12:30**	**Models Systems (In vitro and in vivo approaches)**	***Chairs~James Tomasek and Robert Lafyatis***
11:00	Fibroblast biomechanical cues as key regulators of matrix remodelling	Boris Hinz
11:25	Targeting fibroblasts in vivo	Andrew Leask
11:50	Chemical induced models – strengths and weaknesses	Oliver Distler
12:15	*Discussion*	
12:30	*Lunch*	
Session 11 **1:30-3:30**	**Biomarkers and Disease Classification/New Technology Areas**	***Chairs~Luc Mouthon and Philip Clements***
1:30	The Resolution of Inflammation: Players and Targets	Mauro Peretti
1:55	Contribution of proteomics to the identification of biomarkers in vascular diseases	Luc Mouthon
2:20	Identification of biomarkers in systemic sclerosis	Robert Lafyatis
2:45	Composite measures of disease outcome – lessons from PF-SSc	Lesley Ann Saketkoo
3:10	*Discussion*	
3:30	*END OF SESSION*	
	Afternoon Tea	

WEDNESDAY 27 JULY — *Babbage Lecture Theatre*

Session 12 **09:00-11:00**	**Vascular Pathology and Remodelling in PAH**	***Chairs~Lucie Clapp and Clive Handler***
09:00	Future therapy of pulmonary arterial hypertension: reversing advanced disease	Rubin Tuder
09:25	Molecular mechanism of the TNF superfamily in PAH	Allan Lawrie
09:50	The role of TGFbeta in heritable PAH	Alan Holmes
10:15	Oestrogen and the serotonin system in the development of PAH	Margaret MacLean
10:40	*Discussion*	
11:00	*Morning Coffee*	
Session 13 **11:30-14:40**	**Applying Cell Biology to Human Disease**	***Chairs~Christopher Denton and Ariane Herrick***
11:30	Biomarkers of cancer risk and molecular targets for early Intervention	Jill Siegfried
11:55	Therapeutic opportunities offered by reversible ubiquitination	Michael Clague
12:20	*Discussion*	
12:30	**Future perspectives for the 2013 Workshop**	**Carol Black and Robert Lafyatis**
12:40	LUNCH	
Session 14 **1:00-4:00**	**Scleroderma Clinical Trials Consortium**	***Chair~Peter Merkel***
	Update on the EULAR Points to Consider Project	Dinesh Khanna
	Update on the ACR-EULAR Scleroderma Classification Project	Dinesh Khanna
	Interactive Session – Clinical trial design in scleroderma: Where do we go next?	
	Introduction	Peter Merkel
	DISCUSSION Primary outcome for clinical trials: Skin vs. lung vs. composite	Christopher Denton~ Leader
	DISCUSSION Digital ulcers in scleroderma: Healing vs. repair vs. composite	Lori Chung~Leader
	DISCUSSION Pulmonary arterial hypertension in scleroderma	Oliver Distler~Leader
	DISCUSSION Relatively unstudied aspects of scleroderma: Opportunities for study or of lower priority and feasibility?	Daniel Furst~Leader
	DISCUSSION Summary and wrap up: What are the priorities?	Peter Merkel~Leader
	END OF WORKSHOP	

Five Highest Ranking Abstracts

A GWAS follow-up study reveals the association of IL12RB2 gene with Systemic Sclerosis in Caucasian populations

Lara Bossini-Castillo[1], Jose-Ezequiel Martin[1], Jasper Broen[2], Carmen P Simeon[3], Lorenzo Beretta[4], Madelon C Vonk[2], Jose Luis Callejas[5], Ivan Castellví[6], Patricia Carreira[7], Francisco José García-Hernández[8], Monica Fernandez de Castro[9], and the Spanish Scleroderma Group[#], Marieke JH Coenen[10], Gabriela Riemekasten[11], Torsten Witte[12], Nicolas Hunzelman[13],Alexander Kreuter[14], Jörg H.W. Distler[15], Bobby P Koeleman[16], Alexandre E. Voskuyl[17], Annemie J. Schuerwegh[18], Øyvind Palm[19], Roger Hesselstrand[20], Annika Nordin[21], Paolo Airó[22], Claudio Lunardi[23], Raffaella Scorza[4], Paul Shiels[24], Jacob M. van Laar[25], Ariane Herrick[26], Jane Worthington[26],Christopher Denton[27], Filemon K. Tan[28], Frank C. Arnett[28], Sandeep K. Agarwal[28], Shervin Assassi[28],Carmen Fonseca[27], Maureen D. Mayes[28], Timothy RDJ Radstake[2], Javier Martin [1].

1 Instituto de Parasitología y Biomedicina López-Neyra, IPBLN-CSIC, Granada, Spain. 2 Department of Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. 3 Servicio de Medicina Interna, Hospital Valle de Hebron, Barcelona, Spain. 4 IRCCS Fondazione Policlinico-Mangiagalli-Regina Elena & University of Milan, Allergy, Clinical Immunology and Rheumatology, Milan, Italy. 5 Unidad de Enfermedades Sistémicas Autoinmunes, Servicio de Medicina Interna, Hospital Clínico Universitario San Cecilio, Granada, Spain. 6 Servicio de Reumatología, Hospital Sant Pau, Barcelona, Spain. 7 Servicio de Reumatología, Hospital 12 de Octubre, Madrid, Spain. 8 Servicio de Medicina Interna, Hospital Virgen del Rocío, Sevilla, Spain. 9 Servicio de Reumatología, Hospital Puerta del Hierro, Madrid, Spain. 10 Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. 11 Department of Rheumatology and Clinical Immunology, Charité University Hospital, Berlin, Germany. 12 Hannover Medical School, Hannover, Germany. 13 Department of Dermatology, University of Cologne, Cologne, Germany. 14 Ruhr University of Bochum, Bochum, Germany. 15 Department of Internal Medicine 3, Institute for Clinical Immunology, University of Erlangen-Nuremberg, Erlangen, Germany 16 Section Complex Genetics, Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands. 17 Department of Rheumatology, VU University Medical Center, Amsterdam, The Netherlands. 18 Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands. 19 Department of Rheumatology, Rikshospitalet, Oslo University Hospital, Oslo, Norway. 20 Department of Rheumatology, Lund University Hospital, Lund, Sweden. 21 Karolinska Institute, Stockholm, Sweden. 22 Servizio di Reumatologia ed Immunologia Clinica Spedali Civili, Brescia, Italy. 23 Department of Medicine, Università degli Studi di Verona, Verona, Italy. 24 University of Glasgow, Glasgow, United Kingdom. 25 Institute of Cellular Medicine, Newcastle University, Newcastle, United Kingdom.

26 Arthritis Research UK Epidemiology Unit, The University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom. 27 Centre for Rheumatology, Royal Free and University College Medical School, London, United Kingdom. 28 The University of Texas Health Science Center–Houston, Houston, Texas, USA.

A rare polymorphism in Toll Like Receptor 2 is associated with systemic sclerosisphenotype and increases production of inflammatory mediators

Broen JCA,1 Bossini-Castillo L2, Van Bon L1, Vonk MC1, Knaapen H1, Beretta L,3 Rueda B,2, Hesselstrand R,4 Herrick A,5 Worthington J,5 Hunzelman N,6 Denton C,7 Fonseca C, 7, Riemekasten G,8 Kiener H,9 Scorza PhD,3 Simeon CP,10 Ortego-Centeno N (for the Spanish, Systemic Sclerosis group),11 Gonzalez-Gay MA,12 Airo' P,13 Coenen MJH,14 Martin J,2 and Radstake TRDJ1.

Nijmegen Medical Center, The Netherlands. 2Instituto de Parasitología y Biomedicina, CSIC, Granada, Spain. 3Referral Center for Systemic Autoimmune Diseases, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and University of Milan, Italy. 4Department of Rheumatology, Lund University, S-221 85 Lund, Sweden. 5Rheumatic Diseases Centre, University of Manchester, Salford Royal NHS Foundation Trust, UK. 6Department of Dermatology, University of Cologne, Germany. 7Centre for Rheumatology, Royal Free and University College Medical chool, London, United Kingdom. 8Department of Rheumatology and Clinical Immunology, Charité University Hospital and German Rheumatism Research Centre, a Leibniz institute. 9Department of Internal Medicine, Division of Rheumatology, University of Vienna, Austria.10Servicio de Medinina Interna, Hospital Valle de Hebron, Barcelona, Spain. 11Servicio de Medicina Interna, Hospital Universitario Central de Asturias, Oviedo, Spain. 12Servicio de Medicina Interna, Hospital Clinico Universitario, Granada, Spain. 13Servizio di Reumatologia ed Immunologia Clinica, Spedali Civili, Brescia, Italia. 14Department of Human Genetics, Radboud University Nijmegen Medical Center, The Netherlands.

Dermal Fibrosis in Scleroderma: A Systems Biology Analysis of the SSc Fibroblast Secretome

Miltiadis Paliouras [1, 2, 3], Naif Zaman [4], Paresa Giannopoulos [2, 3], Edwin Wang [4], Murray Baron [1, 2, 3, 5,] and Mark Trifiro [1, 2, 3, 6]

Faculty of Medicine, McGill University, Montreal, QC, Canada, [2] Lady Davis Institute for Medical Research- Jewish General Hospital, Montreal, QC, Canada, [3] Canadian Scleroderma Research Group, [4] Biotechnology Research Institute-National Research Council of Canada, Montreal, QC, Canada, [5] Department of Rheumatology, Jewish General Hospital, Montreal, QC, Canada,[6] Department of Endocrinology, Jewish General Hospital, Montreal, QC, Canada

Cardiac fibrosis and microvascular damage are a hallmark of Systemic Sclerosis heart involvement. Prevalence study using cardiac MRI

T.S. Rodriguez-Reyna, K. Montero-Duarte, S. Rosales-Uvera, C. Martínez-Reyes, C. Reyes-Utrera, J. Vazquez-La Madrid, P. Hernández, J. Morales-Blanhir, M. Morelos-Guzman.

Department of Immunology and Rheumatology. National Institute of Medical Sciences and Nutrition.

Vasco de Quiroga 15. Col. Seccion XVI. Tlalpan. Mexico City, Mexico. CP.14000.

A novel cross-talk between endothelin-1 and adhesion signaling promotes pulmonary fibrosis through focal adhesion kinase activation

[1]David Lagares, [1,2]Oscar Busnadiego, [3]Rosa Ana García-Fernández, [4]Mohit Kapoor, [4]Shangxi Liu, [5]David E. Carter, [6]David Abraham, [6]Xu Shi-Wen, [7]Patricia Carreira, [8]Benjamin A Fontaine, [8]Barry S Shea, [8]Andrew M Tager, [4]Andrew Leask, [1]Santiago, Lamas* and [1,2]Fernando Rodríguez-Pascual**

[1]Centro de Biología Molecular Severo Ochoa (CBMSO), Madrid, Spain, [2]Institut Català de Ciències Cardiovasculars (I.C.C.C.), Barcelona, Spain, [3]Facultad de Veterinaria, Universidad Complutense de Madrid (U.C.M.), Madrid, Spain, [4]University of Western Ontario, London, Ontario, Canada, [5]London Regional Genomics Centre, London, Ontario, Canada, [6]University College London, London, United Kingdom, [7]Hospital Universitario 12 de Octubre, Madrid, Spain.

[8]Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

1. Probiotics for the treatment of Systemic Sclerosis-associated gastrointestinal bloating/distention

T. Frech[1], D. Khanna[2], P. Maranian[2], E. Frech[3], A. Sawitzke[1], M. Murtaugh[4]

[1] Division of Rheumatology, Department of Internal Medicine, University of Utah, Salt Lake City, UT; [2]Division of Rheumatology, David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Mountain West Gastroenterology, Salt Lake City, UT; [4]Division of Epidemiology, Department of Internal Medicine, University of Utah, Salt Lake City, 84132 USA

Background: Gastrointestinal tract (GIT) involvement occurs in approximately 90% of patients with systemic sclerosis (SSc) and is characterized by varying degrees of inflammation, vascular damage, and fibrosis in both the upper and lower GIT [1]. Treatment for SSc is challenging as no immunosuppressive or anti-fibrotic therapy is available for GIT disease with clearly proven efficacy. As such, for GIT disease a focus on symptomatic relief, with anti-reflux measures, rotating antibiotics, and pro-kinetics, is the standard of care [2].

Probiotics are viable, nonpathogenic microorganisms that are able to reach the intestines in sufficient numbers to confer benefit to the host [3]. Disruption of the epithelium barrier can lead to loss of immune tolerance to the microbiota and an inappropriate inflammatory response in the GIT. Of interest, the microbiota instruct immune cells, guides their proper assembly, and contributes to the proper functioning of immunologic inductive sites [4]. The ability of GIT microbiota to modulate the immune system of both local and systemic levels makes the use of probiotics in SSc of interest. Our hypothesis is that GIT symptoms in SSc patients with moderate bloating (a symptom of disturbed GIT homeostasis) would improve with probiotic implementation.

Materials and Methods: Patients were recruited from the University of Utah SSc Clinic. All patients completed a University of California Los Angeles Scleroderma Clinical Trials Consortium Gastrointestinal Tract 2.0 (UCLA SCTC GIT 2.0), http://uclascleroderma.researchcore.org. This 34-item instrument has seven scales: reflux, distention/bloating, diarrhea, fecal soilage, constipation, emotional well-being, and social functioning and a total GI score (a higher score denoted worse HRQOL). Ten patients with a moderate-to- severe distention/ bloating score (1.25-3.00), but otherwise stable disease not requiring any medication adjustment or clinical intervention were offered either Align (bifidobacterium infantis; 10^9 CFU per capsule) or Culturelle (lactobacillus GG;10^9 CFU per capsule). After two months of probiotic initiation, GIT 2.0 was re administered. All analyses were performed using STATA 10.2. We compared 2 samples using paired-t test and calculated effect size. Effect sizes were interpreted as follows: 0.20-0.49 as small, 0.50-0.79 as moderate and >0.80 as large. **Results:**

GIT scales	Before Probiotic Mean (SD)	After 2 Month of Probiotic Therapy - Mean (SD)	Effect Size
Total (0-2)	0.73 (0.35)	0.43 (0.29)**	0.82
Reflux (0-3)	0.74 (0.56)	0.64 (0.48)*	0.18
Bloating/Distention (0-2)	2.15 (0.67)	0.97 (0.77)**	`s
Soilage (0-3)	0.20 (0.42)	0.10 (0.32)	0.24
Diarrhea (0-2)	0.20 (0.42)	0.35 (0.53)	0.36
Constipation (0-2.5)	0.72 (0.89)	0.42 (0.55)	0.34
Social (0-2)	0.30 (0.41)	0.22 (0.42)	0.20
Emotional (0-2)	0.59 (0.87)	0.30 (0.57)*	0.33

*p <0.05 **p<0.01

Conclusions: This pilot study suggests probiotics significantly improve the reflux, distention bloating, and total GIT scales in SSc patients. As hypothesized, the largest effect was seen in distention/ bloating scale (effect size 1.76). Probiotics may be important for modulating SSc pathogenesis.

2. Telomere Length in Systemic Sclerosis Patients by Multiplex Quantitative PCR

T. Frech, N. Hatton, B. Markewitz, R. Cawthon, M. B. Scholand, and A. Sawitzke

University of Utah, Department of Internal Medicine/Divisions of Rheumatology and Pulmonary/Critical Care, 50 N Medical Drive, Salt Lake City, UT, 84132, U.S.A.

Background: An important determinant of cellular senescence is telomere length. There have been contrasting reports of telomere length in SSc. Artlett and colleagues reported a decrease telomere length in a combined cohort of limited SSc (lSSc) and diffuse SSc (dSSc), whereas MacIntyre and colleagues reported increased telomere length and lack of age-related telomere erosion in lSSc (Artlett, 1996; MacIntyre, 2008). For this pilot study, we explore the association of telomere length with clinical features in SSc patients.

Materials and Methods: For this pilot study, we used a monochrome multiplex quantitative PCR (MMQPCR) method to evaluate the relative telomere lengths (t/s ratios) in DNA samples of 33 lSSc (6 male; 27 female) and 10 dSSc (4 male; 6 female) ages 23-81 years, and 107 gender and age-matched healthy controls (HC) ages 23-81years. Analysis of variance (ANOVA) using R was used to assess t/s ratio in lSSc, dSSc, and HC. Gender, age, length of diagnosis (> or < 7 years), presence of interstitial lung disease (ILD), vascular involvement (defined as presence of digital ulcers, renal crisis, or pulmonary hypertension), and serology (ANA, SCL 70, centromeric, PM-1, and RNA polymerase III antibodies) were tested for by comparing the F test statistic.

Results: The average age of patients was 57 years with 38% having vascular involvement and 44% having ILD. The average t/s ratio is 1.406 (SD 0.67) for HC, 1.13 (0.155) for dSSc and 1.148 (0.266) for lSSc patients. The only factor statistically associated to t/s is diagnosis of SSc (p=0.04). A trend towards significance is seen for gender (p=0.06). Serology, presence of ILD or vascular involvement, and length of time of diagnosis (> or < 7 years) were not statistically associated to t/s.

Conclusions: This pilot study suggests that there is a possible difference in relative telomere length in SSc patients and HC. This may be important for disease pathogenesis. Serology, presence of ILD and/or vascular disease, and length of time of diagnosis do not correlate with telomere length. However, future studies should look at a larger SSc population as our reported findings may be due to type 2 error.

3. Comparison of two methods for the assessment of digital microvascular function following cold challenge: Evaluation of the validity and reliability of laser contrast speckle imaging

Pauling J D [1], Shipley JA [1], Raper S [2], Watson M L[3], Ward S G[3], Harris N D [4], McHugh NJ [1, 3]

[1] Royal National Hospital for Rheumatic Diseases, Bath, UK, [2] Department of Mathematical Sciences, University of Bath, UK, [3] Department of Pharmacy and Pharmacology, University of Bath, UK, [4] Department for Health, University of Bath, UK

Background: Laser contrast speckle imaging (LCSI) is a novel non-invasive microvascular imaging modality that has enormous potential for the assessment of vascular dysfunction in scleroderma. We evaluated the validity and reliability of LCSI for the dynamic assessment of digital microvascular function in healthy volunteers.

Materials and methods: Subjects attended 3 times. Simultaneous assessment using LCSI and IRT of perfusion at 3 regions was undertaken (the dorsal and volar aspects of the 3rd fingertips of the right and left hands respectively, and the dorsal aspect of the right 3rd finger middle phalanx). Images were taken at baseline and at 13s intervals for 15 minutes following a standardised local cold challenge. Endpoints included absolute measurements at baseline and following cold challenge, and characteristics of the re-warming curves (maximum % recovery and maximum gradient rewarming). Visits 1 and 2 were undertaken under identical conditions (ambient temperature 23°C) to assess reproducibility. Visit 3 was undertaken at a lower room temperature of 18°C to evaluate responsiveness to total body cooling.

Results: Fourteen healthy participants completed the study. There was greater variability in data generated using LCSI compared with IRT, reflecting greater sensitivity of LCSI to physiological variation and movement artefact. LCSI and IRT correlated well at baseline and following cold challenge for all endpoints (r_s for pooled data between 0.5 to 0.65, p<0.00005). Reproducibility of both IRT and LCSI was excellent (ICCs > 0.75) for absolute assessments, but lower for re-warming curve characteristics. LCSI provides greater spatial resolution than IRT potentially identifying variation in cutaneous perfusion within the digits related to the presence of arteriovenous anastamoses (AVAs). Both techniques were responsive to cooling. Effect sizes were greatest for IRT than LCSI (e.g. -1.17 vs. -0.85 at ROI 1 at baseline), possibly reflecting heat transfer rather than altered vascular perfusion.

Conclusions: In the assessment of digital vascular function, LCSI correlates well with IRT, is reproducible and responsive to total body cooling. Absolute measurements appear preferable to parameters derived from re-warming curve characteristics following cold challenge. The improved temporal and spatial resolution of LCSI may facilitate the development of novel assessment tools of digital cutaneous perfusion and AVA function in scleroderma.

4. Dermal Fibrosis in Scleroderma: A Systems Biology Analysis of the SSc Fibroblast Secretome

Miltiadis Paliouras [1, 2, 3], Naif Zaman [4], Paresa Giannopoulos [2, 3], Edwin Wang [4], Murray Baron [1, 2, 3, 5], and Mark Trifiro [1, 2, 3, 6, 1]

Faculty of Medicine, McGill University, Montreal, QC, Canada, [2] Lady Davis Institute for Medical Research- Jewish General Hospital, Montreal, QC, Canada, [3] Canadian Scleroderma Research Group, [4] Biotechnology Research Institute-National Research Council of Canada, Montreal, QC, Canada, [5] Department of Rheumatology, Jewish General Hospital, Montreal, QC, Canada,[6] Department of Endocrinology, Jewish General Hospital, Montreal, QC, Canada

Background: Pathologically, a principal abnormalitiy in SSc is described as an accumulation of excess extracellular matrix (ECM) secreted by fibroblasts. Our objective is to provide new insights into the molecular pathology of fibrosis in SSc by profiling the secretomes of sera-stimulated cultured diseased fibroblasts. A complete description of the secretome will be discerned by employing a comparative systems biology approach to gain a clearer understanding of the current "machinery" status of fibroblasts pointing to specific activated/dyregulated pathways.

Methodology: *Stimulation/Mass spectrometry:* Stimulation experiments begin with serum starving SSc fibroblast cells for 18 hours. Following serum starvation, samples of the media are collected, and then the cells are stimulated with the subject's own serum for 1hr. After the stimulation, serum-free media was then added and samples collected after a 3hr time point. These supernatant collections will be analyzed mass spectrometry.

Systems biology: To establish subnetwork modules with statistically significant biological functions and processes of our proteomic data, we utilizing a self-constructed protein-interaction database based on functional gene ontological information of each interacting protein. We then comparatively examined the many existing databanks from the substantial public datasets, predominantly derived from different relevant tissue specimens, which are in most cases are microarray/gene expression studies, to determine clinical relevance.

