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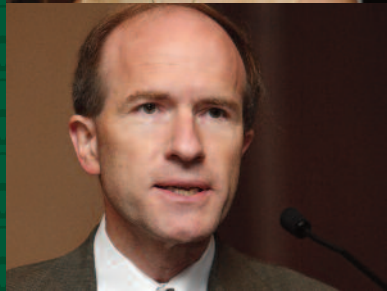
th International
Research Symposium
on Marfan Syndrome



Advances in Pathogenesis and Treatment of
Marfan Syndrome and Related Disorders

11-14 2010
SEPTEMBER

Airlie Center ■ Warrenton, VA ■ U.S.A.



*Supported in part by Genzyme, the March of Dimes, the National Marfan
Foundation and the University of Washington Collagen Diagnostic Laboratory*

8TH INTERNATIONAL RESEARCH SYMPOSIUM
ON THE MARFAN SYNDROME AND RELATED DISORDERS

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8TH INTERNATIONAL RESEARCH SYMPOSIUM
ON THE MARFAN SYNDROME AND RELATED DISORDERS

COMMITTEES

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Co-Chair

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LYNN SAKAI, PhD

Shriners Hospital for Children, Portland, OR, USA

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BART LOEYS, MD, PhD

Ghent University, Belgium

JOSEPHINE GRIMA, PhD

National Marfan Foundation, Port Washington, NY, USA

8TH INTERNATIONAL RESEARCH SYMPOSIUM
ON THE MARFAN SYNDROME AND RELATED DISORDERS

**Advances in Pathogenesis and Treatment of
Marfan Syndrome and Related Disorders**

❧ MEETING OVERVIEW ❧

The International Research Symposia on Marfan Syndrome and Related Disorders have provided a common meeting ground for basic scientists, applied scientists, and clinicians to better understand the molecular etiology of the Marfan syndrome, the biochemical abnormalities produced by the underlying mutations in connective tissue genes, the clinical consequences of these mutations and the medical and surgical management and the effects of these interventions on natural history. These meetings have been held with several year intervals to permit sufficient progress to warrant assessment of the impact of the advances. The last international symposium was held in September, 2005 in Ghent, Belgium.

The symposium brings together a panel of the world's experts for constructive discussions and vibrant debate on the current state of the art research and clinical therapies for Marfan syndrome covering all major disciplines including, cardiology, surgery, orthopedics, genetics and ophthalmology. An exciting three day program is planned for the 8th International Research Symposium on Marfan Syndrome and Related Disorders. The symposium includes invited presentations, presentations based on selected POSTERS and a poster session. We anticipate that this conference will significantly advance knowledge in the area of new therapeutic strategies and investigate the potential meta analysis from several international clinical trials. At this meeting, we are challenging all participants to be provocative and far-reaching in their presentations and questions.

The National Marfan Foundation (NMF) along with the University of Washington Collagen Diagnostic Laboratory, Genzyme and the March of Dimes is hosting the 8th International Symposium for Marfan Syndrome and Related Disorders.

8TH INTERNATIONAL RESEARCH SYMPOSIUM
ON THE MARFAN SYNDROME AND RELATED DISORDERS

PROGRAM

ALL ACTIVITIES WILL TAKE PLACE IN THE FOLLOWING THREE LOCATIONS:

Speaker Presentations: *Federal Room*

Meals: *Airlie Room*

Poster Sessions: *Jefferson Room*

SATURDAY

SEPTEMBER 11, 2010

SESSION
1

THE MARFAN SYNDROME: CURRENT STATUS AND REMAINING PROBLEMS
Chair: Hal Dietz, M.D., Johns Hopkins Hospital, Baltimore, MD, USA

6:30 PM

Welcome and Introduction

Carolyn Levering, President and CEO, National Marfan Foundation,
Port Washington, NY, USA

6:35 PM

Clinical and Biological Perspective: Heterogeneity

Bart Loeys, M.D., Ph.D., Ghent University, Belgium

6:55 PM

Current State of Affairs and Meeting Goals

Hal Dietz, M.D., Johns Hopkins Hospital, Baltimore, MD, USA

7:30 PM

Dinner

SUNDAY

SEPTEMBER 12, 2010

8:00 AM

Breakfast

SESSION
2

CRITERIA FOR CLINICAL AND LABORATORY DIAGNOSIS OF MARFAN SYNDROME:
RATIONALE AND QUESTIONS (*10 min + 5 min Q&A*)

Chairs: Anne De Paepe, M.D., Ph.D., Ghent University, Belgium *and*
Reed Pyeritz, M.D., Ph.D., University of Pennsylvania, Philadelphia, PA, USA