Results: Critically our interaction cluster analysis showed: i) the clinical significance of acquired phenotypes of clinically uninvolved, post-stimulated fibroblasts; and ii) the proteins represented within the clusters analysis include MMPs, TIMPs, cathepsins, cystatins, and ECM proteins, all involved directly in the remodelling of the extracellular microenvironment and linked to fibrosis. To determine the clinical relevance of our SSc protein interaction clusters we assessed and found the transcripts of these proteins in our characterized subnetwork clusters to be enriched in their expression in diseased vs. normal skin tissues in the Milano and colleagues 2008 dataset.

Conclusions: Intriguingly, our data so far suggests that the skin fibrosis phenotype observed in SSc may be a result of dysregulated function of secreted proteases (MMPs and cathepsins) and their endogenous inhibitors (TIMPs and cystatins), leading to abnormal accumulation of ECM. Thus, the amount of ECM in the tissue might be controlled through balance among the ECM production, the ECM degradation by secreted proteases and their inhibition by tissue inhibitors. Too little or too much accumulation of ECM may result in organ fibrosis.

5. Phosphodiesterase inhibitors in the treatment of vascular dysfunction in patients with collagen vascular diseases

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Background: Phosphodiesterase (PDE) inhibitors are enzymes that degrade cellular cAMP and cGMP and are essential for regulating the cyclic nucleotides.

Several reports support a role of c AMP in atherogenesis (vascular inflammations) by modulating endothelial functions , productions of reactive oxygen species, controlling the expression of proinflammatory molecules, expression of metalloproteinases .Progressive widespread vascular dysfunction is mentioned as one important initiating step in pathogenesis and evolution of collagen vascular disease , some of these disease being associated with accelerated atherosclerosis.

The aim was to asses the clinical and biological improvement in vascular function due to PDE inhibition, in patients with systemic lupus erythematosus (SLE) and systemic sclerosis (SS) optimally treated for their diseases.

Material and methods: The study patients were 10 patients with SS (ARA criteria) without pulmonary hypertension, 10 patients with SLE (ARA criteria) and 5 healthy subjects. They were optimally treated for each disease according to European guidelines and also they received Pentoxifilin 1000mg- 1500mg (iv 7days and oral 1 -month).

The established parameters to be measured were :intima media thickness-(IMT) by vascular ultrasonography, diastolic dysfunction of left ventricle assessed by ecocardiography, flow mediated dilatation(FMD)- brachial artery, inflammatory usual tests, attested markers for vascular risk and serum antioxidant capacity :lipoprotein-associated phospholipase A2 (Lp-PLA2),endothelin-1(ET-1),paraoxonase(PON).

Results: Patients with SSc display a more pronounced inflammatory vascular disease than SLE patients, having higher levels of Lp-PLA2, ET-1, and lower levels of PON.Moderately but sustained decreased serum levels are observed after pentoxifilin treatment in these patients (SS,SLE). The vascular involvement includes microvascular and macrovascular changes with progressive formation of thickened neointima.Mean IMT in SSc patients is thicker than in SLE and FMD- is severe depressed in SSc patients.

Conclusions: 1.Collagen vascular disease represent an increased risk of premature atherosclerosis 2.Inhibition of PDE activity by a non selective molecule (Pentoxifilin) is associated with reduction of inflammation related molecules 3.PDEs inhibitors exibit remarkable hemodinamic and inotropic properties, and cytoprotective effects 4.PDEs inhibitors may be a valuable adiacent therapy in patients with collagen vascular diseases.

6. Serum lactate dehydrogenase activity – a possible parameter for vascular involvement in patients with systemic sclerosis

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Background: Vascular abnormalities are a major component of systemic sclerosis (SSc) and microvascular dysfunction is considered to be a key in the pathogenesis of Sc and its complications.

Lactate dehydrogenase release into the extracellular medium is used as a biochemical parameter of cell membrane damage and also as marker for angiogenesis inducing in EC expression of VEGF and modulating its angiogenic potency.

The objective was to evaluate serum LDH activity in SSc patients in relation with biological markers of disease activity and vascular involvement.

Material and methods: We studied 25 patients with SSc (fulfilling ARA diagnostic criteria) 2 males and 23 females and 10 healthy subjects. Patients underwent clinical examination, routine laboratory and immunological tests, echocardiography ,vascular ultrasound assessment , capilaroscopy) Patients were followed-up for a mean period of 5years .LDH activity was measured in absence of myopathy or other factors inducing muscular lesions.There were been assessed some inflammatory markers secreted by cells known to be involved in vascular scleroderma lesions: lipoprotein associated phospholipase A2 (Lp-PLA2), mieloperoxidase (MPO) and p-Selectin – marker for platelet activation.

Results: Serum LDH activity was increased in 82% SSc patients Significant correlations were observed between LDH activity and sP-selectin ($p < 0,01$) , LDH activity and LpPl-A2 ($p<0,01$), Increased serum LDH activity were related to early stages of digital vasculopathy , diastolic left ventricular dysfunction , increased Rodnan score and low FMD ($p<0.01$) No relationship was found between LDH activity and pulmonary involvement . No significant correlation was observed between macrovascular involvement, presence of autoantibodies, and common markers of disease activity (CRP, ESR). In 47% patients LDH activity decrease during treatment with steroids and statins

Conclusions: Increased serum activity of LDH and its correlation with sP-selectin, endothelial dysfunction, digital vasculopathy, diastolic myocardial dysfunction in SSc patients may indicate the important participation of platelets in disease progression, and may be also a possible parameter of microvascular disturbances .Further studies are needed to assess the value of LDH as a simple parameter of angiogenesis.

7. When is a autologous bone marrow transplantation indicated in the treatment of juvenile systemic sclerosis? Results of a multinational survey of Pediatric Rheumatologist

Ivan Foeldvari, Angela Wierk, Dominique Farge

The five year survival of juvenile systemic sclerosis (jSSc) is around 95%. Patients, who died in the two retrospective cohorts, died mostly in the first 24 months of disease course. Autologous bone marrow transplantation (ABMT) seems to be a promising therapeutic approach for adult patients (ASTIS (EU) and SCOT (USA) Trial) with severe disease course. Around 8 patients with jSSc are transplanted according the EBMT registry. Currently no consent based inclusion or exclusion criteria for ABMT in jSSc exists.

Aim of the survey was to get a feeling from paediatric rheumatologists, when they would apply autologous bone marrow transplantation as a treatment option.

Paediatric Rheumatologist - members of the PRES Juvenile Scleroderma Working Group and participants of the Paediatric Rheumatology E-mail Board were asked via Internet to fill out the survey.

22 centres responded, all of them were academic centres. BMT would be considered for 14 of the 22 colleagues after non-response to cyclophosphamide, 10 of 22 after non response to two DMARDs and 12 of 22 after non-response to Rituximab. 19 of 22 would consider transplantation if the CHAQ score ≤ 2, 20 of 22 if the CHQ is less than 40%. 21 of 22 would think about transplantation if the modified Rodnan skin score is more than 30 and 15 of 22 if the DLCO is less than 50%, 18 of 22 if the WHO functional class is 3, 14 of 22 if the FVC less than 60%, 15 of 22 if the pulmonary arterial pressure more than 40 mm/hg and 11 of 22 if left ventricular ejection fraction is less than 40%.

This survey represents an impression, when pediatric rheumatologist would consider ABMT. It is a starting point for a possible evolving ABMT program for this orphan disease involved in vascular scleroderma lesions: lipoprotein associated phospholipase A2 (Lp-PLA2), mieloperoxidase (MPO) and p-Selectin – marker for platelet activation

Results: Serum LDH activity was increased in 82% SSc patients Significant correlations were observed between LDH activity and sP-selectin ($p < 0{,}01$), LDH activity and LpPl-A2 ($p<0{,}01$), Increased serum LDH activity were related to early stages of digital vasculopathy , diastolic left ventricular dysfunction , increased Rodnan score and low FMD ($p<0.01$) No relationship was found between LDH activity and pulmonary involvement . No significant correlation was observed between macrovascular involvement, presence of autoantibodies, and common markers of disease activity (CRP, ESR). In 47% patients LDH activity decrease during treatment with steroids and statins.

Conclusions: Increased serum activity of LDH and its correlation with sP-selectin, endothelial dysfunction, digital vasculopathy, diastolic myocardial dysfunction in SSc patients may indicate the important participation of platelets in disease progression, and may be also a possible parameter of microvascular disturbances .Further studies are needed to assess the value of LDH as a simple parameter of angiogenesis.

8. Preliminary results for 6 minute walk values in healthy German children

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Introduction: 6 minute walk is a primary outcome measure in therapeutic studies for patients with pulmonary hypertension. Currently we have a two of sets of data (Lammers1 et al and Li 2et al) regarding test results in the 6 minute walk test (6MWT) in healthy children with a large span in the norm values in the different age groups.

Aim of the study: To establish norm values for healthy German children for the 6 Minute Walk Test.

Method: The team of an occupational therapist and a study nurse is visiting schools, were previously the parents agreed on the participation of the students on the test. Always just students from one class are invited to participate in the test. The students are performing the test according the international guidelines. The demographic data of the students are collected and the parents fill out a short survey regarding the physical activity and the health condition. Children with chronic diseases, which decrease the stamina are excluded.

Results: Up till now 354 students participated from the age 7 to 12 years. 22 in the age group 6; 49 in the age group of 7; 61 in the age group of 8 years; 64 in the age group of 9 years ; 50 in the age group of 10 years; 51 in the age group of 11 and 57 in the age group of 12. The mean 6 minute walk distance was 449.1 m in the age group of 6, 470 m in the age group of 7; 484 m in the age group of 8 ; 491,6 m in the age group of 9 ; 471,3 m in the age group of 10, 571 m in the age group of 11 and 502,3 m in the age group of 12 years. BMI correlated with the walked distance.

Conclusion: Our results are in the range of the patients from the UK published by Lammers et al 1 and are in significantly lower range than in the Chinese population collected data by Li et al.2. This reflects the importance of this study to gain norm values for our patient population.

References: (1) Lammers et al. Arch Dis Child 2008, 93:464, (2) Am J respir Crit Care Med 2007, 176:174.

9. FAK/src signaling is required for fibrogenic gene expression in normal and scleroderma fibroblasts

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Background: Scleroderma is an autoimmune connective tissue disease of unknown cause, which does not have any treatment. The cell type responsible for fibrosis is a specialized form of fibroblast called the myofibroblast, a highly contractile cell type characterized by activated adhesive signaling. Focal adhesion kinase (FAK)/src mediates adhesive signaling and shows elevated activity in lesional scleroderma fibroblasts.

Objective: To use the FAK/src inhibitor PP2 and FAK knockout fibroblasts to address whether FAK/src contributes to fibrotic gene expression in normal and scleroderma fibroblasts.

Results: Using mRNA (real-time polymerase chain reaction), protein (Western blot) and collagen gel contraction analyses, we showed that, compared to FAK wild-type fibroblasts, FAK knockout fibroblasts or FAK wt cells treated with PP2 (10uM) showed decreased expression of seven major fibrotic genes including collagen type 1, α-smooth muscle actin and CCN2/CTGF. Normal and scleroderma fibroblasts treated with PP2 showed reduced expression of the same genes, and diminished ability to contract a collagen gel matrix.

Conclusions: Blocking FAK signal decreased the levels of several major fibrotic genes and the contractile activity of fibroblasts; FAK inhibitors might be used to control the fibrotic phenotype of lesional fibroblasts in scleroderma.

10. Plasma levels of Angiopoietins 1 and 2 and Tie-2 in Scleroderma

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Background: Vascular injury, tissue hypoxia and insufficient or absent angiogenesis are hallmarks of scleroderma (SSc). The endothelial cell specific receptor tyrosine kinases Tie-1 and Tie-2, and their ligands Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2), critically regulate both vasculogenesis and angiogenesis. Ang-1 mediated Tie-2 signaling is the default adult pathway to control vascular quiescence. Ang-2 antagonizes Ang-1 and responds to endothelial stimulation by several factors including VEGF and hypoxia. Ang-2 may control the transition from resting to activated endothelium and so facilitate the inflammatory response. Ang-2 inhibits Tie-2 signaling and may result in vessel destabilization and regression. The purpose of this study is to assess the associations of Ang-1, Ang-2, and Tie-2 with other angiogenic factors and clinical parameters reflecting the amount of vasculopathy and tissue damage.

Materials and methods: 40 patients with SSc were evaluated and compared to age-matched controls. Plasma levels of soluble sAng-1, sAng-2 and sTie-2 as well as vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and endostatin were measured. Associations between these factors and clinical parameters were assessed.

Results: sAng-1 levels in SSc cases with limited cutaneous disease (lcSSc) were similar to their controls (p=0.3468) but cases with diffuse cutaneous disease (dcSSc) were not (p=0.0026). sAng-2 was higher compared to controls for both lcSSc (p=0.0001) and dcSSc (p=0.0001) and correlated with the modified Rodnan skin score (Spearman r=0.32, p=0.05). In lcSSc cases, sAng-2 correlated with disease duration (r=-0.39, p=0.05), estimated PAP (r=0.34, p=0.01) and DLCO (r=-0.42, p=0.02). Ang-2 correlated with upper body telangiectasia (r=0.39, p=0.0004) and capillary dropout (r=0.33, p=0.044). The ratio of sAng-2 to sAng-1 was higher in lcSSc (p=0.0024) and dcSSc (p=0.0215) compared to their controls. sTie-2 was higher in lcSSc (p=0.0003) and dcSSc (p=0.0125) compared to their controls. sTie-2 was negatively correlated with disease activity, severity and duration. Pro-inflammatory angiogenesis factors VEGF, PlGF and endostatin were elevated in SSc and correlated with Ang-1 (VEGF and PlGF) and Ang-2 levels (PlGF and endostatin).

Conclusions: Levels of sTie-2 are higher in lcSSc only but all SSc cases have elevated levels of sAng-2, suggesting a pro-inflammatory state with the angiogenic balance tilted to an active endothelium.

11. Regulation of CXCR4/CXCXL12-related migration by caveolin-1 in scleroderma

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Background: Scleroderma (SSc) is a complex autoimmune disease characterized by inflammation and fibrosis of the skin, lungs and other organs. Inflammatory cells respond to chemotactic stimuli produced by damaged tissue by displaying a complex repertoire of motility-associated processes and accumulating in the damaged tissue. Because SSc PBM contain much less caveolin-1 and much more CXCR4 than normal PBM and this phenotype is replicated by TGFβ treatment of normal PBM, we compared SSc PBM, control PBM, and TGFβ-treated control PBM in terms of: 1) Migration toward the CXCR4 ligand CXCL12; 2) Motility-associated processes (F-actin polymerization, expression of CXCR4 and its co-receptors CD14, TLR2, and TLR4); and 3) The effect of restoring caveolin activity with the caveolin-1 scaffolding domain peptide (CSD) on these processes.

Materials and Methods: PBM from healthy donors and from SSc patients were isolated by negative selection. Polymerized F-actin was detected using fluorescent phalloidin. Cell migration was assessed in response to CXCL12 +/- TGFβ priming and +/- CSD treatment. TLR2, TLR4, and CD14 levels were estimated by Western blotting.

Results: Phalloidin staining was more intense in SSc and TGFβ-treated PBM than in control cells and was inhibited by CSD. CXCR4 overexpression in SSc and in TGFβ-treated PBM was also reversed by CSD. The percentage of SSc and TGFβ-treated PBM that migrated toward CXCL12 was more than seven-fold enhanced compared to normal PBM and was again inhibited > 80 % by CSD. Finally, when normal PBM were treated with CXCL12, phalloidin staining was greatly enhanced. CSD completely inhibited this effect. To begin to study signaling involving CXCR4 and its co-receptors TLR2, TLR4 and CD14, we examined their expression and found that all three co-receptors are overexpressed on SSc PBM. Moreover, CXCL12 treatment of normal PBM causes co-capping of TLR4 and CD14 that is reversed by CSD.

Conclusions: Caveolin-1 regulates PBM migration in SSc via complex interactions involving the cytoskeleton, several receptors, and their ligands. Lack of caveolin-1 may result in the pathological accumulation of PBM and their derivatives (e.g. fibrocytes) in injured tissue, e.g. fibrotic lung. Current studies further validate CSD as a novel treatment for fibrosing diseases such as SSc.

12. TAK1 signalling is required for fibrotic gene expression in human fibroblasts

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Background: Scars are a normal part of the wound healing process, however too much scarring can cause diseases such as liver cirrhosis, lung fibrosis and the autoimmune disease systemic sclerosis (SSc). Fibrotic diseases, such as SSc, are responsible for nearly 45% of deaths in the developed world (1); there is no cure for fibrosis, and thus finding an appropriate therapy is important. We know that TGFb signalling causes fibrosis, and we believe that TGFb may selectively promote fibrosis through the TGFb Activated Kinase 1 (TAK1) signalling pathway.

Materials and Methods: To identify whether or not the TAK1 pathway is instrumental in the selective modulation of the ability of TGFb to induce expression of profibrotic genes in human dermal fibroblasts we performed Affymetrix genome wide expression profiling of fibroblasts treated with or without TGFb (4 ng/ml, 6 h) in the presence or absence of 5Z-7-Oxozeaenol (300nM).

Results: 331 mRNAs were induced by TGFb (4 ng/ml, 6h) at least 2 fold in the presence of DMSO; of these, 206 were not induced by TGFb when cells were pretreated for 45 minutes with 5Z-7-Oxozeaenol (300nM). DAVID cluster analysis (at least 10 genes/group; $p < 10^{-5}$) revealed that groups of genes involved with cell proliferation, angiogenesis, migration and response to wounding/fibrosis were selectively inhibited by 5Z-7-Oxozeaenol. The gene expression effects were confirmed using real time PCR (RNA) and Western blot (protein) assays and by functional (MTT cell proliferation and scratch/wounding migration) bioassays.

Conclusion: Our results were consistent with the fact that TGFb-induced fibrotic responses are selectively mediated by TAK1. These data support the idea that inhibitors of the TAK1 pathway may be a useful method of treating fibrotic diseases such as scleroderma.

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13. A GWAS follow-up study reveals the association of *IL12RB2* gene with Systemic Sclerosis in Caucasian populations

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Objetive: A single nucleotide polymorphism (SNP) at the *IL12RB2* locus showed a suggestive association signal in a previously published GWAS in systemic sclerosis (SSc). Interestingly, *IL12RB2* encodes IL-12Rβ2, which constitutes the transducing component of the IL-12 receptor (IL-12R). Moreover, the IL-12 pathway plays a key role in autoimmune inflammation and *IL12RB2* polymorphisms have been associated with several autoimmune diseases. Aiming to reveal the possible implication of *IL12RB2* gene in SSc, we conducted fine-mapping GWAS follow-up study in different Caucasian cohorts.

Patients and Methods: The whole analyzed set comprised 15,474 individuals of Caucasian Ancestry (5,991 SSc patients / 9,483 controls). Ten GWAS genotyped single nucleotide polymorphisms (SNPs) in the *IL12RB2* region were analyzed. Then, we included three relevant SNPs in a first follow-up step comprising 7,192 European individuals (3,344 SSc / 3,848 controls). Only the most associated SNP was considered for a second follow-up phase comprising 1,736 US individuals (597 SSc / 1,139 controls). Both follow-up cohorts were genotyped using TaqMan SNP genotyping assays in a real-time PCR System.

Results: After conditioned logistic regression analysis we selected rs3790567 ($P_{MH} = 1.92x10^{-5}$ OR = 1.19) as the genetic variant underlying the observed GWAS peak. After the first follow-up phase, only the association of rs3790567 was consistent ($P_{MH} = 4.84x10^{-3}$ OR = 1.12). Interestingly, the second follow-up phase confirmed this finding ($P_{\chi 2} = 2.82x10^{-4}$ OR = 1.34). It is remarkable that rs3790567 pooled analysis in the whole set of individuals reached a highly statistically significant association ($P_{MH} = 2.82x10^{-9}$ OR = 1.17).

Conclusion: Our data clearly support *IL12RB2* rs3790567 association with SSc, and suggest a relevant role of IL-12 signaling pathway in SSc pathogenesis.

14. A novel cross-talk between endothelin-1 and adhesion signaling promotes pulmonary fibrosis through focal adhesion kinase activation

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Background/Aims: Lung fibrosis, characterised by increased extracellular matrix (ECM) deposition and matrix contraction promoted by activated resident fibroblasts, is a hallmark feature of scleroderma patients. ECM deposition including collagen type I and fibronectin leads to fibroblast activation. Persistence of activated adhesion and adhesive signaling, including focal adhesion kinase (FAK) activation, is an essential characteristicof fibrotic cells. Endothelin-1 (ET-1) has been implicated as an important contributor to fibrosis. However, the potential interplay between adhesive signaling cascades and cellular responses to profibrotic factors remains poorly understood. We hypothesized that FAK serves as a "common signaling node" that controls mechanical cues from the ECM and signals from profibrotic mediators such as endothelin-1 to promote myofibroblast differentiation and lung fibrogenesis.

***Methods*:** The role of FAK in myofibroblast differentiation was examined in vitro using wild type (WT) and FAK-deficient (KO) mouse embryonic fibroblasts upon ET-1 stimulation. FAK activation was tested by western blot (WB). FAK localization within lipid rafts domains was determined by sucrose gradient ultracentrifugation and immunofluorescence (IF). Profibrotic gene expression was assessed by microarray analysis and real-time PCR. In vivo, the therapeutic potential of FAK was investigated in the bleomycin-induced lung fibrosis model. For human samples, FAK expression and activity in lung sections from SSc patients were studied by immunohistochemistry (IHC).

Results: ET-1 induced FAK phosphorylation in a time- and dose-dependent manner as determined by WB. Real time PCR and microarray analysis of WT or KO FAK fibroblasts showed that ET-1 signaling requires FAK to induce a profibrotic gene expression program. Accordingly, the ET-1 receptor antagonist bosentan, reversed the increase in FAK phosphorylation observed in lung fibroblasts from SSc patients. Immunofluorescence studies revealed that activated FAK localized in the vicinity of endothelin receptor A ETAR in the plasmalemma. Consistently, studies in caveolin-enriched fractions demonstrated that FAK is recruited to ETAR to form a trimeric complex with β1-Integrin upon ET-1 stimulation. Furthermore, FAK was immunoprecipitated with ETAR and β1-Integrin, suggesting that both ETAR and β1-Integrin signaling are required to promote FAK activation in confined plasma microdomains. FAK activation by ET-1 was impaired in β1-Integrin-deficient fibroblasts as tested by WB, thus suggesting that this process requires ECM-mediated signaling through β1-Integrin. By using an in vivo model of bleomycin-induced lung injury, we observed that FAK became activated and inhibition of FAK expression by siRNA or pharmacological targeting of FAK with PF-562,271 (Pfizer) resulted in marked abrogation of lung fibrogenesis as quantitated by IHC and collagen content. Finally, FAK and p-FAK were detected in fibrotic foci and were found to be preferentially co-expressed with α-SMA as investigated by IHC in lung sections from SSc patients

Summary (Opcional): FAK functions as a central mediator of fibrogenesis, acting as a key point of convergence integrating cell matrix-adhesion and ET-1 signaling. It is up-regulated in systemic sclerosis and mediates profibrotic responses.

Conclusion: FAK is crucially involved in myofibroblast differentiation and pharmacological or genetic inhibition of FAK results in marked attenuation of experimental lung fibrosis. Thus, targeting FAK signaling pathway may represent a potential therapeutic avenue for the control of fibrosis.

15. Identification of novel genetic markers associated with clinical phenotypes and auto-antibody subsets of systemic sclerosis through a genome wide association strategy

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Background: The aim of this study was to determine the genetic components contributing to different systemic sclerosis (SSc) clinical sub-phenotypes of limited (lcSSc) and diffuse (dcSSc) cutaneous involvement, and with the SSc-specific auto-antibodies, anti-centromere (ACA) and antitopoisomerase I (ATA) through a genome-wide association study (GWAS).

Materials and methods: Four GWAS cohorts, comprising 2,296 SSc patients and 5,171 healthy controls, were meta-analyzed looking for associations in the selected subgroups. Eighteen polymorphisms were further tested in nine independent cohorts comprising an additional 3,175 SSc patients and 4,971 controls. Conditional analysis for associated SNPs in the HLA region was performed to explore their independent association in antibody subgroups.

Results: Overall analysis showed that non-HLA polymorphism rs11642873 in *IRF8* gene to be associated at GWAS level with lcSSc ($P = 2.32 \times 10^{-12}$, OR = 0.75). Also, rs12540874 in *GRB10* gene ($P = 1.27 \times 10^{-6}$, OR = 1.15) and rs11047102 in *SOX5* gene ($P = 1.39 \times 10^{-7}$, OR = 1.36) showed a suggestive association with lcSSc and ACA subgroups respectively. In the HLA region, we observed highly associated allelic combinations in the *HLA-DQB1* locus with ACA ($P = 1.79 \times 10^{-61}$, OR = 2.48), in the *HLA-DPA1/B1* loci with ATA ($P = 4.57 \times 10^{-76}$, OR = 8.84) and in *NOTCH4* with ACA $P = 8.84 \times 10^{-21}$, OR = 0.55) and ATA ($P = 1.14 \times 10^{-8}$, OR = 0.54).

Conclusion: We have identified three new non-HLA genes (*IRF8*, *GRB10*, and *SOX5*) associated with SSc clinical and auto-antibody subgroups. Within the HLA region, *HLA-DQB1*, *HLADPA1/B1* and *NOTCH4* associations with SSc are likely confined to specific auto-antibodies. These data emphasize the differential genetic components of subphenotypes of SSc.

16. Prognostic factors of mortality and 2-year survival analysis of systemic sclerosis with pulmonary arterial hypertension in Thailand

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Background: Pulmonary arterial hypertension (PAH) is a major complication and cause of death in systemic sclerosis (SSc) but natural history of PAH without any treatment has been not identified. Our objective was to identify the predictive factors of mortality and the 2-year survival rate among Thai sufferers of PAH-SSc.

Materials and methods: An historical cohort study was performed among PAH-SSc patients followed up at Srinagarind Hospital, Thailand, between January 2005 and December 2008. Kaplan-Meier and Cox regression analyses were used to estimate the probability of survival and to assess the significant factors associated with death.

Results: PAH was recognized in 60 patients using echocardiographic criteria, right ventricular systolic pressure (RVSP) > 35 mmHg. Two-thirds of the patients were female, > 50 years of age, with the diffuse SSc subtype. Twenty patients (33.3%) died: The mortality rate was 15.6% per 100 person-years. The respective 1-, 2-, 3- and 4-year survival rates were 86.1%, 71.3%, 64.6% and 53.9%. The majority (85%) died without any specific treatment for PAH. Using univariate analysis, the mortality risk was associated with: the World Health Organization functional class (FC) III (Hazard ratio (HR) 27.82, 95%CI 3.17-244.40), visceral organ involvement (HR 5.14, 95%CI 1.19-22.22), esophageal dysmotility (HR 3.08, 95%CI 1.13-8.77) and pericardial effusion (HR 2.84, 95%CI 1.12-7.16). Using Cox regression, the only predictor of death was FCIII. The causes of death in PAH-SSc were related to PAH (60%), infection (30%) and acute renal failure (10%).

Conclusions: Up to one-third of Thai sufferers of PAH-SSc died within 2 years of PAH diagnosis, without any specific treatment being given. Increased mortality risk was found in SSc patients who had FCIII and visceral organ involvement.

17. Pattern of skin thickness progression and clinical correlation in Thai scleroderma patients

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Background: Skin thickness progression in scleroderma (SSc) varies in daily clinical practice observation.

Materials and methods: Our objectives were to define the pattern of skin thickness in SSc and to ascertain the clinical correlation with each skin pattern. An historical cohort with a 3-year follow-up was performed on patients over 15 years of age in Khon Kaen, Thailand, between January 1, 2005 and December 31, 2006. The skin thickness progression rate (STPR) and skin thickness regression rate (STRR) were calculated by the difference in modified Rodnan skin score between the first diagnosis and at the end of follow-up divided by the time of follow up. 'Rapid skin progression' was defined as STPR ≥45 points per year, while intermediate and slow progression was 25-44 and <25, respectively. The rapid skin regression rate (STRR) was STRR ≥45 points per year while intermediate and slow regression were 25-44 and <25, respectively. The Chi-square or Fisher's exact test and student *t* test or Kruskal-Wallis test were used to analyze the association between the clinical characteristics and pattern of skin progression.

Results: SSc cases (117) were included and the female to male ratio was 70**:**47. The mean age of onset was 49.8 years (range, 24.4-75.5). The most common skin pattern was (a) 'slow progression to peak then slow regression' (77 cases; 65.8%) followed by (b) 'continuous slow progression' (37; 31.6%) (c) 'continuous intermediate progression' (2; 1.7%) and (d) 'slow progression to peak then intermediate regression' (1; 0.9%). The respective mean peak skin score and duration of disease at peak skin score was 19.8 points (range, 4-45) and 20.3 months (range, 1-42.2). Only telangiectasia at onset and joints contracture were related to 'continuous slow progression' of skin thickness with p=0.001 and p=0.042, respectively. Neither the type of SSc nor internal organ involvement was correlated with the pattern of skin thickness.

Conclusion: The most common skin pattern in Thai SSc was 'slow progression to peak then slow regression'. Telangiectasia at onset and joint(s) contracture were predictive of continuous progressive skin thickness in the first 3 years.

18. Spontaneous skin regression and predictors of skin regression in Thai scleroderma patients

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Background: Skin tightness is a major clinical manifestation of systemic sclerosis (SSc). Importantly for both clinicians and patients, spontaneous regression of the fibrosis process has been documented.

Materials and methods: Our objectives were to identify the incidence and related clinical characteristics of spontaneous regression among Thai SSc patients. An historical cohort with 4 years of follow-up was performed among SSc patients over 15 years of age diagnosed with SSc between January 1, 2005 and December 31, 2006 in Khon Kaen, Thailand. The start-date was the date of the first symptom and the end-date was the date of the skin score ≤ 2. To estimate the respective probability of regression and to assess the associated factors, the Kaplan-Meier method and Cox regression analysis was used.

Results: 117 cases of SSc were included with a female to male ratio of 1.5**:**1. Thirteen patients (11.1%) experienced regression: the incidence was 0.31 per 100 person-months. The incidence rate of spontaneous skin regression was 0.31 per 100 person-months and the average duration of SSc at the time of regression was 35.9±15.6 months (range, 15.7-60). The factors negatively correlated with regression were (a) diffuse cutaneous type (b) Raynaud's phenomenon (c) esophageal dysmotility and (d) colchicine treatment at onset with a respective hazard ratio (HR) of 0.19, 0.19, 0.26 and 0.20. By contrast, the factor positively correlated with regression was *active alveolitis with cyclophosphamide therapy at onset* with an HR of 4.23 (95%CI 1.23-14.10). After regression analysis, only *Raynaud's phenomenon at onset* and *diffuse cutaneous type* had a significantly negative correlation to regression.

Conclusions: Spontaneous regression of the skin fibrosis process was not uncommon among Thai SSc patients. Factors suggesting a poor predictor for cutaneous manifestation were: *Raynaud's phenomenon*, *diffuse cutaneous type*; while *early cyclophosphamide therapy* might be related to a better skin outcome.

19. Incidence rate and causes of infection in Thai systemic sclerosis patients

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Background: Infection is a cause of death in systemic sclerosis (SSc). The causes of infection could be bacterial, viral, or fungal. Despite of immunosuppressant therapy, there were only few reports of opportunistic infection.

Materials and methods: Our objectives were to estimate the incidence rate of infection, causes of infection, and risk factor of infection in Thai SSc patients. A historical cohort analysis was conducted on SSc patients over 15 years of age, attending the Scleroderma Clinic at Srinagarind Hospital, Khon Kaen University, Khon Kaen, Thailand, between January 1, 2005 and December 31, 2006. The incidence rate with 95%CI was calculated and Odds ratio (OR) was performed to assess the risk of infection and find out the association between type of infection and SSc clinical presentations.

Results: The medical records of 117 SSc patients were reviewed. The female to male ratio was 1.5:1. Of the total 310 person-years under observation, 63 events of infection occurred. The incidence rate of infection was 20.3 per 100 person-years (95%CI 15.6-26.0) and the incidence rate of major infection was 11.0 per 100 person-years (95%CI 8.4-16.5). The mean age and mean duration of SSc at the time of infection was 50.1±11.1 years (range, 25.2-76.6) and 12.9±10.4 months (range, 0.5-34.6), respectively. Urinary tract infection was the most common infection (23.8%), followed by infected ulcer (17.5%) and strongyloidiasis diarrhea (12.7%). Opportunistic infection was found in 1 case (esophageal candidiasis). Esophageal dysmotility was related to a major infection with statistical significant (OR 3.22). There was the clinical association between aspiration pneumonia and esophageal dysmotility (OR 1.23), as well as non-strongyloidiasis diarrhea and gastrointestinal involvement (OR 2.28). However, there was no association between strongyloidiasis diarrhea and gastrointestinal involvement, infected ulcer and Raynaud's phenomenon or digital ulcer, aspiration pneumonia and pulmonary fibrosis, urinary tract infection and renal crisis or female gender, and any infection and immusuppressant therapy or duration of disease. One case was died due to severe bacterial aspiration pneumonia.

Conclusions: Infection was not uncommon in SSc patients, however; opportunisitic infection was rare despite of immunosuppressant therapy. Esophageal dysmotility increased risk factor of major infection particular aspiration pneumonia.

20. Impaired neovascularization capacity of endothelial progenitor cells in systemic sclerosis

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Background: It has been proposed that defective vasculogenesis with reduced and/or dysfunctional endothelial progenitor cells (EPC) plays a role in pathogenesis of systemic sclerosis (SSc), but whether the number of CD45-CD34+CD133+VEGFR2+ 'real' EPC is reduced in SSc patients is a matter of debate. In this study, we established a system for evaluating *in vivo* EPC's capacity to promote neovascularization, and used it to assess functional properties of EPC in SSc patients.

Materials and methods: Peripheral blood samples were obtained from 16 SSc patients (9 diffuse and 7 limited cutaneous SSc) and 12 healthy controls (HC). For evaluating capacity of EPC to promote new blood formation *in vivo*, murine colon carcinoma line CT-26 was transplanted beneath the skin of SCID mice in conjunction with or without immunomagnetically sorted human CD133+ cells. The tumor volume and the number of blood vessels were measured at 10 days. The efficiency of EPC incorporation into the vascular wall was evaluated by double-staining of tumor sections with anti-mouse and anti-human CD31 antibodies.

Results: Co-transplantation of CT-26 with CD133+ cells, but not with CD133- cells, promoted tumor growth. In addition, histological examinations of the tumors showed that co-transplantation of CD133+ cells increased the number of blood vessels compared with transplantation of CT-26 alone. These effects were lost when VEGFR2+ cells were depleted from the transplanted CD133+ cells, indicating a primary role of EPC in neovascularization in our system. Comparisons between SSc and HC showed that tumor growth was slower and blood vessel formation was inferior in the SSc-derived EPC (P = 0.004 and 0.009, respectively), while there was no difference in the proportion of VEGFR2+ cells in the CD133+ cell fraction. The tumor size was correlated with the number of tumor vessels (R = 0.9, P < 0.001). Finally, the tumor vessels incorporating endothelial cells expressing human CD31 but did not express mouse CD31 were fewer in transplantation of SSc-derived versus HC-derived EPC (P = 0.01).

Conclusions: We have established a system to evaluate *in vivo* angiogenic and vasculogenic properties of human EPC. The EPC's ability to be incorporated into vessels and differentiate into endothelial cells is impaired in SSc patients.

21. Local therapy of ischemic lesions with fibrin-conjugated VEGF in UCD-206 chickens, an animal model for systemic sclerosis

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Background: Microvascular damage with intimal proliferation and occlusion of blood vessels is a hallmark of systemic sclerosis (SSc). Vascular repair and angiogenesis are also strongly disturbed. This leads to capillary loss, chronic ischemia, and to clinical manifestations such as fingertip ulcers. Although the expression of vascular endothelial cell growth factor (VEGF) seems to be uncontrolled and chronic, a very high VEGF expression is associated with the lack of fingertip ulcers. Therefore, local administration of VEGF in a form that allows controlled release might be a promising therapeutic approach. The aim of the present study was to proof the concept that cell demanded release of locally applied matrix-bound $VEGF_{121}$ leads to the formation of stable blood vessels, and clinical improvement of ischemic lesions.

Methods: Ischemic comb and neck skin lesions of UCD-206 chickens, an animal model showing all hallmarks of the human disease, were treated locally with $VEGF_{121}$-fibrin, with fibrin alone as a placebo control, or left untreated. After 7 days the clinical outcome was assessed, skin thickness measured, and skin biopsies were taken for further analyses. Perivascular mononuclear cell infiltration was quantitatively analyzed on H&E stained tissue sections using Histoquest. Angiogenesis was studied by indirect immunofluorescence staining using antibodies specific for the endothelial cell marker van Willebrand factor (vWF), and alpha smooth muscle actin (αSMA), which is expressed by pericytes and smooth muscle cells, and serves as a marker for stable blood vessel formation. Endothelial cells, pericytes, and smooth muscle cells were quantified using Tissuequest.

Results: 77,4% of the lesions treated with $VEGF_{121}$-fibrin showed a clear clinical improvement or even complete healing, whereas approximately 80% of the placebo controls showed deterioration. Skin thickness, and perivascular mononuclear cell infiltrates were clearly reduced after treatment with $VEGF_{121}$-fibrin; the number of endothelial cells, capillaries, and mural cells were significantly increased compared to controls.

Conclusions: In the avian SSc model, treatment of ischemic lesions with $VEGF_{121}$-fibrin proofed to be clinically effective by inducing the formation of stable blood vessels. However, long term effects of $VEGF_{121}$, and the effect on fibrosis have to be investigated in a follow up study.

22. Deletion of CCN2 in fibroblasts rescues the fibrotic phenotype observed in pten deficient mice

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Background: Fibrosis is characterized by excessive production of collagens and their contraction by fibroblasts. It is the major cause of internal organ failure. There is no effective therapy till now. The matricellular protein connective tissue growth factor (CTGF/CCN2) is a marker of fibrotic cells and is considered playing an important role in fibrogenesis. Our previous studies showed that (a) mice with specific deletion of CCN2 in fibroblasts were resistant to bleomycin induced skin fibrosis and (b) deletion of pten in fibroblasts resulted in skin fibrosis. We would like to know weather deletion of CCN2 could rescue the fibrotic phenotype caused pten deficiency.

Materials and Methods: Fibroblast specific pten knockout and pten/CCN2-double knockout mice were created by crossing mice carrying floxed pten and floxed CCN2 alleles with mice carrying a tamoxifen-inducible Cre-recombinase under the control of a fibroblast-specific regulatory sequence from the proα2(I) collagen gene. Two months after gene deletion, animals were sacrificed and dermal thickness, collagen production, and α-smooth muscle actin (αSMA) were determined.

Results: Loss of pten resulted in significant increase of dermal thickness, collagen production and the number of αSMA positive myofibroblasts. However pten/CCN2 double knockout mice showed essentially normal dermal thickness, collagen production and number of myofibroblasts.

Conclusion: Our results indicate that CCN2 is required for two models of skin fibrosis, namely bleomycin-induced and PTEN-gene deficiency. These data suggest that therapeutic strategies blocking CCN2 in vivo may be of benefit in combating fibrotic skin disease such as in scleroderma.

23. Novel insights into the PDGF receptor domains recognized by agonistic autoantibodies in SSc

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Background: We confirmed that PDGF receptor (PDGFR) is the target of an autoimmune response in SSc by cloning agonistic and non-agonistic auto-antibodies from PDGFR self-reactive memory B cells of SSc patients. We have elucidated the PDGFR domains bound by these autoantibodies.

Materials and methods: i) We produced a naive-like conformer of PDGFR that was immobilized onto a surface plasmon resonance (SPR) device. This was used to perform binding competition experiments with PDGF and the monoclonal autoantibodies; ii) molecular structures of PDGFR, PDGF and monoclonal autoantibodies were obtained by homology modelling and were used for molecular docking prediction, a structural bioinformatic approach that predicts the statistical probability of a molecule (ligand) to bind to another (receptor) to form a complex; iii) in vitro and in silico data were used to design an "ad hoc" conformational peptide library spanning the PDGFR extracellular domains; iv) peptides bound by PDGF and monoclonal autoantibodies were regenerated from library and pre-incubated with agonistic monoclonal autoantibodies to verify inhibition of antibody biological activity; v) SPR and molecular docking approaches were applied to viral agents exploiting human PDGFR as a co-receptor to infect eukaryotic cells.

Results: We obtained a map of the functional domains of PDGFR extracellular region. The PDGFR epitopes recognized by stimulatory monoclonal autoantibodies are different from those of non-biologically active monoclonal autoantibodies. The epitope bound by PDGF partially overlaps with the epitope of one agonistic monoclonal autoantibody. Pre-incubation of agonistic monoclonal autoantibodies with defined peptides obtained from library, inhibited the antibody stimulation of collagen gene transcription in human fibroblasts. Moreover, we obtained data indicating that part of the epitope "pocket" recognized by the agonistic auto-antibodies is formed by the capsid of a viral agent that binds to PDGFR.

Conclusions: These data shed light on the PDGFR domains linked to SSc pathogenesis and on the formation of agonistic anti-PDGFR autoantibodies. This information may be used to devise new therapeutic strategies to block PDGFR signaling and selectively detect stimulatory auto-antibodies in serum, possibly implicated in early phases of SSc pathogenesis.

24. IQ motif containing GTPase activating protein 1 (IQGAP1): a new player in scleroderma-associated interstitial lung disease (SSc-ILD)

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Background: Scleroderma associated interstitial lung disease (SSc-ILD) is an irreversible and progressive complication of SSc often leading to respiratory failure and death. IQ motif containing GTPase activating protein (IQGAP1) is consistently elevated in lung fibroblasts isolated from SSc-ILD patients and in normal lung fibroblasts exposed to connective tissue growth factor (CTGF). Here we report our latest data demonstrating that IQGAP1 promotes lLD by acting downstream of α5β1 integrin and CTGF signaling pathways in lung fibroblasts.

Materials and Methods: Lung injury was induced in female C57BL/6 mice by a single intratracheal instillation of bleomycin. IQGAP1-siRNA and CTGF-siRNA were delivered by intranasal instillation on days 8, 10, 12, 14, 16, 18, and 20 after bleomycin administration. Mice were sacrificed 3 weeks after bleomycin instillation, and lung tissue and bronchoalveolar lavage (BAL) fluid were investigated. Protein interaction studies were performed in lung fibroblasts using co-immunoprecipitation, 2D-gel electrophoresis, and mass spectrometry methods.

Results: Administration of IQGAP1-siRNA decreased the expression of IQGAP1 by 70% and had no effect on CTGF expression. CTGF-siRNA reduced the expression of CTGF by 80% and IQGAP1 by 40%. Treatment with IQGAP1-siRNA alone and in combination with CTGF-siRNA attenuated the development of bleomycin-induced pulmonary fibrosis. A more profound antifibrotic effect was observed by combinatory treatment with both IQGAP-siRNA plus CTGF-siRNA. IQGAP1 formed a protein-protein interaction complex with α5β1 integrin and CTGF in lung fibroblasts and regulated α-smooth muscle actin (α-SMA) expression.