9:00 AM

The Revised Ghent Nosology for the Marfan Syndrome

S.1

Bart Loeys, M.D., Ph.D., Ghent University, Belgium

9:15 AM

Clinical Care for the Marfan Patient Using a Centralized Database in France

S.2

Guillaume Jondeau, M.D., Hôpital Bichat, Paris, France

9:30 AM

Multidisciplinary Team Approach: The Hamburg Model

S.3

Yskert Von Kodolitsch, M.D., University Hospital Eppendorf, Hamburg, Germany

- 9:45 AM **Aortic Root Replacement in Patients with Marfan Syndrome: Registry Study Update** S.4
Joseph Coselli, M.D., Baylor College of Medicine, Houston, TX, USA
- 10:00 AM **Update on Aortic Root Z-Score Calculation Models** S.5
Richard Devereux, M.D., Weill-Cornell Medical College, New York, USA
- 10:15 AM **The Clinical Spectrum of Complete FBN-1 Allele Deletions** S.6
Yvonne Hilhorst-Hofstee, M.D., Leiden University Medical Center, Germany
- 10:30 a.m. **Coffee Break**
- SESSION 3 **FBN₁ PHENOTYPE/GENOTYPE CORRELATIONS (10 min + 5 min Q&A)**
Chairs: Catherine Boileau, Ph.D., Hôpital Ambroise Paré, Boulogne and Inserm U698, Paris, France, *and*
John Dean, M.D., Royal Hospitals Medical School, Aberdeen, UK
- 11:00 AM **Genotype-Phenotype Correlation in Marfan Syndrome: What Have We Learned From It?** S.7
Laurence Faivre, M.D. Hôpital d'Enfants, Dijon, France
- 11:15 AM **Elucidation of Sensors and Effectors in Matrix Equilibrium Derives Novel Therapeutic Strategies for Scleroderma** S.8
Elizabeth Gerber, B.S., Johns Hopkins University, Baltimore, MD, USA
- 11:30 AM **Poster Session I (No. 1–26, 50–51)**
- 1:00 PM **Lunch**
- SESSION 4 **TGF- β RECEPTOR RELATED PHENOTYPES (10 min + 5 min Q&A)**
Chairs: Bart Loeys, M.D., Ph.D., Ghent University, Belgium and Takayuki Morisaki, M.D., Ph.D., Osaka University, Japan
- 2:00 PM **Loeys-Dietz syndrome vs. Marfan syndrome: Broad Spectra of Aortic/Non-Aortic Phenotypes in Japanese Patients** S.9
Takayuki Morisaki, M.D., Ph.D., Osaka University, Japan
- 2:15 PM **TGFBR1/2 Genotype/Phenotype Correlation** S.10
Eloisa Arbustini, M.D., IRCCS Foundation Policlinico San Matteo, Pavia, Italy
- 2:30 PM **Panel Discussion: Comparing and Distinct Features of Aneurysm Phenotypes That Can Assist in Identification of Effective Therapies**
G. Jondeau, E. Arbustini, B. Loeys, D. Milewicz, H. Dietz, H. Morisaki

SESSION
5

OVERVIEW OF CLINICAL TRIAL PROTOCOLS (10 min + 5 min Q&A)
Chairs: Hal Dietz, M.D., Johns Hopkins Hospital, Baltimore, MD, USA *and*
 Ron A. Lacro, M.D., Children's Hospital, Boston, MA, USA

- 2:50 PM **Pediatric Heart Network (PHN) Randomized Clinical Trial of Atenolol vs Losartan in Marfan Syndrome** S.11
 Ron A. Lacro, M.D., Children's Hospital, Boston, MA, USA
- 3:00 PM **Study of the Efficacy of Losartan on Aortic Dilatation in Patients With Marfan Syndrome** S.12
 Guillaume Jondeau, M.D., Hôpital Bichat, Paris, France
- 3:10 PM **Rationale and Study Design of a Randomized, Double-Blind Trial for the Evaluation of the Effect of Losartan vs. Placebo on Aortic Root Dilatation in Marfan Patients Treated with B-Blockers** S.13
 Julie De Backer, M.D., University of Ghent, Belgium
- 3:20 PM **Marfan Syndrome: The Italian Clinical Trial** S.14
 Eloisa Arbustini, M.D., IRCCS Foundation Policlinico San Matteo, Pavia, Italy
- 3:30 PM **Coffee Break**
- 3:45 PM **Netherlands Trial** S.15
 M. Groenink, M.D., Ph.D., Academic Medical Center, Amsterdam, Netherlands
- 3:55 PM **A Randomized, Open-Label, Losartan Therapy on the Progression of Aortic Root Dilation in Patients with Marfan Syndrome** S.16
 Dr. Hsin-Hui Chiu, M.D., National Taiwan University Hospital, Taiwan
- 4:05 PM **United Kingdom Trial — Aortic Irbesartan Marfan Study (AIMS)** S.17
 John Dean, M.D., Royal Hospitals Medical School, Aberdeen, UK *and*
 Anne Child, M.D., St. George's, University of London, UK
- 4:15 PM **Biomarkers as Outcome Parameters for Clinical Trials** S.18
 (10 min + 5 min Q&A)
 Peter Matt, M.D., University Hospital Basel/Berne, Switzerland
- 4:30 PM **Panel Discussion: Meta Analysis of Trial Data and Potential Outcome Parameters**
- 5:00–6:30 PM **Poster Session II (No. 27–49, 52)**
NMF-sponsored Cocktail Hour
- 6:30 PM **Airlie House Scavenger Hunt**
- 7:30 PM **Dinner**