Conclusions: IQGAP1 forms a signal transduction complex with CTGF via α5β1 integrin in lung fibroblasts, regulates the expression of α-SMA, and promotes pulmonary fibrosis. Inhibition of IQGAP1 has a marked antifibrotic effect in a bleomycin model of pulmonary fibrosis and should be considered as a potential new therapeutic target for the treatment of SSc-ILD.

25. Effect of the Selective Endothelin Receptor Antagonist Ambrisentan on Digital Ulcers in Patients with Systemic Sclerosis: Results of a Prospective Pilot Study

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Background: Previous studies have shown that the dual endothelin receptor antagonist (ETRA) bosentan is useful in the prevention of new digital ulcers (DU) in patients with systemic sclerosis (SSc), but has no effect on the healing of existing ulcers. We sought to evaluate the effect of the relatively selective ETRA ambrisentan on the prevention and healing of DU.

Materials and Methods: This was a prospective open-label single-center study enrolling patients with limited or diffuse cutaneous SSc with at least one active DU located at or distal to the proximal interphalangeal joint. The primary endpoint was the difference in number of new DU that developed in the preceding 4 weeks after 24 weeks of therapy compared with baseline. Secondary endpoints included change in number of DU, mean diameter of DU, physician global assessment of DU severity by visual analogue scale (VAS), and patient assessment of severity of DU and Raynaud phenomenon by VAS. A completers analysis was performed.

Results: 20 patients (80% female, mean age 49.3±13.8 years, 65% dcSSc) with a mean disease duration since first Raynaud symptom of 12.7±10.8 years were enrolled in our study. 12 (60%) received stable doses of a calcium channel blocker and/or other vasodilator throughout the study. 16 patients completed 24 weeks of therapy. 2 withdrew due to lower extremity edema, 2 for scheduling reasons. The mean number of new DU that developed 4 weeks prior to week 24 was not different from baseline (0.44±0.81 vs. 0.45±0.69). However, the total number of DU decreased from 3.1±2.1 to 1.3±1.6 (p=0.004). The mean diameter of all DU also decreased from 3.3±1.6 mm to 1.6±1.5 mm (p<0.0001). Physician and patient assessments of DU severity were significantly improved at week 24 compared with baseline (p=0.015 for both), but patient assessment of Raynaud severity was not significantly different. The most common adverse events were lower extremity edema (50%), anemia (50%), and DU infection (40%).

Conclusions: Ambrisentan may be useful in reducing ulcer burden and healing DU in SSc patients. A larger randomized double-blind placebo-controlled trial is warranted to further evaluate the efficacy of ambrisentan in the prevention and treatment of DU.

26. T helper 17 cells are increased in the skin of systemic sclerosis individuals

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Background: In systemic sclerosis (SSc) inappropriate T cell responses are thought to participate in initiating events ultimately leading to excessive extracellular matrix deposition and fibrosis. The recently described T helper (Th) 17 subset has been shown to be increased in the peripheral blood of SSc individuals. The aim of our study was to assess the presence of Th17 cells in the SSc and healthy skin.

Material and methods: Upon informed consent and approval by the ethical committee, skin samples were obtained from 4 SSc and 8 healthy donors used as controls (ctrl). T cell lines from the skin of all the individuals were grown in the presence of IL-2, in 4 independent replicates. Intracellular localization of IL-17A, IL-22, IL-4, and IFN-γ in CD4+ T cells as well as the surface expression of chemokine receptors were assessed by multiparametric FACS analysis. Paraffin embedded skin biopsies were stained for CD3/IL-17 and positive cells frequency determined by laser-scanning confocal-microscopy.

Results: In T cell lines generated from the skin 79.1%±10.5 and 73.7%±16.9 were CD4+ T cells in SSc and ctrl, respectively. Production of IL-17A, IL-22, IL-4, and IFN-γ was detected in all T cell lines. Statistically significant differences were observed in the subsets co-producing IL-17A and IL-22 (5.6%±7.9 vs 2.5±1.6) as well as IFN- and IL-4 (7.8%±4.6 vs. 2.4%±1.8), which were more frequent in SSc compared to ctrl. As expected, T cell lines generated form the skins were skewed for high expression of CCR4 than counterparts from the peripheral blood. In addition, the percentage of CXCR3+ cells was higher in SSc (48.1%±17.9) compared to ctrl (29.5%±13.0). Finally, histological identification of CD3+IL-17+ was possible in both SSc and ctrl skin, and Th17 cells tended to be more frequent in SSc (3.5 cells/field) than ctrl (1.7 cells/field) (p=0.09).

Conclusions: Our preliminary data indicate that Th17 co-producing IL-17A and IL-22 are increased in SSc skin, which may suggest a role for these cells in the pathogenesis of the disease. These results provide new rationale for targeting IL-17 and the Th17 differentiation pathway as novel approaches to harness the clinical course of SSc.

27. A novel automated longitudinal in vivo assessment of bleomycin induced pulmonary fibrosis in mice using in vivo high resolution micro-CT

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Background: In vivo high resolution µCT imaging in animal models of lung disease allows longitudinal observations of pathological processes and image-based measurements of functional parameters. Current µCT image based algorithms for fibrosis quantification are characterized by lower spatial resolutions, insufficient correlation to histopathological or physiological data, and are labor intensive.

Materials and Methods: Lung fibrosis was induced in 8-week old male C57/Bl6 mice, by intratracheal instillation of 0.05U bleomycin (BLM) or control phosphate buffered saline (PBS). Mice were treated daily with imatinib (50mg/kg/d via daily i.p. injection) or vehicle control (AD). A 'scanning only' group evaluated scan toxicity (all n=6). Freely breathing, isoflurane sedated mice were scanned weekly at 35 µm resolution (SkyScan® in-vivo µCT) with retrospective respiratory gating. 4 weeks after induction, invasive pulmonary function tests were performed (Flexivent® SCIREC). Tracheotomised mice were euthanized and lungs were scanned for pressure-volume loop construction, by externally applying decreasing pressures (30-0 cmH_2O). A fully automated algorithm calculated aerated lung volumes and densities. Lungs were collected for histopathological scoring (Ashcroft score). The hydroxyproline assay quantified total collagen content.

Results: Serial scanning caused no abnormalities. Imatinib attenuated fibrosis by 30%, based on Ashcroft score and lung compliance. Untreated BLM-challenged lungs had significantly lower aerated lung volumes, calculated upon µCT images, reflecting restrictive pulmonary disease. Imatinib-treated BLM-challenged animals had lung volumes intermediate between untreated BLM challenged and the control groups. Calculated lung volumes correlated to Ashcroft score and hydroxyproline content. Constructed pressure-volume loops agree with invasive pulmonary function tests. Plotted mean density histograms discriminate between our 5 experimental groups and show the dynamic progression of fibrosis in the longitudinal setup. The reconstructed 3- and 4D images provide critical information on topographical distribution of fibrosis.

Conclusions: We present a fully automated in vivo µCT fibrosis analysis protocol, resulting in quantitative measurement of pulmonary volumes in the bleomycin induced pulmonary fibrosis model. We show that the resulting volumes correlate with histopathological scores, total collagen contents and invasive pulmonary function tests. We demonstrate that this imaging technique and consecutive automated analysis is suitable for serial imaging of individual animals and is sensitive enough to detect modest treatment effects.

28. Use of the EQ-5D as a measure of health quality in systemic sclerosis

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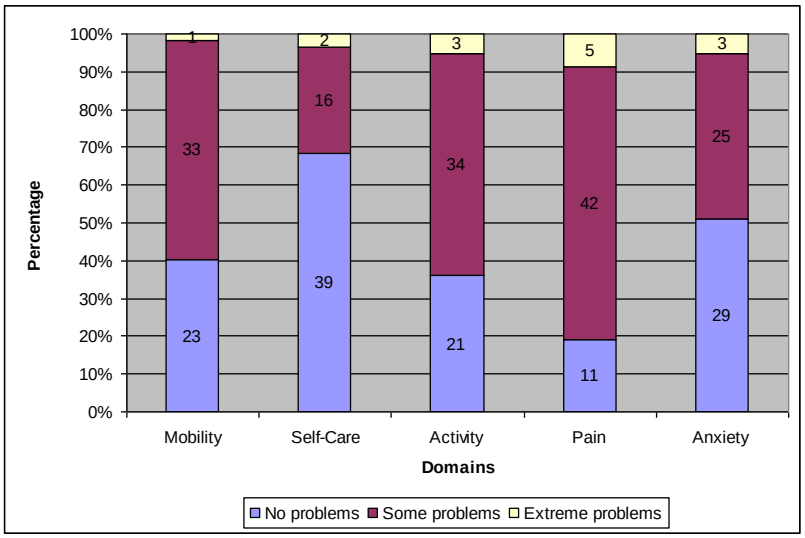
Objectives: The EQ-5D™ is a simple, self-reported, standardised measure of health outcome. It comprises 5 domains (mobility, self-care, activity, pain/discomfort and anxiety/depression) for which patients can report no problems, some problems or extreme problems. This provides a simple descriptive profile which can be converted into a single index value for health status using a general population-based value set (the time trade-off value, TTO; lower TTO indicative of worse health). There is an additional patient global assessment (0-100 visual analogue scale [VAS], 100 indicative of best imaginable health state). Few studies have evaluated the EQ-5D in systemic sclerosis (SSc).

Methods: Fifty-eight patients with SSc completed the EQ-5D and Scleroderma Health Assessment Questionnaire (SHAQ) in response to a postal survey (~70% response rate). A descriptive analysis was undertaken along with an evaluation of the influence of patient demographics, clinical phenotype, serology and correlation with the SHAQ.

Results: Pain was the most prominently reported health problem affecting 81% of patients in contrast to self-care, for which only 31.6% reported problems (P<0.0001, Table 1.). The mean VAS was 67.8 (SD 20.4). The mean TTO was 0.62 (SD 0.31) and correlated well with the VAS (r=0.51, P<0.001) and HAQ-DI (r=-0.75, P<0.001). The TTO correlated well with the scleroderma-VAS scores for Pain (r=-0.54, P<0.001), Intestinal (r=-0.52, P<0.001), Respiratory (r=-0.53, P<0.001), Raynaud's (r=-0.62, P<0.001) and Patient Global Assessment (r=-0.53, P<0.001), but less well for Digital Ulceration (r=-0.3, P=0.03). There was no correlation between the TTO and the TLco, FVC or highest Rodnan Skin Score. Similarly, the TTO was not influenced by gender, serology, disease duration (< vs. > 5years), or subtype (limited vs. diffuse). Of the SSc organ-specific manifestations, only upper gastro-intestinal involvement (n=41/58) was associated with a significantly lower mean TTO (0.55 vs. 0.8, P=0.005).

Conclusions: This is the first study to evaluate the influence of patient demographics, disease characteristics and SHAQ on the EQ-5D in SSc. The EQ-5D generally correlates well with the SHAQ. The apparent lower health quality of patients with upper gastro-intestinal complications may reflect the presence of additional organ-specific complications in this population. The EQ-5D should be considered for future therapeutic trials and cost-effectiveness analyses in SSc.

Table 1 Descriptive profile of the 5 domains of the EQ-5D™ reported by 58 patients with SSc (n=57 for mobility, self-care, and anxiety



29. Impaired angiogenesis in Fli1 deficient endothelial cells

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Background: Endothelial cell (EC) injury is an initiating event in the vascular disease component of Systemic Sclerosis (SSc). Although the mechanisms of the injury remain poorly understood, there is evidence that transcription factor Fli1, which is downregulated in ECs in the skin of SSc patients, plays an important role in development and maintenance of SSc vasculopathy. Our previous study showed that mice with a conditional knockout of Fli1 (Fli1CKO) in ECs display abnormal skin vasculature and markedly increased vessel permeability suggesting that Fli1 is involved in regulation of genes critical for vascular remodeling (Asano et al 2010). The aim of this study was to investigate mechanisms responsible for modulating expression level of Fli1 and functional consequences of its deficiency in ECs.

Materials and methods: Human dermal microvascular endothelial cells (HDMECs) were isolated from foreskin tissues. Fli1 knockdown was performed using siRNA Fli1 adenovirus. Expression levels of Fli1 were determined by qPCR and western blot. Proliferation was examined with CellTiter 96® AQueous One Solution Cell Proliferation Assay. Angiogenic potential was assessed in the mouse aortic ring assay in Fli1CKO mice. IFN-α (100U/ml), TLR2 (2μg/ml), TLR3 (25μg/ml), TLR4 (2.5μg/ml).

Results: Fli1 mRNA expression was significantly decreased in response to IFN-α (3.57-fold p=0.001) and TLR4 ligand (5-fold p=0.001). In the absence of Fli1 proliferation of HDMECs was markedly decreased and no further significant responses to immune mediators was observed. However, in control cells treated with scrambled RNA, responses to the above treatments were preserved indicating that the proliferative responses of HDMECs are Fli1-dependent. We observed statistically significant decrease of total number of branching tubules in Fli1CKO mice vs. WT aortas.

Conclusion: This work demonstrates that Fli1 function is required for the homeostasis of HDMECs. Additionally, our preliminary observations indicate that IFN-α, as well as TLR4 ligand reduce expression levels of Fli1 in ECs suggesting that TLR activation directly or via IFN pathway contributes to Fli1 downregulation and a subsequent endothelial cell dysfunction. Furthermore Fli1 deficiency results in disrupted angiogenesis in cultured ECs and in the aortic ring model.

30. A rare polymorphism in *Toll Like Receptor 2* is associated with systemic sclerosisphenotype and increases production of inflammatory mediators

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Aim: To investigate whether polymorphisms in *toll like receptor* (*TLR*) genes, previously reported to be associated with immune mediated diseases are implicated in systemic sclerosis (SSc).

Methods: We genotyped 14 polymorphisms in the *TLR 2, 4, 7, 8* and *9* genes in a discovery cohort comprising 452 SSc patients and 537 controls and a replication cohort consisting of 1170 SSc patients and 925 controls. Furthermore we analyzed 15 year follow-up data from 964 patients to assess the potential association of *TLR* variants with the development of disease complications. Next to this, we analyzed the functional impact of the associated polymorphism on monocyte derived dendritic cells.

Results: Exploiting the discovery cohort, we observed that a rare functional polymorphism in *TLR2* (Pro631His), was associated with anti-topoisomerase positivity (p=0.003 OR 2.24 95%CI:1.24-4.04). This observation was validated in the replication cohort (p=0.0001 OR 2.73 95%CI:1.85-4.04). In addition, the replication cohort also revealed an association between the *TLR2* variant with the diffuse subform of the disease and the development of pulmonary arterial hypertension, respectively (p=0.02, Log-Rank p=0.003, Cox proportional hazards ratio: 5.61 ((95%CI 1.53-20.58)). Functional analysis revealed that monocyte derived dendritic cells carrying the Pro63His variant produce more inflammatory mediators (TNFalpha and IL-6) upon TLR2 mediated stimulation (both p<0.0001).

Conclusion: The rare TLR2 Pro631His variant is robustly associated with anti-topoisomerase positivity, diffuse SSc and the development of PAH. Besides, this variant influences TLR2 mediated cell responses. Further research is necessary to reveal the precise role of TLR2 in the disease pathogenesis of SSc.

31. Polymorphisms in the Interleukin 4, Interleukin 13 and corresponding receptor genes are not associated with Systemic Sclerosis and do not influence gene expression.

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Abstract Aim: PolymorphismsIn the genes encoding interleukin 4 (IL4), interleukin 13 (IL13) and their corresponding receptors have previously been associated with multiple immune mediated diseases. In this study we aim to validate these previous observations in systemic sclerosis (SSc) patients and scrutinize their effectson gene expression in various populations of peripheral blood leukocytes.

Patients and Methods: We genotyped acohort of 2488 SSc patients and 2246 healthy controls from The Netherlands, Spain, United Kingdom, Italy, ermany and France. Taqman assays were used to genotype SNPs in the following genes: 1) *IL4* (*−590C/T*/rs2243250), 2) *IL4 receptor alpha (IL4RA)* (*Q576R*/rs1801275) 3) *IL13* (*R130Q* (rs20541) and *---1112C/T* (rs1800925)) and 4) *IL13 receptor alpha 1* (*IL13RA1)* (*43163:G/A* (*rs6646259)).* In addition, the effect of these polymorphisms on expression of the corresponding genes was assessed using quantitative RT---PCR on cDNA samples derived from peripheral blood B---cells, T- -cells, plasmacytoid dendritic cells, monocytes and myeloid dendritic cells. Moreover, we investigated whether these polymorphisms influence development of pulmonary complications over 15 years of disease.

Results: None of these polymorphisms were enriched in the SSc population or in any SSc clinical subtype. In addition, we did not observe any effect on transcript levels in the cell subtypes or on development of pulmonary complications.

Conclusions: Our data show that these polymorphisms do not play a role in SSc and do not influence the expression of their corresponding transcript in peripheral blood cells.

32. Non-calcemic natural vitamin D analogue, 20(OH)D_3, protects mice from bleomycin induced scleroderma

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Background: The purpose of the present study is to assess anti-fibrotic effects of a novel vitamin D analogue. 1α, 25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] regulates calcium homeostasis and bone remodeling. It is also known to inhibit collagen synthesis by some types of fibroblasts, has been shown to inhibit interstitial fibrosis in a renal model and may improve bone marrow fibrosis in patients with myelofibrosis. Use of 1,25$(OH)_2D_3$ as a therapeutic anti-fibrotic agent is limited by its inherent ability to induce hypercalcemia. Recently our group identified a novel secosteroid, 20$(OH)D_3$, that does not induce calcemia. We found that 1,25$(OH)_2D_3$ and 20$(OH)D_3$ at 10^{-10} and 10^{-9} M significantly inhibited (from $\geq$ 90%) total collagen and hyaluronan synthesis by cultured human dermal fibroblasts stimulated with TGF-β1 (5 ng/ml) with no cytotoxic effect.

Materials and Method: In the present study we administered 20$(OH)D_3$ or sesame oil vehicle i.p. daily for 21 days at a dose of 3 µg/kg to C57BL/6 mice receiving subcutaneous injection of bleomycin to induce localized dermal fibrosis, a standard murine model of scleroderma. At day 22 mice were euthanized and skin obtained for histology and quantitation of total collagen content using a Sicrcoll Collagen Assay.

Results: 20$(OH)D_3$ treatment of mice led to marked significant ($p=0.019$) reduction in total collagen content (ng/mg of tissue) of skin compared to Sesame Oil Vehicle treated mice that approached collagen content of Bleomycin Vehicle Control: (576 $\pm$ 70)=20$(OH)D_3$ + Bleomycin; (1326 $\pm$ 236)=Sesame Oil + Bleomycin; (503 $\pm$ 85)=Sesame Oil + Saline. Furthermore, 20$(OH)D_3$ inhibited type I collagen synthesis by scleroderma and normal donor skin fibroblasts cultured with TGF-β1 as well as Col1A1 mRNA.

Conclusion: These data suggest that 20$(OH)D_3$ identified by us with no hypercalcemic property retains the anti-fibrotic properties of 1,25$(OH)_2D_3$ in vivo in a scleroderma mouse model and *in vitro* in cultured scleroderma and normal fibroblasts.

33. Notch Regulates Hypoxia-induced Angiogenesis

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Systemic sclerosis is a complex connective tissue disease characterised by ischaemic vasculopathy which may result in hypoxic tissues.

Objective: To examine the effect of hypoxia on Notch signalling pathway components in human dermal endothelial cells (HDEC).

Methods: Expression and regulation of Notch-1 and downstream signalling components (Hey1, Hey2 and DLL-) and HIF-1α under normoxic and hypoxic conditions (3%) were assessed. Notch-1 siRNA, was used to inhibit Notch signalling pathway. Microvascular endothelial cell (HDEC) activation, in the presence of Notch-1 siRNA and the γ-secretase inhibitor DAPT, under normoxic and hypoxic conditions was assessed by matrigel tube formation assay, migration assay, invasion assay and MM2/9 zymography.

Results: Exposure of HDEC to 3% hypoxia (mimicking the *in vivo* joint environment) induced HIF-1α and Notch-1 IC protein expression and Notch-1 IC, its ligand DLL-4 and downstream signalling components (Hrt1 and Hrt2) mRNA expression. Furthermore, 3% hypoxia significantly increased angiogenic tube formation, EC migration and invasion, pro-MMP-2 and-9 activity, effects of which were inhibited using Notch-1 siRNA and the γ-secretase inhibitor DAPT.

Conclusion: This study demonstrates activation of the Notch signalling pathway components in HDEC. Furthermore, we show that hypoxia (3% oxygen) induces HIF-1α, NICD, DLL-4 and NICD signalling pathway components. Finally we demonstrate that hypoxia-induced angiogenesis inhibition with DAPT and Notch-1 siRNA, indicating hypoxia-induced angiogenesis is at least partly dependent on Notch signalling pathway.

34. HLA-B35 and TLR3 cooperate in the induction of ET1 via activation of ATF4 pathway in endothelial cells

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Background: HLA-B35 (Human Leukocyte Antigen class I) has been associated with an increased risk for developing scleroderma-associated pulmonary hypertension (PHT). We have recently reported that the presence of HLA-B35 contributes to endothelial cell (EC) dysfunction via ER (Endoplasmic Reticulum) stress and UPR (Unfolded Protein Response) (1). TLR3, a key mediator of the innate immunity, has also been implicated in Scleroderma pathogenesis (2). The goal of our study was to determine the molecular mechanisms that mediate induction of ET-1 by ER stress and TLR3 in ECs.

Materials and methods: Human dermal microvascular endothelial cells (HDMECs) were transduced for 48 hours with Adenovirus expressing HLA-B35 or control virus HLA-B8. Cells were treated with Thapsigargin (TG) (10pM), a known ER stress inducer, and with TLR3 ligand (PolyI:C) (2,5μg/ml) for 24 hours alone or in combination. Using siRNA technology the depletion of a UPR mediator was performed. After the treatments, quantitative real-time polymerase chain reaction (qPCR) and western blot were performed to check upregulation of ET1 and ER stress mediators.