MONDAY

SEPTEMBER 13, 2010

8:00 AM Breakfast

SESSION
6

LESSONS FROM MARFAN-RELATED DISORDERS (*10 min + 5 min Q&A*)
Chairs: Dianna Milewicz, M.D., Ph.D., University of Texas at Houston, USA and
 Suneel Apte, Ph.D., Cleveland Clinic Foundation, IL, USA

9:00 AM Novel Genetic Variants Predisposing to Sporadic Thoracic Aortic Disease S.19
 Dianna Milewicz, M.D., Ph.D., University of Texas at Houston, USA

9:15 AM Mechanistic Insights Regarding Filaminopathies and Aneurysm S.20
 David Kim, M.S., Johns Hopkins Hospital, Baltimore, MD, USA

9:30 AM The Disease Phenotypes and Mechanisms of LTBP4 Mutations S.21
 Zsolt Urban, Ph.D., University of Pittsburgh, PA, USA

9:45 AM Null Mutations in LTBP2 Cause Primary Congenital Glaucoma S.22
 Manir Ali, M.D., Leeds Institute of Molecular Medicine, St. James's University
 Hospital, Leeds, UK

10:00 AM Identification of ADAMTS10 and ADAMTSL2 Mutations in the Acromelic S.23
 Dysplasia Group
 Valerie Cormier-Daire, M.D., Ph.D., Hôpital Necker Enfants Malades and
 Inserm U781, Paris, France

10:15 AM Isolated Ectopia Lentis: Report of a New Deletion in the ADAMSL4 Gene S.24
 and Evidence for Genetic Heterogeneity of the Autosomal Recessive Form
 of the Disease
 Nadine Hanna, Ph.D., Hôpital Ambroise Paré and Inserm U698, Paris, France

10:30 AM Coffee Break

10:45 AM Role of ADAMTSL4 Mutations in FBN1 Mutation Negative Ectopia Lentis S.25
 Patients
 Anne Child, M.D., St. George's, University of London, UK

11:00 AM ADAMTSL6 β Rescues Microfibril Disorder in Marfan Syndrome Through S.26
 The Promotion of Fibrillin-1 Assembly
 Saito Masahiro, D.D.S., Ph.D., Tokyo University of Science, Chiba, Japan

11:15 AM A Novel Genetic Pathway Underlies Weill-Marchesani Syndrome S.27
 Gerhard Sengle, Ph.D., Oregon Health & Science University, Portland, OR, USA

11:30 AM Group Discussion and Comments

12:00 PM Lunch

SESSION
7

ANIMAL MODELS OF FIBRILLINOPATHIES AND RELATED DISORDERS WITH REGARD TO CELL SPECIFIC COMPARTMENTS IN DEVELOPMENT OF AORTIC DISEASE
(10 min + 5 min Q&A)

Chairs: Francesco Ramirez, Ph.D. Mount Sinai School of Medicine, NY, USA *and* Lynn Sakai, Ph.D., Shriners Hospital for Children, Portland, OR, USA

- 1:00 PM **Aortic Disease in Germline, Vascular Smooth Muscle Cell (VSMC) Specific and Endothelial Cell (EC) Specific FBN-1 Mutant Mice** S.28
Lynn Sakai, Ph.D., Shriners Hospital for Children, Portland, OR, USA
- 1:15 PM **A New Mouse Model for Marfan Syndrome Presents Phenotypic Variability Associated with the Genetic Background, and Overall Levels of FBN-1 Expression** S.29
Lygia Pereira, Ph.D., Instituto de Biociências, University of São Paulo, Brazil
- 1:30 PM **Investigation of Loeys-Dietz Syndrome Using an Allelic Series of Mutant Mice** S.30
David Loch, Ph.D., Johns Hopkins Hospital, Baltimore, MD, USA
- 1:45 PM **ADAMTSL2 Binds Fibrillin-1, Accelerates Fibrillin-1 Microfibril Biogenesis and Is Mutated in Canine Musladin-Lueke Syndrome, A Heritable Connective Tissue Disorder with Extensive Skin Fibrosis** S.31
Suneel Apte, Ph.D. Cleveland Clinic Foundation, IL, USA
- 2:00 PM **Postnatal Function of Transforming Growth Factor β 2 in Cardiovascular Disease** S.32
Mohamad Azhar, Ph.D., University of Arizona, Tucson, AZ, USA
- 2:15 PM **Characterization and Treatment of Osteopenia in Mice with Severe Marfan Syndrome** S.33
Luca Carta, Ph.D., Mt. Sinai School of Medicine, NY, USA
- 2:30 PM **Conditional Inactivation of Fibrillin-1 in Aortic Tissue Compartments** S.34
Jason Cook, B.A., Mt. Sinai School of Medicine, NY, USA
- 2:45 PM **Coffee Break**

SESSION
8

FUNDAMENTAL MECHANISMS AND SIGNALING (10 min + 5 min Q&A)

Chairs: Dan Rifkin, Ph.D., New York University, USA, *and* Lynn Sakai, Ph.D., Shriners Hospital for Children, Portland, OR, USA

- 3:15 PM **Smooth Muscle Contractile Function and Thoracic Aortic Disease** S.35
Dianna Milewicz, M.D., Ph.D., University of Texas at Houston, TX
- 3:30 PM **Epigenetic Control of Aortic Smooth Muscle Cells in Marfan and Non-Marfan Thoracic Aneurysms** S.36
Delphine Gomez, M.D., Hôpital Xavier Bichat and Inserm U698, Paris, France

3:45 PM	Canonical and Non-Canonical TGF β Signaling Jef Doyle, MBBChir MHS MA, Johns Hopkins Hospital, Baltimore, MD, USA	S.37
4:00 PM	Molecular and Structural Characterization of the Interactions Between The N- and C-Terminal Regions of Human Fibrillin-1 Penny Handford, Ph.D. University of Oxford, UK	S.38
4:15 PM	Investigation of the Signaling Pathway Altered in a Mouse Model of Ascending Aortic Aneurysms Hiromi Yanagisawa, M.D., Ph.D., University of Texas Southwestern Medical Center, Dallas, TX, USA	S.39
4:30 PM	Extracellular Regulation of BMP Signaling By Fibrillin Microfibrils Gerhard Sengle, Ph.D., Shriners Hospital for Children, Portland, OR, USA	S.40
4:45 PM	Regulatory Role of Fibrillin in Bone Homeostasis Dieter P. Reinhardt, Ph.D., McGill University, Montreal, CN	S.41

6:00 PM Dinner

Special Evening Session

SESSION
9

AORTIC AND MITRAL VALVE DEVELOPMENT (*10 min + 5 min Q&A*)
Chair: Hal Dietz, M.D., Johns Hopkins Hospital, USA

7:30 PM	Molecular Mechanisms of Valve Development Katherine Yutzey, Ph.D.	S.42
7:45 PM	Aortic Development — Developmental Perspective Magdi Yacoub, M.D., Imperial College of London, UK	S.43
8:00 PM	Developmental Mechanisms of Adult Heart Valve Diseases (VHD) Roger Markwald, Ph.D., Medical University of South Carolina, Charleston, SC, USA	S.44
8:15 PM	Developmental Underpinnings of Acquired Aortic Aneurysm in Marfan Syndrome Mark Lindsay, M.D., Johns Hopkins Hospital, Baltimore, MD, USA	S.45

TUESDAY

SEPTEMBER 14, 2010

8:00 AM Breakfast

SESSION
10EMERGING NEW THERAPEUTIC STRATEGIES (10 min + 5 min Q&A)
Chair: Bart Loeys, M.D., Ph.D., Ghent University, Belgium9:00 AM Therapeutic Strategies in Marfan Syndrome S.46
Jennifer Pardo-Habashi M.D., Johns Hopkins Hospital, Baltimore, MD, USA9:15 AM Calcium Channel Blockers Exacerbate Aortic Disease and Cause Premature Lethality in Marfan Syndrome S.47
Jef Doyle, MBBChir MHS MA, Johns Hopkins Hospital, Baltimore, MD, USA9:30 AM Doxycycline and Losartan Combination Treatment Further Delays Aneurysm Rupture in a Mouse Model of Marfan Syndrome Compared to Single Drug Treatment S.48
B. Timothy Baxter, M.D., University of Nebraska Medical Center, Omaha, NE, USA9:45 AM Comparison of Pravastatin, Losartan and Doxycycline for Attenuation of Aortic Root Dilation in a Murine Model of Marfan Syndrome S.49
Darren McLoughlin, MRCS, Royal College of Surgeons in Ireland, Dublin10:00 AM Angiotensin II Infusion Promotes The Formation of Ascending Aortic Aneurysms S.50
Alan Daugherty, M.D., University of Kentucky, Lexington, KY, USA10:15 AM Vascular Smooth Muscle Expression of S100A12 Induces Aortic Aneurysm Formation S.51
Marion Hofmann Bowman, M.D, Ph.D., University of Chicago, IL, USA10:30 AM Increased T-Helper Cell Response in Marfan Syndrome Is Modified by Losartan S.52
Teodora Radonic, M.D., Academic Medical Center, Amsterdam, Netherlands