Results: Analysis of known ER stress/UPR pathways showed that HLA-B35 or TG unregulated ET1 expression which correlated with increased phosphorylation of PERK and eIF2α and increased expression of transcription factor ATF4. Interestingly TLR3 treatment showed activation of the same pathway suggesting that ER stress and TLR3 may share a common signaling pathway to induce ET1. Furthermore we tested the effects of TLR3 ligand in combination with HLA-B35 (and TG) and we observed cooperation in the induction of ET1 via synergistic enhancement of ATF4 activation. In order to determine whether ATF4 is involved in the regulation of ET1 gene we examined the effect of ATF4 siRNA. Depletion of ATF4 reduced basal expression level and prevented upregulation of ET1 mRNA in response to HLA-B35 and TLR3 suggesting a key role of ATF4 in ET1 regulation.

Conclusions: Our data have established that PERK/eIF2α/ATF4 pathway plays a pivotal role in regulation of ET1 gene expression in response to TLR3 and ER stress. PERK/eIF2α/ATF4 pathway may represent a novel pathogenic mechanism in PHT in SSc patients.

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35. Cardiac fibrosis and microvascular damage are a hallmark of Systemic Sclerosis heart involvement. Prevalence study using cardiac MRI

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Background: Heart involvement has been described in 37-80% of systemic sclerosis (SSc) patients; prevalence rates vary depending on the study methodology. Our aims were to determine the prevalence of heart involvement in a cohort of patients with SSc, to describe the patterns of heart involvement using cardiac MRI, and to correlate these data with disease subsets and target organ involvement.

Methods: We included patients from our SSc cohort, without cardiovascular risk factors. They underwent clinical evaluation; also EKG, CPK, CPK-MB, ultrasensitive-CRP, ESR, SSc-specific autoantibodies, antiphospholipid antibodies, coronary angiotomography and cardiac MRI were performed.

Results: We included 62 SSc patients (29 with diffuse cutaneous SSc (dcSSc) and 33 with limited cutaneous SSc (lcSSc)); 60 of them were female; mean time of evolution was 9.7 years; mean LVEF was 59.4%; 45% showed myocardial fibrosis (17.8% with patchy distribution, 35.7% in bands, 10.7% subendocardic, 28.5% with mixed patterns and 7.1% transmural), with higher prevalence in dcSSc than in lcSSc (58.6% vs 33.3%; p=0.04); the percentage of cardiac fibrosis was significantly higher in dcSSc (6.7%) than in lcSSc (1.6%; p=0.02). Cardiac fibrosis was more prevalent in basal anteroseptal (27%) and inferoseptal (12.9%) segments, as well as in the middle segments of the anteroseptal wall (19.4% and 12.9% in segments 8 and 7, respectively). Ninety three percent of coronary angiotomographies were normal (mean Ca score 2.9). Cardiac fibrosis was associated with lower LVEF (55.8 vs 66.5%, p<0.0009) and inversely associated with vascular involvement (86.7 vs 100%, p=0.03). Microvascular damage (measured as subendocardic perfusion defect) was present in 79% of patients and it was associated with higher CRP (1.28 vs 0.22; p=0.001) and positive anti β2 glycoprotein 1 (aβ2GP) IgG antibody (p=0.001). There was no association of cardiac fibrosis or microvascular damage with atherosclerosis.

Conclusions: Patients with systemic sclerosis show high frequency of cardiac fibrosis and subendocardic concentric perfusion defects, related to microvascular damage. Cardiac damage in SSc is not associated to ischemic heart disease. Cardiac MRI is a sensitive, noninvasive method to detect heart involvement in SSc. Elevated CRP and positive anti-aβ2GP IgG antibodies may help to identify patients at risk for SSc heart involvement.

36. Adenosine A_{2A} receptor occupancy promotes dermal fibrosis by modulating Fli1 and CTGF expression

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Background: Increased production of extracellular matrix in the skin is the hallmark of scleroderma. We have previously reported that adenosine, acting at A_{2A} receptors, enhances dermal collagen production both *in vitro* and in a murine model of dermal fibrosis although the mechanisms by which adenosine receptor stimulation promotes collagen production are not clear. Fli1 is a known transcriptional repressor of fibrillar collagen genes and connective tissue growth factor (CTGF/CCN2) in dermal fibroblasts. To further clarify the mechanism by which A_{2A} receptor stimulation induces dermal collagen accumulation, we explored the effects of A_{2A} receptor occupancy on Fli1 and downstream mediators of fibroblast matrix production.

Methods: Primary human dermal fibroblasts were stimulated with the selective adenosine A_{2A}R agonist CGS21680 (1μM) for varying time periods and message levels (real time-RTPCR) for Fli1 and CTGF were quantitated. In addition we measured cell-associated and supernatant levels of collagen I and nuclear levels of Fli1 by Western blot.

Results: Adenosine A_{2A} receptor stimulation for 4 hours reduced Fli1 mRNA expression by 47.0±18.2% (4 hrs, p<0.05 vs. control, n=4) and reduced nuclear protein levels of Fli1 by 31.9±12.8% (24 hrs, p<0.05, n=4). CTGF mRNA level was increased by 1.57-fold following A_{2A} receptor stimulation with CGS21680 (1μM) for 10 hours. CTGF protein secretion was increased to 4-fold control (24 hours, p<0.05, n=4). As expected A_{2A} receptor stimulation increased collagen type I protein secretion (1.52-fold, p<0.05, n=3) and the increase in collagen I production was completely abrogated by an antibody to CTGF (1.07-fold of control, p<0.05 vs.CGS21680 alone, n=3) but not by control antiserum.

Conclusion: A_{2A}R occupancy promotes dermal matrix production by suppressing expression of the transcriptional factor Fli1. The resultant increase in CTGF expression acts in an autocrine fashion to stimulate collagen I production. These findings suggest that modulation of A_{2A}R function may be a novel therapeutic target for limiting skin fibrosis in such conditions as scleroderma.

37. Adenosine mediates murine bleomycin-induced dermal fibrosis

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Background: Genetic deletion of CD39/73 results in vastly decreased adenosine in tissues, protecting against bleomycin-induced pulmonary fibrosis. To characterize the contribution of endogenous adenosine to fibrogenesis in the skin, we determined its impact on murine bleomycin-induced dermal fibrosis.

Methods: Male CD39/CD73-double deficient mice (CD39/73KO) were injected with bleomycin (0.1U sc qodx18d) and compared to wild-type littermates. Dermal morphometry was assessed on 6mm skin punch biopsies. Hydroxyproline levels were determined and adenosine levels assessed by HPLC. To further characterize the contribution of endogenous adenosine to skin fibrosis and the key parts of this pathway, we also looked at individual knockouts of CD39 and CD73.

Finally, we examined the expression of the transcription factor Fli1 (Friend leukemia integration-1), a repressor of key matrix-producing genes, in our mice. Deficiency of Fli1, downregulated in SSc fibroblasts, has been shown to reproduce the fibrotic changes of systemic sclerosis (SSc) in animals.

Results: CD39/CD73KO mice showed lower dermal thickness (0.29±0.05 vs. 0.36±0.10mm), skin-fold thickness (0.79±0.16 vs. 0.97±0.37mm), tensile strength (198.2±7.3 vs. 248.7±7.0g) and hydroxyproline content (21.7±1.2 vs. 26.5±1.1µg/mg tissue) (n=6, $p<0.01$ for each) compared to controls after bleomycin treatment; correlating with a decrease in adenosine levels, and associated with a mild increase in Fli-1 immunostaining.

CD73KO mice showed no statistically significant difference in dermal thickness (0.59±0.26 vs. 0.52±0.62mm), skin-fold thickness (1.08±0.06 vs. 1.07±0.09mm), or tensile strength (270.2±72.9 vs. 248.0±38.3g) (n=3, $p>0.05$ for each) compared to controls without bleomycin treatment, with no detectable changes in Fli-1 staining.

Conclusion: This work shows that extracellular adenosine, possibly acting in part through Fli-1, is necessary for the sclerosing effects of bleomycin. Blockade of adenosine or its receptors may be useful in the treatment of diseases such as scleroderma where dermal fibrosis is a prominent manifestation.

38. Chi3L1, a potential early disease marker for scleroderma

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Background: Scleroderma (SSc) is characterized by fibrosis in the connective tissues of the skin and organs of the body. Dermal fibroblasts are one of the main effector cells involved in the development of fibrotic lesions, and their biological activity is regulated by a variety of inflammatory cytokines and growth factors, many of which have been implicated in the progressive development of these lesions in SSc patients. Chi3L1 is part of the innate stress response of connective tissue cells and elevated serum levels have been observed in SSc. Therefore we investigated the capacity of skin cells from SSc patients and from healthy individuals to synthesize Chi3L1 and the regulation of this process by growth factors and cytokines.

Materials and Methods: Fibroblasts were isolated from skin biopsies taken from the forearm and the abdomen, maintained in monolayer cell culture, and stimulated with cytokines and growth factors: IL1, IL6, IL-17, OSM, TGF-ß and PDGF in the absence of serum. Chi3L1 in culture media was analyzed by SDS-PAGE and immunoblotting. Cells actively producing Chi3L1 were identified by immunolocalization.

Results: Chi3L1 was not produced endogenously by normal skin fibroblasts, nor was it inducible in these cells by the cytokines and growth factors tested in this study. In contrast, fibroblasts from biopsies of SSc patients produced variable amounts of Chi3L1 endogenously, and exposure to IL-1 or OSM resulted in a strong upregulation of Chi3L1 expression. Four subgroups of SSc patients were identified based on the capacity of the skin fibroblasts from their arm and abdomen for endogenous production of Chi3L1 and inducibility by OSM. The group of patients endogenously expressed Chi3L1 in both the arm and abdomen, and in whom Chi3L1 expression was also inducible by OSM at both sites, were in the early phase of disease.

Immunolocalization showed that only a fraction of the total cell population produced Chi3L1 spontaneously and/or in response to OSM treatment. These cells did not stain positively for α-smooth muscle actin, which identifies myofibroblasts. The cells producing Chi3L1 were either actively producing nestin, CD73 and STRO-1(all of which are mesenchymal stem cell markers) or were in close association with such cells. Nestin, CD73 or STRO-1 positive cells were not detected in normal skin fibroblast preparations.

Conclusions: Induction and up-regulation of Chi3L1 by IL-1 and OSM in some SSc skin cells may occur during inflammation, where it may help protect the cells from damage. The capacity for Chi3L1 production identifies a cell-population in skin of SSc patients which may be associated with the recruitment or function of undifferentiated stem cells to the lesions. To what extent these cells contribute to the fibrotic process in SSc remains to be determined.

39. Morphoea-related tenosynovitis: An example of the relationship between inflammation and fibrosis

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Background: Localised scleroderma ('morphoea') is important not only because of its association with significant musculoskeletal pain and disability, but also because understanding its pathogenesis is highly relevant to systemic sclerosis (SSc) research. Improved imaging techniques, especially magnetic resonance imaging (MRI), have the potential to increase our understanding of morphoea pathophysiology and treatment response.

Method: We present four patients (two males aged 49 and 62 at presentation and two females, 47 and 37) with tenosynovitis deep or in close proximity to areas of morphoea.

Results: All four of our patients had areas of skin thickening involving both upper and lower limbs sparing the fingers and toes. All had restriction of finger and wrist movements and two had limited elbow movements. The degree of restriction suggested involvement of periarticular tissue in addition to skin thickening. One patient had Raynaud's phenomenon and was ANA positive, but the clinical picture was not one of SSc. Of the other three patients, none had any clinical or laboratory features of SSc apart from one being weakly ANA positive (1/100). Skin biopsies in all four patients were consistent with morphoea. All had active tenosynovitis on either MRI (three patients) or ultrasound (one patient). On this basis all were treated with prednisolone with varied starting doses (15, 30, 40 and 60mg). All reported improvement within two months and were subsequently found to have objective improvement in both finger to palm distance and skin thickening. One patient was able to discontinue steroids after 20 months. Two remain on low dose steroids after 18 and 30 months. The fourth patient required methotrexate as a steroid sparing agent and continues on this after six years, together with low dose prednisolone.

Conclusion: (1). Morphoea can be associated with tenosynovitis, which should be suspected in patients with disproportionate restriction of movement. (2). Tenosynovitis can be demonstrated on MRI and ultrasound and if diagnosed early is steroid responsive.

(3). Understanding the temporal relationships between fibrosis and inflammation in morphoea (using sequential MR imaging) may provide new insights into the pathogenesis of SSc.

40. Identificaion of potential markers of skn fibrosis using targeted disruption of TGFBeta signaling in transgenic mice

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Background: TGFβ is a profibrotic cytokine involved in tissue repair and wound healing. We have demonstrated that deletion of TβRII selectively in fibroblasts is antifibrotic. Previous studies show that mice from this strain are highly resistant to bleomycin induced experimental fibrosis. We have used this model to identify potential key mediators of fibrotic scar formation in the skin. These may include novel markers that could be used to assess disease activity in systemic sclerosis (SSc).

Methods: We have deleted TβRII in fibroblasts of mice post-natally, using a Cre-Lox strategy that circumvents the embryonic lethality of a conventional knock-out for this gene by the administration of tamoxifen to activate Cre recombinase and hence deletion of TβRII. Gender matched littermates were studied and 4mm dermal punch wounds performed. Wound histology was assessed for collagen deposition using Sirius red and Masson's trichrome stains. Dermal fibroblasts cultured from wildtype or mutant mice were treated with recombinant TGFβ1 (2ng/ml) and illumina microarray gene profiling performed.

Results: Healing of full thickness skin wounds was severely impaired in mice after fibroblast-specific deletion of TβRII. For wildtype wounds at 14 days mean (±sd) diameter was 0.57 mm (±0.29) compared with 1.75 mm (±0.28) for TβRII-null-fib wounds (p=0.001). In 7 day null wounds there was reduced expression of markers of wound healing including alpha-SMA. Illumina microarray analysis defined a TGFbeta induced gene cohort in wildtype cells and confirmed that mutant fibroblasts were essentially refractory. Key marker genes for myofibroblast phenotype were not induced and induction of several candidate profibrotic mediators including CTGF and endothelin-1 was diminished. Representative data for 5 key genes is summarised in *Table 1*.

Conclusions: Our findings confirm that an intact TGFbeta response in resident dermal fibroblasts plays a key role in normal skin wound healing. Our observations are potentially relevant to strategies that aim to attenuate fibrosis since these same approaches may have detrimental effects on connective tissue repair. This mouse strain provides a platform for better understanding human fibrotic disease such as SSc and genes that show differential expression may be candidate markers of scar formation and fibrosis.

Table 1. Normalised gene expression in skin fibroblasts from wildtype and mutant mice

	WILDTYPE (N=6)						**NULL (N=4)**					
GENE	BASAL		TGFb1				BASAL		TGFb1			
	Mean	SD	Mean	SD	Fold Change	P Value	Mean	SD	Mean	SD	Fold Change	P Value
alphaSMA	2260	1329	**9363**	3176	**4.1**	0.00009	**3136**	707	**962**	1220	**1.1**	0.23
Transgelin	1231	870	**4748**	1267	**3.9**	0.00005	**1505**	564	**518**	695	**1.2**	0.20
Endothelin-1	352	108	**1115**	304	**3.2**	0.00002	**356**	79	**109**	71	**1.1**	0.21
Myosin Heavy Chain	324	221	**985**	642	**3.0**	0.01021	**408**	93	**126**	108	**1.1**	0.18
CTGF	5783	3196	**12198**	5210	**2.1**	0.01014	**4352**	3006	**1840**	4426	**1.1**	0.11

41. Ciprofloxacin has antifibrotic effects in SSc fibroblasts via up-regulation of Fli1

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Background: Increased collagen and CCN2 deposition, and lower levels of MMP1 characterize SSc dermal fibroblasts. The transcription factor Fli1 is downregulated in SSc dermal fibroblasts (Kubo et al, 2003), and histone deacetylation of the Fli1 promoter contributes to this downregulation. Furthermore, levels of histone deacetylase 1 (HDAC1) were upregulated in SSc (Wang et al, 2006). Fli1 is a repressor of collagen and CCN2 and has also been implicated in the regulation of MMP1 gene expression.

Ciprofloxacin is a quinolone antibiotic with antifibrotic effects on the articular cartilage via up-regulation of MMP1 and a decrease in collagen deposition. In a recent clinical trial on SSc patients, ciprofloxacin significantly reduced the modified Rodnan skin score (Ruben et al, 2010). However, the exact mechanism for the antifibrotic effects of Ciprofloxacin in SSc is currently unknown.

Materials and methods: SSc and normal dermal fibroblasts and were treated with ciprofloxacin (5-100μg/ml) for 48 to 96 h. Expression levels of collagen type I, CCN2, MMP1, COMP, Fli1, and HDAC1 were analyzed by western blot for the protein levels and by quantitative RT-PCR for the mRNA levels.

Results: Ciprofloxacin treatment downregulated in a dose dependent manner the mRNA levels of the profibrotic markers CCN2 (up to 70%) and COMP (up to 30%) in SSc cells, while it up-regulated MMP1 gene expression by approximately 4 fold. Similar effects were observed in control fibroblasts. The protein levels of collagen type I and CCN2 were significantly downregulated in SSc and normal cells (up to 80%). There was no effect on Smad pathway, and a 3 fold increase in the expression of Fli1 protein in ciprofloxacin-treated SSc fibroblasts, with minimal or no differences in control cells. The enhanced Fli1 expression in SSc fibroblasts after antibiotic treatment correlated with a significant decrease in the protein levels of HDAC1.

Conclusions: This study shows for the first time that ciprofloxacin is a negative regulator of CCN2 and COMP gene expression and that in SSc dermal fibroblasts its antifibrotic effects could be mediated via up-regulation of Fli1. Our results also suggest an epigenetic regulation of Fli1 by ciprofloxacin via inhibition of HDAC1.

42. The DNA methylation inhibitor 5-azacytidine up-regulates PTEN levels in scleroderma fibroblasts

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Background: More than a decade of research has clearly established the importance of PTEN as a tumor suppressor gene and as a negative regulator of the PI3/Akt pathway. Recent investigation revealed that the tumor suppressive activity of the PTEN protein in malignant cells can be altered at multiple levels through hypermethylation, an important epigenetic process.

In vivo and in vitro studies provided evidence that PTEN protein levels are reduced in both cultured scleroderma fibroblasts and lesional scleroderma skin in vivo (Bu et al, 2010). A novel function of nuclear PTEN as a co-factor of the Smad2/3 phophatase, PPM1A, has been recently demonstrated (Bu et al, 2008). We have previously shown concomitant downregulation of PPM1A and PTEN in scleroderma fibroblasts. The mechanism of down-regulation of PTEN and PPM1A in scleroderma remains unknown. There is accumulating evidence suggesting that DNA methylation processes are involved in the formation of the scleroderma phenotype (Wang et al, 2006). In the present study, we have investigated the hypothesis that the decreased PTEN and PPM1A levels in scleroderma cells are related to epigenetic factors, specifically promoter hypermethylation.

Methods: Dermal fibroblast from 4 different cell lines from scleroderma patients were treated with a low dose of the DNA methylation inhibitor 5-azacytidine (5-azaC) 5μM for 7 days. We then analyzed the total PTEN protein and PPM1A levels by Western blot and mRNA expression by using RT-PCR.

Results: We observed a 2 fold increase in PTEN levels in 5-azaC-treated fibroblasts compared to control, suggesting that hypermethylation might me a mechanism explaining decreased levels of PTEN in scleroderma fibroblasts. There was no change observed in the levels of PPM1A in scleroderma cells compared to control.

Conclusion: These results suggest that decreased levels of PTEN in scleroderma could be due to epigenetic alterations, specifically hypermethylation of the PTEN promoter. Even though PPM1A levels have been found to be coordinately decreased in scleroderma cells in relation with PTEN, inhibition of methylation did not restore the PPM1A levels, suggesting a different mechanism explaining the decreased PPM1A expression in scleroderma.

43 Systemic sclerosis patients responsive to *mycophenolate mofetil* (MMF) treatment map to the 'inflammatory' intrinsic gene expression subset

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Background: Heterogeneity among patients with systemic sclerosis (SSc) has confounded clinical trials. We previously identified 'intrinsic' gene expression subsets by microarray analysis of SSc and control skin biopsies. Each intrinsic subset is defined by expression of unique genes representing different cellular infiltrates and pathway activation, and is stable when analyzed longitudinally. Here we test the hypothesis that patients who respond clinically to *mycophenolate mofetil* (MMF) would also show a specific gene expression response. We further test the hypothesis that patients responding to MMF map to the same intrinsic subset.

Methods: RNA isolated from skin biopsies of 7 SSc patients before and after MMF treatment was analyzed by DNA microarray hybridization. Intrinsic subset assignment was determined by combined analysis of the MMF treated patients along with an independent cohort of lesional and non-lesional skin biopsies from an additional 15 dSSc patients and 10 healthy controls from the Northwestern registry. A total of 83 biopsies were analyzed. Intrinsic subsets were defined as previously described. Changes in gene expression before and after treatment were determined using a paired t-test with correction for multiple hypothesis testing.

Results: After treatment with MMF for at least 6 months, four of seven patients showed improvement in mRSS ε5 and ε20% improvement from baseline. Analysis of the intrinsic gene expression signatures in this cohort (FDR < 3%) recapitulated the previously identified intrinsic gene expression subsets (diffuse-proliferative, inflammatory and normal-like). All four MMF responders were classified as the inflammatory subset, while the non-responders were classified as the normal-like or diffuse-proliferation subset. Comparison of the gene expression between baseline and post-treatment biopsies in the four responders identified a 610-gene biomarker response signature ($p<0.01$, FDR<10%) that was absent in the non-responders.