10:45 AM Coffee Break

SESSION
11ASURGICAL MANAGEMENT OF MARFAN SYNDROME (10 min + 5 min Q&A)
Chair: Joseph Coselli, M.D., Baylor College of Medicine, Houston, TX, USA11:00 AM Fate of the Distal Aorta in Patients with Marfan Syndrome S.53
Joseph Coselli, M.D., Baylor College of Medicine, Houston, TX, USA11:15 AM Long term Results after Aortic Root Replacement in Patients with Marfan Syndrome S.54
Alexander Bernhardt, M.D., University Heart Center Hamburg, Germany

11:30 AM	Aortic Valve-Sparing in Marfan Syndrome: Midterm Results with David Operation (Stanford Modification) Alberto Forteza, M.D., Hospital Universitario, Madrid, Spain	S.55
11:45 AM	Midterm Results of Valve-Sparing Aortic Root Replacement for Annulo-Aortic Ectasia Nawata Kan, M.D., Ph.D., University of Tokyo Hospital, Tokyo, Japan	S.56
12:00 PM	Lunch	
SESSION 11B	MEDICAL MANAGEMENT OF MARFAN SYNDROME (10 min + 5 min Q&A) <i>Chair:</i> Anne De Paepe, M.D., Ph.D., Ghent University, Belgium	
1:00 PM	Assessment of Dural Ectasia in Marfan Syndrome Rigmor Lundby, M.D., Rikshospitalet University Hospital, Oslo, Norway	S.57
1:15 PM	Ocular signs and symptoms of Marfan syndrome Bart Leroy, M.D. Ph.D., Ghent University, Belgium	S.58
1:30 PM	Spine and Joint Issues, Brace or Not, Feet Management Paul Sponseller, M.D., Johns Hopkins University, Baltimore, MD, USA	S.59
1:45 PM	Pain Management for Marfan Syndrome Sabine Kost-Byerly M.D., Johns Hopkins University, Baltimore, MD, USA	S.60
2:00 PM	Health Related Quality of Life in Marfan syndrome; A Cross-Sectional Study of SF-36 in 84 Adults With a Verified Diagnosis. Svend Rand-Hendriksen, M.D., TRS National Resource Centre for Rare Disorders, Sunnaas Rehabilitation Hospital, Nesoddtangen, Norway	S.61
SESSION 12	PSYCHOSOCIAL AND ELSI ISSUES (10 min + 5 min Q&A) <i>Chair:</i> Reed Pyeritz, M.D., Ph.D., University of Pennsylvania, Philadelphia, PA, USA	
2:15 PM	Comprehending the Other Side: Patient and Family Perspectives Brigid C. Guttmacher, M.A., L.P.C., Washington, DC, USA	S.62
2:30 PM	Wrap-up and Summary Hal Dietz, M.D., Johns Hopkins Hospital, Baltimore, MD, USA	

8TH INTERNATIONAL RESEARCH SYMPOSIUM
ON THE MARFAN SYNDROME AND RELATED DISORDERS

POSTER LIST

POSTER SESSION 1: Sunday, September 12, 11:30 AM – 1:00 PM

Posters 1–26, 50–51

POSTER SESSION 2: Sunday, September 12, 5:00 PM – 6:30 PM

Posters 27–49, 52

POSTER No.

- 1 Gene Test Priority in Marfan Syndrome Screening Clinic
Child, Anne
- 2 Marfan Syndrome and Associated Disorders (MSAD) Databases: 15 Years of Experience
Collod-Beroud, Gwenaelle
- 3 Towards the Dissection of Marfanoid Syndromes with Mental Retardation
Faivre, Laurence
- 4 The Clinical Spectrum of Complete FBN1 Allele Deletions
Hilhorst-Hofstee, Yvonne
- 5 Quantitative Sequence Analysis of FBN1 Premature Termination Codons Provides Evidence
for Incomplete NMD in Leukocytes
Magyar, Istvan
- 6 The Spectrum of Clinical Manifestations in Marfan Syndrome
Rybczynski, Meike
- 7 The Diagnostic Value of the Facial Features of Marfan Syndrome
Ting, Beverlie
- 8 Is it Really Marfan Syndrome? Differential Diagnoses at the Brisbane Marfan Clinic
West, Malcolm
- 9 Musculoskeletal Findings of Loeys-Dietz Syndrome
Sponseller, Paul D
- 10 The Natural History of Dural Ectasia in Marfan Syndrome
Mesfin, Addisu
- 11 Longterm Results After Aortic Root Replacement in Patients with Marfan Syndrome
Bernhardt, AMJ
- 12 Mitral Valve Surgery in Patients with Marfan Syndrome
Bernhardt, AMJ

- 13 Minimizing Paraplegia in Patients with Marfan Syndrome Undergoing Descending and Thoracoabdominal Aortic Aneurysm Repair
Bischoff, MS
- 14 Evaluation of Left Ventricular Function in 200 Patients with Marfan Syndrome using Conventional Doppler-Echocardiography, Tissue Doppler and 2D Strain Imaging
Detaint, D
- 15 Proteinuria and Microalbuminuria as Predictors of Renal Dysfunction in a Cohort of Forty Eight Marfan Syndrome Patients
Forteza, Alberto
- 16 Aortic Valve-Sparing in Marfan Syndrome: Midterm Results with David Operation (Stanford Modification)
Forteza, Alberto
- 17 Midterm Results of Valve-Sparing Aortic Root Replacement for Annulo-Aortic Ectasia
Kan, Nawata
- 18 Nursing and Research Working Together
Radojewski, Liz
- 19 Mechanical Properties of Ascending Aorta with Marfan and Loeys-Dietz Syndromes
Rojo, Francisco J
- 20 Frequency of Sleep Apnea in Adults with Marfan Syndrome
Rybczynski, Meike
- 21 Patent Foramen Ovale and Marfan Syndrome: A New Association with Differences Between Children and Adults
Sanchez, V
- 22 Biventricular Performance in Patients without Significant Valvular Disease: Comparison to Normal Subjects and Longitudinal Follow-Up
Scholte, Arthur
- 23 The Age-Related Regional Changes of Aortic Compliance in the Marfan Syndrome: Assessment with Velocity-Encoded MRI
Scholte, Arthur
- 24 Augmentation Index Predicts Cardiovascular Disease in Adults with Marfan-like Features
Sheikhzadeh, Sara
- 25 Improved Screening for Aortic Root Dilation by Transthoracic Echocardiography
Shiran, Hadas
- 26 Protrusio Acetabuli and Total Hip Arthroplasty (THA) in Patients with Marfan Syndrome
Thakkar, Savyasachi
- 27 An ACTA2 Genetic Variant in a Family Presenting with a Thoracic Aortic Dissection and a Carotid Aneurysm
Baudhuin, Linnea M

- 28 Role of ADAMTSL4 Mutations in FBN1 Mutation Negative Ectopia Lentis Patients
Child, Anne
- 29 Isolated Ectopia Lentis: Report of a New Deletion in the AdamsL4 Gene and Evidence for Genetic Heterogeneity of the Autosomal Recessive Form of the Disease
Hanna, Nadine
- 30 Hemizygous Deletion Comprising COL3A1 and COL5A2 Causes Aortic Dissection
Meienberg, Janine
- 31 Ascending Aneurysms with a Bicuspid Aortic Valve — What Can We Learn from Marfan Syndrome?
Mohamd, SA
- 32 A Novel Genetic Pathway Underlies Weill-Marchesani Syndrome
Sengle, Gerhard
- 33 Postnatal Function of Transforming Growth Factor Beta2 in Cardiovascular Disease
Azhar, Mohamad
- 34 Role of Transforming Growth Factor — Beta3 in Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia Type 1 (AVRD1)
Azhar, Mohamad
- 35 Characterization and Treatment of Osteopenia in Mice with Severe Marfan Syndrome
Carta, Luca
- 36 In Vivo Deletion of the First Hybrid Domain in Fibrillin-1
Charbonneau, Noe
- 37 Conditional Inactivation of Fibrillin-1 in Aortic Tissue Compartments
Cook, Jason R
- 38 Dynamic Regulation of the Fibrillin-Like FBN1 Protein
Frاند, Alison
- 39 Aortic Disease in a New Mouse Model of Marfan Syndrome
Manalo, Elise C
- 40 ADAMTSL6 β Rescues Microfibril Disorder in Marfan Syndrome through the Promotion of Fibrillin-1 Assembly
Masahiro, Saito
- 41 Transgenic Mice Overexpressing AdamTSL-6 in Cartilage Exhibit Dwarfism and Craniofacial Abnormalities
Tsutsui, Ko
- 42 Tissue and Developmental-Specific Variability in FBN1 Isoform Expression
Burchett, Mary
- 43 Increased T-Helper Cell Response in Marfan Syndrome is Modified by Losartan
Radonic, Teodora