Conclusions: Patients with SSc who show a clinical response to MMF have baseline gene expression different than in the non-responders. We find that this difference in baseline gene expression among the responders is consistent with the inflammatory intrinsic subset. Our results show a consistent change in gene expression after MMF treatment in four responders that is absent in the three non-responders. *Supported by NIH/NICHD 5K12HD055884 to MH, a grant from the Scleroderma Research Foundation to MLW and by NIH U01AR055063 to RL and MLW.*

44. Oral Imatinib for the Treatment of Scleroderma Pulmonary Involvement: preliminary results of a pilot study.

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Background: Pulmonary involvement (PI) represents a major cause of death of scleroderma patients. Cyclophosphamide showed a small beneficial effect in RCTs, but a large portion of patients appeared to be totally refractory. Since activation of tyrosine kinases may be involved in the pathogenesis of scleroderma, we have decided to test the effect of imatinib mesylate, a tyrosine kinases inhibitor, on pulmonary and skin fibrosis in a cohort of scleroderma patients refractory to conventional therapy.

Materials and methods: This study, a phase II multicentric open trial, is focused on 30 consecutive patients with active pulmonary involvement defined as: grade 2 dyspnea (Mahler Dyspnea Index) plus interstitial alveolitis (CT scan showing ground-glass in two lung segments OR neutrophilic/eosinophilic alveolitis), refractory to cyclophosphamide (6g or more for a minimum of three months). The study follows a 'Simon's two-stage design': ten patients are enrolled in a first phase. A "good response to the treatment" is required in ≥10% of patients before starting the second phase of enrolment (20 patients). The drug will be rejected if the final observed response rate will be in less than 30% of patients. Patients are treated with oral Imatinib, 200 mg/d for 6 months. A "good response" is defined as the improvement of PI measured trough predefined clinical, functional and radiological criteria in presence of a fair drug tolerance. Secondary outcomes are: cutaneous involvement (mRSS) and laboratory evidence of reduced skin fibroblast activation.

Results: In the first phase three patients (30%) matched the criteria for a "good response": 2 patients (diffuse, late SSc) have shown an increase≥15% in FVC rate and 1 patient (limited, early SSc) an increase≥15% of DLCO and an improved CT-scan pattern after 6 months of treatment. Four severe adverse effect, all judged as unrelated to the study drug, were recorded. No data about cutaneous involvement are still available and no laboratory investigation on biological samples have been performed so far. The second phase is under way and the results will be available in few months.

Conclusions: If the available data will be confirmed by the final assessment, imatinib mesylate appears effective and tolerable in a subgroup of scleroderma patients with pulmonary involvement refractory to conventional treatment. These results warrant further investigations and a randomized placebo controlled trial.

45. Role of NOX homologues NOX 4 and DUOX 2 in scleroderma fibrosis

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Background: Oxidative stress plays an important role in the development of fibrosis under various pathological conditions. NADPH oxidase (NOX) is a multicomponent enzyme that catalyzes the reduction of molecular oxygen to superoxide. Among the seven members of the NOX family are key differences in their activation, subunit composition, localization and expression. Scleroderma (SSc) is a clinically heterogeneous and often lethal acquired disorder of the connective tissue characterized by vascular, immune/inflammatory and fibrotic manifestations. We have recently identified in the serum of scleroderma patients the presence of stimulatory anti-PDGF receptor (PDGFR) auto-antibodies (SScIgG) that are able to induce ROS production and fibrosis.

Objectives: The goal of the present study is to explore the differential contribution of NOX homologues in the disease progression.

Methods: Total IgG were isolated from serum of SSc patients and normal controls by affinity chromatography. Total RNA was isolated from normal and scleroderma fibroblasts and reverse transcribed, according to the manufacturer's instructions (Bio-Rad). Quantitative real-time PCR reactions were performed using SYBR Green PCR Master Mix (Bio-Rad). The relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. SSc skin fibroblasts were transiently transfected with the siRNA against NOX4 and DUOX2 with lipofectamine (Invitrogen).For protein expression, cells were lysed with RIPA buffer and subjected to western blot with specific antibodies.

Results: PDGF and SSc IgG induces intracellular ROS followed by collagen1a1 and a-sma gene overexpression in normal human fibroblasts. Knockdown of NOX4 and DUOX2 expression with siRNA decreases PDGF and SSc IgG induced ROS and collagen expression. Compared to healthy skin fibroblasts, dermal fibroblast from scleroderma patients show enhanced expression of NOX1 and NOX 4 subunits.NOX4 and DUOX 2 knockdown can affect basal ROS levels and block collagen protein expression.

Conclusions: Our findings suggest that SSc IgG initiates a NOX4/DUOX2 mediated reactive oxygen cascade and clarifies the role of these subunits in the progression of the fibrosis.

46. Pilot study to assess optical coherence tomography as a marker of disease for systemic sclerosis

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Introduction: Systemic sclerosis (SSc) is a connective tissue disease characterised by fibrosis and ischaemia. Our aim was to validate optical coherence tomography (OCT) as a technique to measure skin thickness, and relate to perfusion.

Methods: 20 patients with SSc and 20 healthy controls were recruited. Thickness of the epidermis (representative of disease severity) was assessed (distal digit, proximal digit, dorsum of hand, forearm, upper arm) using OCT, high-frequency ultrasound (HFUS, previously validated for measuring skin thickness) and modified Rodnan skin score. Laser Doppler imaging (LDI, measuring microvascular function [difference in perfusion between distal phalanx and dorsum of hand]) and nailfold capillaroscopy (NC, measuring microvascular structure [capillary density]) were measures of vascular involvement of disease.

Results: Epidermal thickness could be measured by both OCT and HFUS and was statistically significantly higher in patients at the digits, dorsum and forearm as measured by HFUS and at the proximal digit and dorsum as measured by OCT. HFUS and OCT showed correlation with skin score when all sites were grouped together; $R=0.40$ $p<0.001$, $R=0.47$, $p<0.001$. LDI and NC confirmed concomitant functional and structural microvascular abnormality (Table 1).

Conclusion: Validating the measurement of skin thickness with OCT and correlation of measured thickness with skin score is a first step in validating the use of OCT as a measurement tool in SSc.

47. NOX4 and ROS implication on biological effects mediated by anti PDGF receptor autoantibodies in human pulmonary artery smooth muscle cells.

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Background: Microvasculature damage is one of the earliest events in the onset of Scleroderma (SSc, Systemic sclerosis). Oxidative stress has been implicated in the pathogenesis of SSc and reactive oxygen species (ROS) have been recognized as important signaling molecules in the vascular wall. The NADPH oxidase (NOX) family is the major source of ROS in the vasculature. Between the various existing NADPH oxidase isoformes, NOX4 is the predominant form in vascular smooth muscle cells. Since we have already identified the presence of anti PDGF receptor (PDGFR) stimulatory antibodies in patients with Systemic Sclerosis, in this study we sought to investigate the effects of these autoantibodies on human vascular smooth muscle cells(HPASMC), in order to understand the biological effects and the implication of ROS and NOX4**.**

Methods: Serum immunoglobulins were purified by affinity chromatography. Intracellular ROS generation was determined using 2',7'-dichlorofluorescein diacetate. Proliferation and migration were studied using BrdU based proliferation ELISA and migration scratch test respectively. PDGFR and immunoglobulins interaction was studied with immunoprecipitation. NOX4 and Collagen I (α1) gene expression were analyzed by Real time PCR. NOX4 was silenced using siRNA. Immunohistochemistry was carried out on paraffin embedded skin sections. Nox4 protein expression was studied by FACs analysis.

Results: We were able to show ex vivo that SSc is characterized by excessive oxidative stress and upregulation of NOX4 within the vascular wall. In vitro, Scleroderma immunoglobulins induce ROS production in HPASMC. This effect is mediated through the PDGFR and NOX4. Furthermore PDGF and SSc immunoglobulins mediate NOX4 upregulation which in turns determines a ROS mediated mitogenic and pro-migratory effect on human pulmonary artery smooth muscle cells and a significant induction in collagen I (α1) gene expression.

Conclusions: Anti PDGFR autoantibodies may contribute to vascular damage observed in Scleroderma through their effects on ROS production and NOX4 expression.

48. Evaluating determinants of fatigue in systemic sclerosis assessed using the Functional Assessment of Chronic Illness Therapy Fatigue scale (FACIT-F)

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Objectives The Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System is a collection of health-related quality of life (HRQOL) questionnaires. The FACIT-F questionnaire is a 13-item questionnaire of fatigue which is translated into a summary score (0-52, higher scores indicative of lower fatigue). Fatigue is a debilitating, yet poorly understood symptom affecting patients with systemic sclerosis (SSc). Few studies have evaluated the validity and determinants of the FACIT-F in SSc.

Methods Sixty patients with SSc (52 female, mean age 63.0 [SD 11.7]) completed the FACIT-F, EuroQual-5Domain health questionnaire (EQ-5D) and Scleroderma Health Assessment Questionnaire (SHAQ) in response to a postal survey (~70% response rate). Case-notes were scrutinised for details including patient demographics, serology and clinical phenotype. A descriptive analysis was undertaken along with an evaluation of potential associations of fatigue in SSc.

Results: The mean FACIT-F score was 29.1 (SD 16.2). The mean FACIT-F score was higher in limited (n=41) cutaneous disease (32.5 vs. 21.6, p=0.014) which was also reflected in a moderate correlation between the FACIT-F and highest previous Rodnan skin score (r=-0.33, p=0.047). There were strong correlations between the FACIT-F score and health quality (r=0.74 and 0.72 for EQ-5D VAS and time trade-off respectively, p<0.001). There was moderate correlation with function assessed using HAQ-DI (r=-0.41, p=0.001) and the SHAQ visual analogue scores (VAS) for intestinal (r=-0.4, p=0.001), Raynaud's (r=-0.34, p=0.009) and respiratory (r=-0.28, p=0.034) symptoms. In contrast, no association was found between FACIT-F score and either age, disease duration, most recent pulmonary function test results (forced vital capacity and gas transfer), gender, smoking history, serology, organ-specific complications, or the SHAQ-VAS scores for pain, digital ulceration and patient global assessment.

Conclusions: The burden of fatigue in our study was similar to that reported in previous studies of rheumatoid arthritis (mean FACIT-F 28.8), although our study design was vulnerable to selection bias. Diffuse cutaneous disease, reduced functional capacity and lower patient-reported health quality (EQ-5D) were all associated with higher levels of fatigue. Higher self-report indices of Raynaud's severity, breathing and intestinal problems from the SHAQ were also associated with higher fatigue levels.

49. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a novel antifibrotic factor implicated in systemic sclerosis (SSc)

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Background: Transforming growth factor-beta (TGF-β) stimulates collagen synthesis and myofibroblast differentiation and is implicated in fibrosis in SSc. Ligands of the nuclear orphan receptor PPAR-gamma abrogate profibrotic TGF-ß responses. Nrf2 is a cap-and-collar transcription factor that orchestrates the antioxidant/cytoprotective response. The synthetic Peroxisome Proliferator-Activated Receptor-gamma (PPAR-γ) ligand CDDO induces Nrf2 in normal fibroblasts. The objective of the current study is to investigate the expression, regulation and role of Nrf2 in fibrosis.

Materials and methods: Nrf2 expression in SSc skin biopsies was examined by IHC. Effects of Nrf2 loss-of-function and gain-of-function were evaluated in vitro and in vivo. Regulation of Nrf2 expression and activity was examined by Western blot, immunofluorescence and reporter assays in normal and SSc fibroblasts. Effects of multiple Nrf2 inducers on profibrotic responses were evaluated in human skin fibroblasts in vitro and in mice in vivo.

Results: Nrf2 expression was reduced in SSc skin biopsies. Nrf2 on its own blocked stimulation of collagen synthesis and myofibroblast differentiation induced by TGF-β in human skin fibroblasts. In contrast, Nrf2 knockdown resulted in constitutively enhanced collagen gene expression. Mice with Nrf2 deletion showed heightened susceptibility to bleomycin-induced skin fibrosis. The PPAR-γ ligand CDDO induced accumulation of Nrf2 by preventing its degradation. In normal fibroblasts, CDDO potently inhibited the stimulation of fibrotic responses induced by TGF-ß, and normalized elevated collagen production in SSc fibroblasts. The anti-fibrotic effect of CDDO occurred in a PPAR-gamma-independent Nrf2-dependent manner. In vivo, CDDO attenuated bleomycin-induced skin fibrosis in mice.

Conclusions: Nrf2 serves as a novel endogenous antifibrotic factor in normal fibroblasts. Deficient Nrf2 expression in patients with SSc might contribute to the progression of fibrosis. Pharmacological induction of Nrf2 via protein stabilization potently inhibited TGF-ß induced profibrotic responses in vitro and prevented bleomycin-induced fibrosis in vivo. Therefore Nrf2 is a novel target for anti-fibrotic therapy, and pharmacological modulation of its expression or activity might have a therapeutic potential in SSc.

50. Clinical and Serological Features of Systemic Sclerosis in a Chinese Cohort

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Objective: We investigated clinical manifestations and serological features of systemic sclerosis (SSc) in a Chinese cohort.

Methods: A total of 381 patients meeting the criteria of SSc of the American College of Rheumatology were enrolled in a multicenter study of Chinese SSc. Patients were evaluated for degree of skin fibrosis, other organ involvement and SSc specific autoantibodies. Fisher's exact and chi-square tests were used in the studies associations between autoantibodies and clinical manifestations.

Results: The ratio of women to men in the Chinese SSc cohort is 5.3 to 1. Anti-nuclear autoantibodies (ANA) were present in 91.6% of subjects, anti-topoisomerase I in 53%, anti-centromere in 10.2%, anti-RNP in 21.4%, while anti-RNA polymerase III autoantibodies were found in only 1.9% of Chinese SSc cohort. Specific auto-antibodies appeared to be associated with clinical manifestations. Particularly, the presence of anti-topoisomerase I was in 59.2% of diffuse SSc versus 47.5% of limited form of SSc, in 57.5% of patients with pulmonary fibrosis versus 28 % without pulmonary fibrosis, in 60.5% patients with versus 39.8% without dysfunction of esophagus. The presence of anti-centromere autoantibodies occurred in Chinese SSc patients with less pulmonary fibrosis and less involvement of esophagus dysfunction (Table 1).

Conclusion: Chinese SSc patients examined in this cohort characteristically showed a high occurrence of anti-topoisomerase I and low occurrence of anti-RNA polymerase III. The presence of anti-topoisomerase I was associated with diffuse SSc, lung fibrosis and dysfunction of esophagus. In contrast, the presence of anti-centromere autoantibodies showed opposite associations.

Table 1. Associations between autoantibodies and clinical features of SSc.

Table 1 Associations between autoantibodies and clinical features of SSc

		anti-topo I(%)		p-value	Odds ratio	ACA (%)		p-value	Odds ratio
		+	-			+	-		
lcSSc		58(17.7)	64(19.5)			15(4.6)	107(32.6)		
dcSSc		122(37.2)	84(25.6)	**0.04**	1.6	20(6.1)	186(56.7)	0.463	1.3
Lung	+	127(45.7)	94(33.8)			15(5.4)	206(74.1)		
	-	16(5.8)	41(14.7)	**7.8×10^{-5}**	**3.46**	17(6.1)	40(14.4)	**1.2×10^{-6}**	**0.17**
esophagus	+	86(35.1)	56(22.9)			12(4.9)	130(53.1)		
	-	41(16.7)	62(25.3)	**0.001**	**2.32**	19(7.7)	84(34.3)	**0.02**	0.41

51. Low levels of S-nitrosothiol in patients of Systemic Sclerosis and Raynaud's in relation to Nitric Oxide

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Objectives: Plasma S-nitrosothiols (RSNO) are circulating nitric oxide (NO) donors. In systemic sclerosis (SSc) endothelial NO synthesis is impaired but inducible macrophage nitric oxide synthase is activated. The levels of RSNOs in SSc and Primary Raynauds' (RP) patients were measured which were not reported.before.

Introduction: S-nitrosothiols (RSNOs) are biological metabolites of NO which forms covalent bonds to the free sulphydryl groups of proteins, peptides and cysteine and acts as a reservoir of NO. RSNOs may protect against cellular toxicity associated with oxidative stress. The immediate breakdown product of RSNOs in human plasma is nitrite (NO_2^-). The function of a broad spectrum of proteins is regulated by S-nitrosylation.

Patients and methods: Venous blood was collected in tubes from patients (78% female) with approval of the ethics committee (16 Primary Raynaud's, 34 limited SSc and 11 diffuse SSc patients). Twenty six healthy subjects were used as controls. Concentrations of NO_2^- and RSNOs were detected by a chemiluminescence. The values were correlated with biological age, skin score, capillary score and duration of disease.

Results: A striking significant reduction in the level of detectable RSNOs was observed in lSSc (1.31. ± 0.37 nM, n = 34) and dSSc patients (0.73 ± 0.41, nM) compared to controls (6 ±0.8 nM, n = 26). They were also lower in some RP patients. Nitrite levels in PRP and SSc were not significantly different from those of controls. RSNO levels decreases in the early years of SSc progression. In the controls, S-nitrosothiols correlated with age, decreasing significantly after the age of thirty five to forty, but not in patients. It also decreased with higher skin scores for lSSc and, in RP, with increased capillary score.

Conclusion: The study provides further evidence for a dysfunction in NO metabolism in SSc.

52. Structural, smooth muscle and endothelial dysfunction in the thoracic aorta of the Tight-Skin 1 (Tsk-1/$^+$) mouse

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Objective: Systemic sclerosis (SSc) is a disease that features excessive collagen overproduction, fibrosis, as well as large and small vessel dysfunction. Previously we have shown that nitric oxide (NO), an important physiological signalling molecule and vasodilator has abnormal metabolism in the skin of SSc patients and in the scleroderma-like syndrome of the tight-skin 1 (Tsk-1/$^+$) fibrillin-1 mutant mouse. The present study investigates contractile function and also the role of NO, in the thoracic aorta of the Tsk-1/$^+$ mouse.

Methods: Thoracic aortae from heterozygous Tsk-1/$^+$ mice (age: 4, 8, 12 months) were compared with pallid littermates as control. Histology was used to stain sections for collagen or elastin expression. Vessel wall structure was further assessed by EM microscopy, mouse gene array (Illumina MouseWG-6 v2), and western blotting. TGF-β plasma levels were measured by ELISA. Vascular isometric tension measurements of contractile function were studied using an organ bath. Potassium chloride or phenylephrine (PE) agonists were used to induce vasoconstriction in aortic vascular rings. Vascular rings were also pre-incubated with L-NAME, a non-specific NO synthase (NOS) inhibitor. Aortic cGMP levels (an indirect marker of NO production) was measured by ELISA.

Results: In Tsk-1/$^+$ thoracic aorta, using histological staining and EM microscopy we observed thickening of the aortic arch wall, disruption of the elastic fiber architecture, and elevated levels of collagen in the aortic adventitia. Both gene and protein expression of the smooth-muscle α-1 adrenergic receptor, as well as calponin, caldesmon, and α-SMA protein were decreased in the Tsk-1/$^+$ group compared to controls. Profibrotic proteins such as CTGF and ET-1, as well as TGF- levels and collagen were increased in Tsk-1/$^+$ aortae. Isometric tension measurement revealed that both KCl and PE-induced contractions were reduced in aortic Tsk-1/$^+$ vessels at all ages studied. Inhibition of endogenous NO production in aortic control vessels by pre-treatment with L-NAME increased PE-induced contractions, but had no significant effect in the aortic Tsk-1/$^+$ group, indicating reduced basal NO bioavailability. Aortic Tsk-1/$^+$ vessels also showed decreased gene and protein expression of eNOS, and reduced cGMP content.

Conclusion: The pathogenesis of the Tsk-1/$^+$ mouse exhibits vasomotor dysfunction of the thoracic aorta that could be associated with increased collagen deposition, smooth muscle dysfunction, and reduced NO bioavailability.

53. Sequential nailfold video-capillary analysis in scleroderma reveals both destructive and reparative capillary changes

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Background: Scleroderma (SSc) is a rare and progressive heterogeneous disease of unknown aetiology characterised by widespread microvasculopathy, extensive fibrosis and autoimmunity. Previous studies indicate that vascular injury is an early event in scleroderma with an unknown initial insult. The purpose of this study was to characterise sequential morphological changes to nailfold capillaries in scleroderma using video capillaroscopy.

Methods: The nailfold capillary images of the 4th digit bilaterally were recorded using a Capiscope (KK Technology) in 9 patients with scleroderma and 4 healthy controls, monitored regularly over periods up to 12 months. The capiscope technology allows the creation of a computerised mosaic image of the nailfold capillary bed allowing precise identification of individual capillaries over time. Mean capillary density in each nailfold was also assessed.

Results: Nailfold capillary morphology and density was observed to be relatively stable in healthy controls. In contrast, patients with scleroderma showed evidence of evolving gross morphological capillary change including capillary dilatation, and contraction, capillary ghosting, recurrent capillary bleeding, capillary infarction, capillary drop-out and capillary angiogenesis. Morphological changes were apparent in some patients with scleroderma in a period as short as 6 weeks. Further, mean capillary density decreased in 4 patients, increased in 2 patients and remained unchanged in 3 patients over the period of study.

Conclusion: In health, nailfold capillary morphology and density is stable over time. In scleroderma, the nailfold capillary bed is a dynamic tissue with evidence of multiple and evolving changes in capillary calibre, capillary bleeding, capillary destruction and angiogenesis. The cause of these capillary morphological changes is not known.