- 44 Regulatory Role of Fibrillin in Bone Homeostasis
Reinhardt, Dieter P
- 45 Differential Effects of Neonatal and Classical Marfan Mutations in Fibrillin-1
Reinhardt, Dieter P
- 46 Candidate Modifiers of FBN1 Activity May Be Associated with Variable Phenotype in Marfan Syndrome
Summers, Kim M
- 47 Marfan Syndrome and Bicuspid Aortic Valve Aneurysm Ultrastructure Abnormalities
Do, Hong-Lien
- 48 Comparison of Pravastatin, Losartan and Doxycycline for Attenuation of Aortic Root Dilation in a Murine Model of Marfan Syndrome
McLoughlin, Darren
- 49 Prenatal and Preimplantation Diagnoses in Marfan Syndrome: The Point of View of French Patients and Geneticists
Faivre, Laurence
- 50 FBN1, TGFBR1, TGFBR2, and SLC2A10 Mutation Analyses in Patients with Suspected Marfan Syndrome: A Swiss Study
Henggeler, Caroline
- 51 Clinical Significance of Unusual Horizontal Striae of the Back in Children with Possible Connective Tissue Disorder
Powell-Hamilton, Nina
- 52 Deletion of Acta2 Leads to Increased Smooth Muscle Cell Proliferation and Neointimal Formation in Mice
Papke, Christina

ABSTRACTS OF INVITED SPEAKERS



Listed in Order of Presentation

S. 1**THE REVISED GHENT NOSOLOGY FOR THE MARFAN SYNDROME**

B Loeys¹, B. Callewaert¹, J. De Backer¹, L Faivre², G Jondeau³, R Devereux⁴, R Pyeritz⁵, P. Sponseller⁶, P. Wordsworth⁷, D. Milewicz⁸, Y. Hilhorst-Hofstee⁹, A. Braverman¹⁰, H. Dietz⁶ and A. De Paepe¹

¹Ghent University, Belgium, ²CHU Dijon, France, ³AP-HP, Hopital Bichat, Paris, France, ⁴Weill Medical College of Cornell University, New York, ⁵University of Pennsylvania, Philadelphia, ⁶ Johns Hopkins University, Baltimore, ⁷University of Oxford, UK, ⁸University of Texas, Houston, ⁹ Leiden University Medical Center, Leiden, Washington University School of Medicine, Saint-Louis¹⁰.

The diagnosis of Marfan syndrome (MFS) relies on a set of international criteria, outlined by expert opinion. In 1996, the initial Berlin nosology was revised because of the risk of overdiagnosis and redefined as the Ghent nosology, a more stringent set of major and minor criteria.

These Ghent criteria have proven to work well since with improving molecular techniques, confirmation of the diagnosis is possible in over 95% of patients. However, concerns with the Ghent criteria are that some of the diagnostic manifestations have not been validated as “hinge points” (eg. dural ectasia) and others necessitate cumbersome imaging studies. Moreover, in the absence of aortic dilation, the diagnosis can be stigmatizing, hamper career aspirations and restrict life-insurances opportunities. The label “MFS” may cause psychosocial burden by “restricted exercise permission” and situational depressions.

Following an international expert meeting, we propose a revised Ghent nosology in which aortic root aneurysm and ectopia lentis are cardinal features. In absence of any family history, the presence of these two manifestations is sufficient for the unequivocal diagnosis of MFS. In absence of any of these two, the presence of bonafide *FBN1* mutation or a combination of systemic features is required. For the latter a new scoring system has been designed and validated. In this way *FBN1* testing is not mandatory but useful when available.

The proposed new nosology puts more weight on the cardiovascular manifestations of the disease. We anticipate that the new nosology can delay a definitive diagnosis of MFS but decreases the risk of premature or mis-diagnosis and facilitates discussion of risk and follow-up / management guidelines.

S. 2

CLINICAL CARE FOR THE MARFAN PATIENT USING A CENTRALIZED DATABASE IN FRANCE

Guillaume Jondeau, Centre National de Référence Pour Le Syndrome de Marfan et Apparentés. Hôpital Bichat, Université Paris 7, Paris, France.

In France a political decision has been taken to set up national networks to provide care to patients suffering from rare diseases including Marfan syndrome and related disorders. For Marfan syndrome and related disorders, 1 Referent Centre has been defined and is completed by several (7) Competence Centres throughout the territory. All are working in network. This has led to the development of a on-line medical chart that is available from internet and allows all participants to gather and access clinical information on patients from wherever when necessary. This also allows prospective completion of a database on a large multicentre population.