54. Serum levels of platelet-derived growth factor are reduced in patients with systemic sclerosis who are at risk of pulmonary arterial hypertension

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Background: The role of various angiogenic and fibrogenic cytokines in the pathogenesis of systemic sclerosis (SSc) is controversial. NT-Pro-BNP has shown the most consistent results with increased levels in patients with pulmonary arterial hypertension (PAH). No specific biomarker has been demonstrated to predict which patients are most at risk of PAH.

Aim: To investigate the levels of various angiogenic and fibrogenic cytokines in patients with SSc with different clinical phenotypes.

Methods: Fifty six patients with SSc were included in this pilot study. They were further stratified into three main disease phenotypes: 1) SSc patients with PAH (n=14), 2) SSc patients with dis-proportionate fall in DLCO (>20%) with normal FEV1 and FVC but no evidence of PAH on 2D echocardiography and/or right heart catheter studies (n=17) and 3) SSc patients without known lung disease (n=20). There was also one small patient cohort with Interstitial lung disease (ILD) (n=5) investigated in this pilot. Twenty two healthy subjects with no evidence of connective tissue disease were included. Fractalkine, FGF2, PDGF AB/BB, VEGF and GM-CSF levels were measured using a Milliplex MAP Kit (Millipore, Billerica, MA, USA). NT-Pro-BNP was measured by electrochemiluminescence (ECLIA) immunoassay on a COBAS E (Roche Diagnostics GmbH, D-68298 Mannheim) immunoassay analyser. Statistical analysis used a non-parametric method, the Kruskel-Wallis test.

Results: Fractalkine, FGF-2 and NT-Pro-BNP levels were increased in SSc patients compared with healthy controls ($p<0.05$). PDGF AB/BB levels were significantly reduced in SSc patients compared with healthy subjects ($p<0.05$). There was no difference in VEGF and GM-CSF levels between SSc patients and healthy subjects. Among the SSc patients, NT-Pro-BNP levels were significantly different in all the three sub-groups (group 1 > group 2 > group 3) with the highest levels seen in patients with PAH ($p<0.001$). PDGF AB/BB levels showed the opposite trend with decreasing levels in the three subgroups (group 1 < group 2 < group 3) ($p<0.05$). The levels of fractalkine and FGF-2 did not differ among the SSc patient subgroups.

Conclusion: The findings from this pilot study suggest that in addition to elevated levels of NT-Pro-BNP, reduced PDGF AB/BB could be used to identify SSc patients at risk of developing PAH. The low levels of PDGF AB/BBand increased levels of FGF-2 in SSc patients may be linked to impaired angiogenesis. Further studies are required to validate these initial observations and to evaluate the usefulness of serial measurements of these cytokines to predict patients at risk of developing PAH.

55. T cell derived IL-6 and IL-13 drive fibroblast fibrosis: implications for Systemic Sclerosis

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Systemic Sclerosis (SS) is an autoimmune disease of unknown aetiology that is characterised by inflammation, vasculopathy and excessive extracellular matrix deposition. The extracellular matrix deposition is primarily in the skin and organs and can lead to organ failure. The immune abnormalities in the disease include T and B cell activation and a host of proinflammatory cytokines that may mediate the fibrotic response characteristic of the disease. Tumour necrosis-α (TNF-α) is a pro-inflammatory cytokine that may be involved in disease pathogenesis and has been demonstrated to be upregulated in SS. TNF-α signals through two receptors causing a variety of downstream effects that depends on cell type and context. The aim was to investigate the role of TNF-α in T cells and the role of pro-inflammatory cytokines in scleroderma and matrix deposition. We used T cells from scleroderma and controls and analysed these for the TNF-α receptor using flow Cytometry to examine expression, both in skin and PBMCs. Specific mutant ligands that are recombinant for TNF-α receptor subtypes or soluble TNF was used to examine downstream effects. T cell conditioned medium was added to normal dermal fibroblasts and markers of fibrosis were examined including Collagen Type I by Real Time Polymerase Chain reaction (RT-PCR). T-cell-derived cytokines were measured using ELISA and subsequent cytokines neutralised with antibodies or isotype controls and collagen I measured. **Results:** T cells were present in high numbers in the skin of patients. Also TNF-αR II was elevated in T cells from both the skin of affected patients and also T cells from peripheral blood compared to healthy controls. Mutant ligands to receptor subtypes leads to elevated Interleukin-6 and also IL-13 expression from healthy and scleroderma donors. However scleroderma donors have a much higher constitutive level of both cytokines without the addition of TNF-α ligands suggesting activation of T cells. Conditioned medium leads to upregulated α-smooth muscle actin content in dermal fibroblast and also upregulated Collagen I expression by 20 fold after incubation with TNF-R subtypes both R1 and R2. Suppression of T cell derived cytokines IL-6 and IL-13 in combination by neutralising antibodies leads to an attenuated increase in collagen I mRNA expression as compared to the relevant matched isotype controls, indicating a pivotal role of these cytokines in fibrogenesis.

Conclusion: Scleroderma T cells expressed elevated TNF-R 2 expression, this maybe an activation marker in scleroderma. T cells are activated 'in vivo' and secrete the cytokines IL-6 and IL-13. IL-6 and IL-13 work in a synergistic fashion leading to enhanced extracellular matrix deposition.

56. Fli1 is involved in Bleomycin-induced Lung Fibrosis and Inflammation

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Background/Rationale: Fli1, a member of the Ets family of transcription factors, is known to play a role in megakaryopoeisis, and exerts negative regulation on collagen synthesis. Homozygous deletion of Fli1 in mice is embryonic lethal at E11.5 due to a hemorrhagic phenotype. Although homozygous disruption of Fli1 is lethal, Fli1 heterozygous (Fli1+/-) mice are viable with minor defects in hematopoeisis. Disruption of Fli1 in mice has been shown to contribute to elevated collagen and dermal fibrosis; however, lung fibrosis has not been assessed. The goal of this study is to determine if Fli1+/- mice display more severe inflammation and fibrosis of the lung after bleomycin administration.

Methods: Fli1+/- or wild type (WT) control male mice (4-5 months) were injected subcutaneously with 100uL of bleomycin (1mg/mL) or saline daily for 4 weeks. Lung and skin were examined at 28d after the first injection. Total RNA was extracted and examined for expression of Fli1, Col1a1, Col1a2, Col3a1, Col5a1 and Col5a2. Total collagen in tissue was examined by Gomori's Trichrome stain and a colorimetric assay. Inflammation was determined by H+E, mRNA analysis of inflammatory markers and immunohistochemistry.

Results: Basal collagen levels were elevated in lungs of Fli1+/- (saline injected) mice by qPCR and Trichrome stain when compared to WT mice. WT mice develop fibrosis only after bleomycin challenge. Similarly, Fli1+/- saline injected mice show elevated cell infilatration by H+E; whereas WT mice develop inflammation only upon bleomycin administration. Fli1 mRNA levels were decreased 50% in lungs of Fli1+/- mice and further decreased after bleomycin administration. Importantly, Fli1 is also significantly decreased in WT mice after Bleomycin (63%, p=0.007, n=2).

Conclusion: Both groups of mice show evidence of lung fibrosis after bleomycin challenge; whereas Fli1+/- mice show evidence of lung fibrosis at the basal level. This supports the role of Fli1 deficiency in fibrosis of the lung and suggests a role for Fli1 as a key mediator in the pathogensis of both spontaneous and bleomycin-induced fibrosis.

57. Examination of the pre-fibrotic and fibrotic disease in Tsk2/+ mice shows early ECM changes are attributable to elastin dysregulation

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Rationale: The Tight Skin (Tsk) 2 mouse model of systemic sclerosis (SSc) has many features of the human disease including tight skin, excessive collagen deposition, alterations in the extracellular matrix, and occurrence of antinuclear antibodies (ANA) with age. Because current treatments only ameliorate disease symptoms, studies of the Tsk2/+ mouse are important for understanding of disease pathology and progression, and the potential unmasking of new therapeutic targets. This model was used to develop a novel timeline of disease progression and to evaluate ECM changes essential for fibrosis and disease development that are difficult to study in human individuals with SSc.

Methods: Skin samples from Tsk2/+ and WT littermates in our colony of B6.Tsk2/+ congenic mice (N4-N8) (2 – 24 weeks old) were examined for ECM alterations and cellular infiltrates with age. Neonatal fibroblasts were obtained from Tsk2/+ and WT mice and evaluated for collagen and elastin protein production *in vitro*. Fibroblasts were obtained from patients suffering from SSc and healthy controls and evaluated for collagen and elastin transcript levels. Blood samples were collected serially from individual mice and examined by indirect immunofluorescence for the presence of antinuclear antibodies.

Results: *In vivo*: Using histological and total protein analysis, we show that collagen accumulation increases with age in Tsk2/+ mice, with significant increases as compared to WT littermates not occurring until 10 weeks of age ($N = 10$; $p < 0.01$). This age is fully 8 weeks after the development of the "tight" phenotype. In contrast, histological examination of the ECM of the dermis revealed a significant 2.5-fold increase of elastin fibers at 2 weeks of age ($N = 6$; $p < 0.001$) that continued throughout adulthood. Skin from 2 week old Tsk2/+ males have a 2.5-fold increase in production of elastin transcripts compared to WT males ($N = 4$; $P < 0.05$). The first occurrence of antinuclear antibody production in Tsk2/+ mice was at 17 weeks of age and the presence of antinuclear antibody overall was not different between Tsk2/+ and WT littermates.

In vitro: We show that neonatal fibroblasts isolated from Tsk2/+ mice produce significantly more collagen than WT fibroblasts ($N = 6$; $p < 0.001$). SSc skin fibroblasts also have increased production of collagen and elastin transcripts compared to healthy controls ($N = 3$; $p = 0.03$).

Conclusions: Our studies reveal a novel timeline of disease development in the Tsk2/+ mouse and show that the disease state is progressive with initiating ECM changes occurring months before fibrosis. The progressive disease state in this model is valuable for examination of early, pre-fibrotic disease pathology that is otherwise impossible to examine in human disease. New data on the cellular autonomy of ECM dysregulation *in vitro* suggest that the Tsk2/+ mutation is capable of acting very early in mouse development, a finding that corroborates the embryonic lethality seen in Tsk2 homozygotes.

58. IL-6 overexpression in early dcSSc associates with poor clinical outcome and may drive fibrotic response

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Introduction: We previously reported that thrombocytosis may identify a subgroup of diffuse cutaneous systemic sclerosis (dcSSc) with elevated IL-6 and high modified Rodnan skin score (mRSS). In this study, we explore the dermal expression of IL-6 in SSc and evaluate its potential profibrotic effects and downstream signalling pathways.

Methods: Expression levels of IL-6 were examined using immunohistochemistry and Western blot analysis. Skin biopsies were obtained from patients with early and established dcSSc, limited cutaneous SSc (lcSSc) and healthy controls. The effect of IL-6 and soluble IL-6 receptor on extracellular matrix (ECM) production was evaluated in cultured normal and SSc fibroblasts. Interplay between signalling pathways regulated by IL-6 trans-signalling was examined using pharmacological inhibitors to induce activation of ECM protein expression. To explore the potential link between IL-6 levels and clinical outcomes in dcSSc, serum IL-6 levels from 39 patients was measured by ELISA and cases were categorised into high IL-6 (≥10pg/ml) and low IL-6 cohorts. Association between serum IL-6 levels at presentation and mRSS at 36 month from disease onset was determined by Pearson's correlation. Difference in survival between cohorts was examined using Kaplan-Meier analysis.

Results: There was greater dermal IL-6 expression in patients with early dcSSc compared to established dcSSc. Prominent staining for IL-6 was associated with vascular structures and mononuclear inflammatory infiltrate in 8/10 patients with early dcSSc. In contrast, there was minimal fibroblastic staining for IL-6 in patients with lcSSc and established dcSSc.

There was upregulation of collagen synthesis in normal fibroblasts (34.3±2.45 vs 9.88±1.54 Densitometry Image Unit (DIU) controls, $p < 0.05$) in response to IL-6 (25ng/ml) and sIL-6R (20ng/ml). Similar induction of SMA and CTGF by 12-fold and 15-fold respectively were observed in normal fibroblasts. The IL-6 trans-signalling activation of ECM production in normal fibroblasts was abrogated by AG490 and S3I-201 that targets JAK2 and STAT3 signalling pathways respectively.

Serum IL-6 levels at presentation positively correlated with mRSS at 36 months followup in dcSSc cases with available data ($r=0.81$, $p<0.01$, $n=16$). Kaplan-Meier analysis showed that the 5-year survival was 93% and 81% in the group with low and high IL-6 levels respectively ($p=0.02$, log rank test).

Conclusions: These results confirm overexpression of IL-6 in selected cases of dcSSc and IL-6 trans-signalling may activate markers of fibrosis. Our study provides support for the potential benefit of targeting the IL-6 ligand-receptor axis in early dcSSc.

59. N-TproBNP as a potential outcome predictor in Scleroderma Renal Crisis

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Background: Scleroderma renal crisis (SRC) is a life-threatening complication of Systemic Sclerosis (SSc), clinically characterised by acute renal failure and hypertension with significant mortality and morbidity. Therefore, novel biomarkers to identify patients at high risk of poor renal outcome would be invaluable.

The N-terminal fragment of Brain Natriuretic Peptide (N-TproBNP) has an important role as biomarker in heart failure and pulmonary arterial hypertension. Notably, N-TproBNP has shown clinical utility also in the setting of renal impairment. In this study, we assess the role of N-TproBNP in a retrospective cohort of SRC patients.

Methods: 19 SRC patients (16 dcSSc and 3 lcSSc) were enrolled in this study. All patients had normal pulmonary artery systolic pressure. Three patient subgroups were identified based on renal outcomes (no dialysis, temporary dialysis and permanent dialysis). Kruskal-Wallis test was used to compare N-TproBNP levels among the subgroups. Logistic regression analysis was performed to investigate the relationship between N-TproBNP levels and renal outcome. N-TproBNP levels were compared at SRC presentation and at six-month followup using Wilcoxon matched-pair test. Associations between N-TproBNP and creatinine, haemoglobin (Hb) and disease duration at SRC were determined by Spearman's coefficient.

Results: There was significant difference in N-TproBNP levels among three subgroups of patients based on renal outcome (median: 'no dialysis' 119, 'temporary dialysis' 1729.5 and 'permanent dialysis' 3373 pmol/L; $p=0.003$). At logistic regression analysis, N-TproBNP>1494 pmol/L was strongly predictive of requirement for dialysis (OR 70, $p<0.005$, 95%CI 3-1317). Among the eleven patients (57.9%) who had N-TproBNP levels repeated at six-month followup, there was a significant reduction in N-TproBNP values ($p= 0.0029$). As expected, N-TproBNP levels strongly correlated with serum creatinine ($\rho= 0.6105$, $p= 0.0055$) and negatively with eGFR ($\rho=-0.7446$, $p= 0.0009$). N-TproBNP levels were negatively correlated with Hb levels ($\rho=-0.7123$, $p=0.0006$) and disease duration ($\rho=-0.7083$, $p=0.0007$). Similar correlation was observed between N-TproBNP and left ventricular ejection fraction but this didn't meet significance ($\rho=-0.4594$, $p=0.0734$).

Conclusion: These data strongly suggest that N-TproBNP may be a useful biomarker in risk-stratification of renal outcome among SRC patients, and selectively identifies patients who are likely to require renal replacement therapy.

60. Estimation of cellular states from SSc gene expression reveals heterogeneity of pathway expression.

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Background: Genome-scale gene expression profiles for SSc and healthy control skin biopsies have identified molecularly distinct subsets of SSc. These subsets have been linked to disease severity and may respond differently to therapy. It is less clear which biochemical pathways are dysregulated to produce the different disease states. Due to the cost and complexity of perturbative experiments in disease models, bioinformatics approaches in SSc must use inference tools based on "static" gene expression profiles from patient skin biopsies to build hypotheses to test in models. Given the heterogeneity of scleroderma, we hypothesized that multiple canonical pathways would be dysregulated and that meaningful patterns of co-regulation could be statistically identified from gene expression in whole tissue.

Materials and methods: We collected a database of canonical gene pathways comprised of KEGG, BioCarta, and Reactome annotated pathways. To identify pathways that showed differential regulation, we employed a clustering method called "Pathway-based Clustering" on a publicly available SSc skin microarray data set. To infer the cellular state corresponding to the identified modes of regulation, we fit Gibbs distributions, which allow for the identification of gene-gene interactions and novel co-regulation, to the expression profiles of the clusters.

Results: Over 100 of the 880 pathways used in this study, including disease implicated pathways like sphingolipid metabolism, showed significant differential regulation. Surprisingly, clustering often identified more than two modes of differential regulation. In the TGFb signaling pathway, for example, five distinct modes of regulation were found. Thus, skin tissue in SSc patients shows that parts of canonical pathways are a regulated differently in separate subsets of patients. Furthermore, the fit Gibbs distributions identify putative co-regulation within pathways that is not annotated in the standard pathway.

Conclusions: Canonical gene pathways show complex modes of regulation in SSc skin tissue with different parts of the pathway regulated differently. The mechanisms behind this regulation are unknown, but the present study has established a framework for future pathway-based studies on gene expression in SSc.

61. Forced activation of Wnt signaling in dermal fibroblasts causes avascular fibrosis

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Background: The canonical Wnt/β-catenin signaling pathway is required for dermal cell specification in mouse embryo. In adult tissue, Wnt/β-catenin activity promotes fibroblast proliferation during wound healing and is dysregulated in the tight-skin mouse and human systemic sclerosis skin. We hypothesized that activation of Wnt/β-catenin signaling is sufficient for pro-fibrotic behavior in dermal fibroblasts.

Materials and methods: To constitutively activate β-catenin in dermal fibroblasts, inducible *HoxB6Cre-ER* transgenic females were crossed with males carrying *R26R-YFP/R26R-YFP* lineage tracer and an allele of *β-catenin* with floxed exon 3 (*β-catenin* ^{stabilized}). One dose of tamoxifen administered to pregnant females carrying E16.5 embryos was sufficient to induce Cre-ER-mediated recombination of the *R26R-YFP* lineage tracer. Mutant mice and littermate controls were born and skin was analyzed at postnatal timepoints up to 6 weeks of age by H&E, Masson's trichrome stain, immunohistochemistry, and quantitative RT-PCR. Immunohistochemistry was done with antibodies against YFP, β-catenin, CTGF, and the endothelial cell marker MECA-32. Flow cytometry was used to sort for YFP- and PECAM-positive cells collected from perinatal control dermis.

Results: Flow cytometry of *HoxB6CreER/+; R26R-YFP/+* skin verified that YFP-expressing cells possessed dermal, not endothelial, identity. The ventral skin of *HoxB6CreER/+; R26R-YFP/+; β-catenin*^{stabilized}*/+* mutant mice, compared to littermate controls, had gradually increased dermal thickening, collagen deposition, and CTGF-expressing fibroblasts. Mutant mice also had an ectopic subcutaneous layer of progressively thickening fibrotic tissue that was completely avascular, indicating a non-cell-autonomous anti-angiogenic effect of canonical Wnt signaling. Lineage analysis demonstrated a significant contribution by mutant dermal cells to the dermis and the subcutaneous fibrotic tissue. Cells in the mutant dermis and underlying fibrotic tissue demonstrated stably increased β-catenin activity, signified by nuclear β-catenin in dermal fibroblasts. Variable increases in nuclear β-catenin were observed in skin from patients with early- and late-stage systemic sclerosis.

Conclusions: Stabilization of Wnt/β-catenin signaling in mouse dermal fibroblasts resulted in development of progressive dermal fibrosis and subcutaneous avascular fibrosis. This genetic model supports a pro-fibrotic role for dermal canonical Wnt signaling and provides a framework to investigate the mechanisms of fibrosis in the dermis at the genome-wide level.

62. Up-regulation of collagen type V mRNA in a model of systemic sclerosis

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Background: For a better understanding of the pathophysiology of systemic sclerosis (SSc), animal models are important tools. We have described an animal model of SSc induced by type V collagen (COLV) that resembles the human disease. Considering that tissue fibrosis is one of the hallmarks of SSc the aim of this study was to investigate the early disease in the lung of this model with special emphasis on collagen deposition and mRNA collagen synthesis.

Methods: Female rabbits from New Zealand lineage were immunized with COLV plus Freund´s adjuvant (FA). Animals immunized only with FA were used as controls. Seven days, 75-days and 210-days after the first immunization, the animals were sacrificed and the lungs submitted to immunofluorescence, real-time qPCR and biochemical examination to determine collagen content, morphology and mRNA expressions of COL I, III and V, and the quantity of collagen-specific amino acid hydroxyproline.

Results: The immunolabeling for the COL I, III and V by immunofluorescence showed an intense expression of COLV in the bronchovascular interstitium near the inflammatory infiltrate in lung tissue of COLV-rabbits at day-210. Additionally, morphologic analysis demonstrated that the progressive remodeling of the pulmonary extracellular matrix observed in this group of animals was characterized by thickened COLV deposition with distorted fibrils. In accordance with these observations, the real-time qPCR revealed markedly up-regulation of COLV mRNA of the COLV-group at 210-day comparing with controls ($p<0.001$). Importantly, the content of hydroxyproline was compared for COLV-group and controls at day-7, day-75 and day-210, suggesting that the day-210 group represents an early disease stage without established fibrosis.

Conclusions: We found an early up-regulation of COLV mRNA in the lung tissue of SSc experimental model induced by COLV immunization emphasizing the role of this protein in the pathogenesis of SSc. In addition, our results reinforce the importance of this model, emerging promising to study the early stages of pulmonary fibrosis in this severe disease.

63. Clinical Significance of Defined ANCA Positivity in Systemic Sclerosis

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Purpose: While an atypical ANCA is reported in 20-30% of patients who have systemic sclerosis (SSc), this is not usually clinically significant. Conversely, patients with systemic sclerosis who have confirmed ANCA positivity with either myeloperoxidase (MPO) or proteinase-3 (PR3) antibodies on ELISA and clinical vasculitis are rarely reported in the literature. We have interrogated our database of patients with SSc who also have listed a clear clinical or histological diagnosis of vasculitis to look for the prevalence of overlap disease with ANCA-associated vasculitis (AAV) and whether these patients have altered disease features.

Methods: We examined a clinical database of 2200 patients with either limited or diffuse cutaneous SSc. Patients who had a clear clinical or histological diagnosis of vasculitis of any description had their serology investigations reviewed and repeated if possible to look for ANCA positivity with either MPO or PR3 reactivity. The clinical features, serology and histology of those patients who had both SSc and AAV were examined in detail.

Results: From our SSc cohort, 35 patients (1.6%) had a current or previous history of vasculitis, in whom the distribution of antibodies characteristically associated with the SSc features of their disease was comparable to those previously published from our cohort. For example, 20% were anti-centromere positive, 20% anti-topoisomerase-1 positive and 6% anti-fibrillarin antibody positive. Of these 35 patients, 8 (0.4% of total cohort) were either anti-MPO or anti-PR3 antibody positive. Both patients from the 35 who carried anti-fibrillarin antibodies, usually associated with overlap disease and increased incidence of renal and cardiac complications, were ANCA positive. No patients with vasculitis and centromere antibodies were ANCA positive. Of the 8 ANCA positive patients, 7 had limited cutaneous SSc and anti-MPO antibodies and one had diffuse disease with anti-PR3 antibodies. The latter patient had clinical features of microscopic polyangiitis with renal, neurological and skin involvement. None of the MPO positive patients had granulomatous disease. All but one had glomerulonephritis, and 6 had pulmonary fibrosis.

Conclusions: SSc in overlap with ANCA-associated vasculitis is rare, and clinical features are more mixed than when either of these two conditions occurs separately. From our database, anti-fibrillarin antibodies may be more associated with overlap AAV than the other scleroderma-specific antibodies.

64. Absence of epithelial to mesenchymal transition despite activation of keratinocytes in scleroderma skin.

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Background: Recently, we found that scleroderma (SSc) epithelial cells show activated phenotype similar to the one found in wound healing. Keratinocyte-fibroblast cross talk is important in health and disease including epithelial to mesenchymal transition (EMT). EMT is now identified as an important mechanism contributing to lung, liver, and kidney fibrosis. In the SSc epidermis we found active HGF signalling via c-Met, and SMAD phosphorylation consistent with TGFβ signalling, both mechanisms implicated in driving EMT. Also we found increased vimentin levels in whole skin biopsy proteomics. Therefore, we decided to look for evidence of EMT in the skin of scleroderma patients to determine if the skin fibrosis in scleroderma might be driven by the EMT process.

Materials and methods: Forearm skin biopsies taken from scleroderma patients diffuse subset (n=6) and age matched healthy controls (n=6) were analysed by immunohistochemical staining using antibodies against epithelial markers, K14 and E-cadherin as well as mesenchymal cell markers such as: vimentin, FSP-1, α-SMA. Collagen IV was also identified in the sections to determine integrity of the basement membrane.

Results: Immunohistochemistry results showed activated skin phenotype. Epidermal thickness was increased in SSc skin (p=0.005), so was the area of basal and spinous layer keratinocytes (p=0.0016, p=0.0038). However, higher number of ki67 positive cells in SSc epidermis was not significant (p=0.08). We did not observe any loss of E-cadherin or gain of vimentin in basal keratinocytes. Cells in the subepidermis of healthy controls had increased vimentin staining. The collagen IV layer in the basal membrane was not compromised. However, we noticed increased levels of FSP-1 expression in scleroderma skin when compared with healthy control skin.

Conclusions: Our results indicate that despite keratinocytes activation and HGF signalling, EMT is not taking place in scleroderma skin. The findings are consistent with the clinical observation that skin cancers are not seen at increased frequency in scleroderma patients. However, more investigations should be done to definitely exclude the EMT process from pathogenesis of scleroderma skin.

65. Vesicular trafficking in a model of systemic sclerosis

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Background: Activated fibroblasts are a hallmark of systemic sclerosis and exhibit increased secretion and deposition of extracellular matrix (ECM) proteins, which alters growth factor and integrin signalling, promoting tissue contraction and fibrosis. A key determinant regulating the extent and progression of fibrosis is intracellular vesicular trafficking, which governs the exocytic transport of ECM proteins and is under the control of autologous as well as external cues. We hypothesise that vesicular trafficking is altered in fibrotic fibroblasts.

Materials and methods: Wild type and tight-skin heterozygote mouse (TSK+/-) fibroblasts were used as model system and plated on glass coverslips coated either with Matrigel or collagen type I. After labelling with NBD-ceramide, a fluorescent lipid analogue that accumulates in the Golgi complex, live fibroblasts were observed by Total Internal Reflection Microscopy. Movement of NBD-ceramide labelled vesicles close to the glass-plasma membrane interface was observed at 200 ms time intervals for 30 seconds. Vesicle trajectories were detected and average velocity, final displacement as well as mean square displacements (MSD) calculated for each trajectory.

Results: TSK+/- and wild type cells displayed similar vesicular velocities, however, average displacement was higher in TSK+/- cells (4 µm compared to 2.7 µm). Furthermore, diffusion coefficients were higher in TSK+/- cells (1.5 compared to 0.9 µm/s). MSD plots confirmed these observations and showed that vesicles in TSK+/- cells move with higher velocities compared to those present in wild type cells. Interestingly, these results were independent of the substrate the cells were plated on. These differences are not due to structural changes in the Golgi apparatus between control and TSK+/- cells. In fact, Golgi areas were determined by measuring the two-dimensional area occupied by the fluorescent label and normalising it to cell perimeter after visualisation of the actin cytoskeleton. No significant differences were observed between control and TSK+/- cells.

Conclusions: Differences in vesicular velocity in TSK+/- and wild type fibroblasts indicate that trafficking is altered in disease cells and it is likely to contribute to the extended deposition of ECM observed in systemic sclerosis fibroblasts.

66. Identification of stable housekeeping genes for real-time PCR in human pulmonary fibroblasts

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Background: Quantitative real time PCR is an important tool in investigating gene transcription levels under different biological conditions. Accurate results, however, rely on controlling for differences in mRNA quantity and quality between samples. This is commonly achieved by normalisation to expression of reference genes, also known as housekeeping genes (HKGs). Expression stability of the HKGs used is critical, but to date no systematic study has been performed in pulmonary fibroblasts.

Materials and Methods: Microarray data of gene expression in explanted fibroblasts from SSc-ILD (systemic sclerosis interstitial lung disease biopsies, n=8) and control (normal periphery of resected tumors, n=10) lung tissue was used to assess variation in expression of commonly used HKGs.The most stable HKGs, with no difference in expression according to disease status and <15% expression variability between all samples, were tested by qRT-PCR in an independent experiment in which SSc-ILD (n=3) and control (n=3) fibroblasts were cultured in 0.1% BSA for 24hrs, and a further 24hrs with or without IFNγ (10ng/mL). A measure of expression stability (M), the average pairwise variation with each of the other studied genes (with a recommended maximum value of 1.5) for each HKG, was calculated using the program geNorm (Vandesompele, J. *et al.* Genome Biology 2002;3:34.1-12).

Results: A significant ($p<0.05$) difference in expression levels between patients and controls were found for *ACTB*, *ALAS1*, *B2M*, and *GAPDH. HPRT1*, *RPL32, SDHA*, *TBP*, *YWHAZ*, and *UBC*, were identified as the most stably expressed according to the microarray data. When tested further in geNorm these six HKGs all had an expression stability of $M<1.0$; the most stable gene was *HPRT1* (M=0.66) followed by *YWHAZ* (M=0.68), *RPL32* (M=0.72), *SDHA* (M=0.82), *TBP* (M=0.84), and *UBC* (M=0.92).

Conclusion: We have shown that four genes commonly used for normalisation of mRNA expression levels are not appropriate for use when studying control and SSc pulmonary fibroblasts. We have also identified a number of HKGs which are suitable for qRT-PCR normalisation in human lung fibroblasts under the conditions tested. This data demonstrates the necessity for empirical identification of HKGs.

67. The Role of Epithelial to Mesenchymal Transition in Systemic Sclerosis

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Background: Systemic Sclerosis (SSc) is a connective tissue disease of unknown etiology that is characterized by inflammation and autoimmunity, vasculopathy and interstitial remodelling, which results in tissue scarring and fibrosis. During fibrosis, tissue structure is progressively replaced by abnormal collagen-rich extracellular matrix (ECM) and the fibroblast is a principle cell type in ECM synthesis. Therefore, there is growing interest in the different cell types from which fibroblasts may derive, and the factors that activate and recruit these cells to lesions and promote their *in situ* differentiation into myofibroblasts. Epithelial cells are one of the cells types which may contribute to the fibroblastic pool through a process termed epithelial to mesenchymal transition (EMT). Previous work have shown that transgenic mice with fibroblast-specific over-expression of connective tissue growth factor (CTGF/CCN2) or constitutively-active transforming growth factor-β (TGF-β) type I receptor (ALK5) develop tissue fibrosis, primarily in the skin and lung. Our *in vivo* data suggests that over-expression of CTGF or activation of TGF-β signalling also results in pathological changes in the adjacent epithelium.

Materials and methods: Type II alveolar epithelial cell lines from human (A549) and rat (T2) were cultured in DMEM supplemented with 10% foetal calf serum until confluent. Cells were serum-starved over-night before stimulation with TGF-β or CTGF. To assess whether the cytokine treatments were able to induce EMT, the prototypical epithelial cells marker, E-cadherin, and mesenchymal cell markers including CTGF/CCN2, SNAIL, α-SMA, collagen type I and fibronectin were examined by Western blotting.

Results: TGF-β induced A549 and T2 type II alveolar epithelial cells to undergo a change in morphology from a prototypical cuboidal shape to a mesenchymal-like morphology. TGF-β also induced expression of a range of EMT molecular markers, including decreased E-cadherin expression, and increased expression of mesenchymal markers such as SNAIL, CTGF, α-SMA and collagen type I.

Similarly, addition of exogenous CTGF to T2 cells resulted in decreased E-cadherin expression and induced α-SMA expression.

Furthermore, CTGF-specific siRNA dose-dependently suppressed TGF-β-induced CTGF and SNAIL protein expression towards basal levels in A549 cells.

Conclusions: The current data suggest that epithelial to mesenchymal transition may play an important role the fibrosis. In SSc lung disease, over-expression of CTGF/CCN2 by stromal fibroblasts may drive the phenotypic switching increasing the number of pro-fibrotic, matrix-producing mesenchymal cells.

68. Analysis of single nucleotide polymorphisms (SNPs) in the IL7R and IL7 gene in patients with systemic sclerosis (SSc)

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Background: Systemic sclerosis (SSc) is characterized by vascular damage, autoimmunity and fibrosis. The interplay between these processes is likely to be pivotal to pathogenesis of SSc. Clinical heterogeneity is a hallmark of SSc and it is likely that this is determined at least in part by genetic factors. In particular differences in expression and signaling through IL7 receptor (IL7R) have been identified as factors determining clinical activity in other autoimmune rheumatic diseases such as SLE and systemic vasculitis but no detailed studies of genetic alterations of IL7 and (IL7R) in SSc have been undertaken. SSc represents a prototypic, chronic, non-relapsing, progressive autoimmune disease; we investigate whether genetic alterations also exist in SSc patients that may associate with clinical phenotypes.

Patients and Methods: Patients with SSc (n=728) and healthy controls (n=260) were genotyped for 15 SNPs in the gene region of IL7R and 7 polymorphisms in the region of IL7 and we determined the association with specific clinical and serological characteristics. All patients and controls were UK-Caucasian. Genotyping was performed by the KASPar system (allele specific PCR, KBiosciences, UK).

The statistical analysis was performed using logistic regression analysis to compare the distribution of IL7R/IL7 polymorphisms.

Results: No significant differences in the genotype distribution were observed between the patient group and healthy controls, all of which were in Hardy-Weinberg equilibrium. However, there was a significant difference between SSc patients being positive versus negative for anti-topoisomerase I antibodies (ATA) in four SNPs located in the IL7R region, rs11567685 (p=0.0075, odds ratio (OR) for CC genotype 1.469, 95%-confidence interval (CI) 1.11-1.95), rs11567751 (p=0.007, OR for TT 1.467, 95%-CI 1.11-1.94), rs987107 (p=0.0081, OR for TT 1.456, 95%-CI 1.10-1.92) and rs3194051 (p=0.0072, OR for GG 1.466, 95%-CI 1.11-1.94).

Conclusion: Here we report that homozygous carriers of the minor allele in four SNPs of the IL7R gene region were significantly stronger associated with a positive ATA-status in SSc patients. This gene has been described to be associated with immune regulation in other autoimmune diseases opening a possibility of a common autoimmune genetic pathway. In addition it is possible that IL-7 expression of signalling may prove a useful candidate biomarker in disease assessment.

69. Analysis of cutaneous wound healing and fibrosis in a Platelet-derived growth factor receptor knockout mouse

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Background: Platelet derived growth factors (PDGFs) are potent activators of migration and proliferation in mesenchymal cells such as fibroblasts. PDGFs have been implicated in the pathogenesis of scarring and fibrosis. Recent data showing that PDGFR auto antibodies are present in SSc, plus ongoing clinical trial using tyrosine kinase small molecular inhibitors, have suggested that PDGFR may have an important role in SSc. Additionally, it may have a specific role in mediating the accelerated fibrotic response associated with SSc. There are five different PDGF ligands: PDGF-AA, PDGF-BB, PDGF-CC, PDGF-DD, and PDGF-AB, which bind three dimeric receptors: PDFGRαα, PDGFRββ and PDGFRαβ, with varying specificities and affinities. However, it is not entirely clear which ligand/receptor combinations are expressed in SSc and contribute to the wound healing and fibrotic response

Objectives: To generate a fibroblast-specific conditional inducible PDGFR knockout mouse. To analyse the impact of PDGFRα deletion in fibroblasts on cutaneous wound healing and associated scarring.

Methods: We generated a conditional, fibroblast specific tamoxifen inducible PDGFRα knockout mouse. The resultant mice that were PDGFRα $Flox^{+/+}$ $Cre^{+/-}$ were treated with 1.0mg tamoxifen or corn oil by IP injection for 5 days. Two weeks after the treatment was completed, the animals were wounded using a 4mm dermal punch biopsy. The wounds were re-biopsied after 3, 7, or 10 days and the wounds analysed for wound closure, PDGFRs and fibrotic markers. Depletion of PDGFRα was shown by western blot analysis of dermal fibroblasts from explants cultures of tamoxifen treated mice.

Results: The dermal fibroblasts of tamoxifen treated PDGFRα $Fl^{+/+}$ $Cre^{+/-}$ mice were successfully depleted of PDGFRα, as shown by western blot. The mean wound size at day 3 post wound was 1352μm (sem+/- 282.3μm) for the control compared to the PDGFRα deleted mice 1956μm (sem+/- 250.4μm). Analysis of the dermal boundary revealed no significant difference between the PDGFRα deleted mice and controls at day 3 or 7 seven post wound. However, there does appear to be a difference in the dermal boundary at day 10; PDGFRα deleted 1299.93μm (sem+/- 128μm) compared to 713.44μm (sem+/- 226μm); $p>0.05$. There was no significant difference in the depth of the dermis.

Conclusions: Specific deletion of PDGFRα from mouse dermal fibroblasts appears to slow the rate of cutaneous wound closure.

70. Overexpression of $VEGF_{165}b$, an inhibitory splice variant of vascular endothelial growth factor, leads to insufficient angiogenesis in patients with systemic sclerosis

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Objective: Systemic sclerosis (SSc) is a chronic connective tissue disorder characterized by widespread microangiopathy, fibrosis, and autoimmunity that affects the skin and internal organs. Although in SSc there is a lack of sufficient angiogenic response to chronic tissue ischemia culminating in the loss of capillary vessels, the expression of vascular endothelial growth factor-A (VEGF) has paradoxically been shown to be upregulated in SSc skin and circulation. However, previous studies in the field did not distinguish between the proangiogenic $VEGF_{165}$ and antiangiogenic $VEGF_{165}b$ isoforms that are generated by alternative splicing in the terminal exon of VEGF pre-RNA. In the present study, we investigated whether VEGF isoform expression could be altered in skin and circulation of SSc patients.

Methods: The expression of $VEGF_{165}b$, pan-VEGF, VEGF receptor-2 (VEGFR-2), transforming growth factor-β1 (TGF-β1) and serine/arginine protein 55 (SRp55) splicing factor were investigated in skin biopsies from patients with SSc (n=35) and healthy subjects (n=23) using RT-PCR, quantitative real-time PCR, Western blotting, immunohistochemistry and confocal microscopy. Circulating levels of $VEGF_{165}b$ and pan-VEGF were measured by ELISA in plasma samples from SSc patients (n=61) and healthy controls (n=30). VEGFR-2 phosphorylation, intracellular signaling and capillary morphogenesis on Matrigel were studied in microvascular endothelial cells (MVECs) isolated from SSc (n=6) and healthy control (n=6) skin.

Results: $VEGF_{165}b$ splice variant was selectively overexpressed at both the mRNA and protein levels in SSc skin. Elevated $VEGF_{165}b$ expression correlated with increased expression of profibrotic TGF-β1 cytokine and SRp55 splicing factor in keratinocytes, fibroblasts, endothelial cells, and perivascular inflammatory cells. Circulating levels of $VEGF_{165}b$ were significantly higher in SSc patients than in control subjects. MVECs isolated from SSc skin (SSc-MVECs) expressed and released higher levels of $VEGF_{165}b$ than healthy MVECs (H-MVECs). TGF-β1 upregulated the expression of $VEGF_{165}b$ and SRp55 in both SSc- and H-MVECs. In SSc-MVECs, VEGFR-2 was overexpressed, but its phosphorylation and ERK1/2 downstream signaling were impaired. Recombinant human $VEGF_{165}b$ and SSc-MVEC–conditioned medium inhibited $VEGF_{165}$-mediated VEGFR-2 phosphorylation, ERK1/2 activation and capillary morphogenesis in H-MVECs. The addition of anti-$VEGF_{165}b$ blocking antibodies abrogated the antiangiogenic effect of SSc-MVEC–conditioned medium. Capillary morphogenesis was severely impaired in SSc-MVECs and could be ameliorated by treatment with recombinant $VEGF_{165}$ and anti-$VEGF_{165}b$ blocking antibodies.

Conclusions: In SSc, a switch from proangiogenic to antiangiogenic VEGF isoforms may have a crucial role in the insufficient angiogenic response to chronic ischemia. The combination of proangiogenic $VEGF_{165}$ administration and $VEGF_{165}b$ neutralization might represent a potential therapeutic strategy to promote effective angiogenesis and capillary regeneration in SSc.

Keywords: systemic sclerosis, peripheral vascular disease, angiogenesis, VEGF.

71. Intermittent systemic VEGF inhibition induces pulmonary arterial hypertension in a transgenic mouse model of scleroderma

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Purpose: PAH complicates up to 15% of SSc cases and occurs throughout the disease suggesting that a second vascular event occurring in the context of a systemic disease may be responsible. We have previously shown that a transgenic mouse model develops many features of SSc inclusing susceptibility to systemic vasculopathy and lung fibrosis. A role for altered VEGF signaling in PAH-SSc is supported by data that correlate circulating VEGF with mPAP at diagnosis. High circulating VEGF levels may be a marker of repair in response to vascular injury. VEGF signaling is upregulated in the TBRIIdk-fib mouse model of SSc, which has evidence of a constitutive pulmonary vasculopathy. We have inhibited VEGF signaling using SU5416 to induce endothelial apoptosis in this model.

Methods: The transgenic mouse strain TβRIIΔk-fib expresses a kinase-deficient type II TGFβ receptor driven by a fibroblast-specific promoter leading to balanced ligand-dependent upregulation of TGFβ signalling. The constitutive pulmonary vasculopathy was confirmed by histological assessment of vessel architecture, isolated organ bath and *in vivo* haemodynamic studies. Biochemical analysis of the VEGF signaling axis by quantitative PCR and Western blotting was performed using cultured pulmonary artery smooth muscle cells, and by immunostaining of tissue sections. In vivo SU5416 administration to transgenic and wildtype animals was compared to vehicle administration alone (n=6 each group). Post mortem RV mass index measurements were taken and histological and immunohistochemical stains (H&E, SR, CD31) were performed.

Results: Within the transgenic pulmonary arterial circulation, hypertrophy of the smooth muscle layer was increased (mean wildtype vessel thickness: circumference ratio 0.66±0.02, mean transgenic 0.88±0.04, $p<0.05$). Pulmonary arterial ring responses to direct and receptor-mediated contractile stimuli were reduced in the transgenic animals (in response to endothelin contraction at 10^{-5}M wildtype 1.10mN±0.02, transgenic 0.62±0.12, $p<0.05$) and right ventricular pressures were elevated in transgenic animals (wildtype mean 29mmHg±4, transgenic mean 37mmHg±3, $p<0.05$). Explanted transgenic PASMC showed upregulation of VEGF and VEGFR1. RV mass index in transgenic animals was increased after treatment with SU5416 (transgenic, vehicle only 0.19±0.01, SU5416 treated 0.29±0.03, $p<0.05$). Histological and immunohistochemical analysis revealed evidence of obliterative endothelial proliferation in transgenic SU5416 treated animals similar to human plexiform lesions which was not seen in any other group.

Conclusion: Treatment with SU5416 exacerbates the underlying constitutive pulmonary vascular defect of this transgenic mouse model and replicates the key histological and patho-physiological features seen in human PAH-SSc. These findings support a role for perturbed TGFβ and VEGF activity in the pulmonary circulation in SSc, supporting the concept of a second pulmonary endothelial injury leading to PAH in SSc. This model may provide a valuable platform for future therapeutic studies in vivo as well as providing insight into pathogenic mechanisms.

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