Practical difficulties are related to security standards that are varying from centre to centre, and will probably need to change from client-server using 4D, to https protocol available on standard browsers (e.g. i-explorer or firefox)

This database has been used for the description of natural history of pathologies associated with TFGBR2 mutation, the determination of the normal values for aortic diameter in children, the evolution of clinical practice over the years, the event rate associated with a given aortic diameter in Marfan patients, etc...

S. 3

MULTIDISCIPLINARY TEAM APPROACH: THE HAMBURG MODEL

von Kodolitsch, Yskert; MD, MBA

Department of General and Interventional Cardiology at the University Hospital Eppendorf, Hamburg, Germany

Objective: Marfan patients require specialized interdisciplinary care for optimal out-come and life quality.

Methods: The Hamburg Marfan Center was founded in 1996 and spans the experience with ≈700 patients. Our report focuses on our response to organizational challenges that we encountered when applying medical care to individual patients.

Results: Geographic challenges: 22% of our patients travel ≥100 kilometers to our center. Thus, we usually provide ambulatory care during a single day. Moreover, local medical care is provided by local practitioners with whom we maintain a collaborative network structure. 2. Multidisciplinary challenge: for initial diagnosis each patient sees a cardiologist, a heart surgeon, an orthopedist, a geneticist, an ophthalmologist and a radiologist. Follow-up examinations are usually performed only by a cardiologist, a heart surgeon and a radiologist. When special problems arise, we contact gynecologists, neonatologists, and psychologists with experience with Marfan patients. We maintain separate teams for children and for adults. Financial challenges: during 2008 we spent 389.16 Euro/patient for clinical diagnostics and imaging procedures. Health insurances had to pay for these expenses only due to a novel legal directive that was pushed by the German Marfan foundation. Challenges by individuality: together with patient representatives we developed measures both to consider social and psychological patient requirements and to increase patient adherence to medical recommendations.

Conclusions: Multidisciplinary medical teams need to organize centers that are capable to respond to numerous organizational challenges specific of their patients and of their environment in order to optimize the quality of care for Marfan patients.

S. 4**AORTIC ROOT REPLACEMENT IN PATIENTS WITH MARFAN SYNDROME: REGISTRY STUDY UPDATE**

Joseph S. Coselli, MD; Division of Cardiothoracic Surgery, Michael E. DeBakey Department of Surgery, Baylor College of Medicine; and Cardiovascular Surgery Service, Texas Heart Institute at St. Luke's Episcopal Hospital; Houston, Texas

Objective: In 2005, a prospective, international registry study was initiated to provide comparative data about short-term clinical outcomes after aortic valve-sparing (AVS) and aortic valve-replacing (AVR) root operations in patients with Marfan syndrome. The purpose of this report is to provide an update of the study enrollment and outcomes.

Methods: The study currently has 303 participants, who represent 96% of the target enrollment (316 participants). This report focuses on the 252 participants for whom 30-day outcome data are available. All enrollees met strict diagnostic criteria for Marfan syndrome based on the original Ghent nosology and underwent AVS (n=183) or AVR (n=69) root operations at one of 19 participating centers. Clinical data, biological samples, and echocardiographic and computer tomographic images were collected for study participants.

Results: Of the 252 participants, 2 (0.8%) died within 30 days, 1 death occurring in the AVR group and 1 in the AVS group, ($P=0.5$). There were no significant intergroup differences in valve-related complications, which occurred in 4 AVR participants and 5 AVS participants ($P=0.3$). These complications included 4 bleeding events, 2 embolic events, and 3 instances of nonstructural valve dysfunction. **One AVS participant required an aortic-root reoperation because of coronary artery kinking.**

Conclusions: Despite the complexity of valve-sparing root replacement, this approach did not result in any demonstrable adverse early outcomes. The 30-day mortality and valve-related complication rates were low, with no significant intergroup difference.

S. 5**UPDATE ON AORTIC ROOT Z-SCORE CALCULATION MODELS**

Richard Devereux, M.D., Weill-Cornell Medical College, New York, USA

Nomograms to predict normal aortic root diameter for body surface area (BSA) in broad ranges of age have been widely used and adopted in guidelines, but have been limited by lack of consideration of gender effects, jumps in upper limits of normal aortic diameter at transitions to older age strata, and limited data on appropriate normal limits for older teenagers.

In a recent study, we used echocardiograms to measure aortic root diameter at the sinuses of Valsalva by American Society of Echocardiography convention in normal-weight, non-hypertensive, non-diabetic individuals ≥ 15 years old without aortic valve disease from clinical or population-based samples. Analyses of covariance in general linear model and linear regression with assessment of residuals identified determinants and develop predictive models for normal aortic root diameter and its 95% confidence interval.

A total of 1,207 apparently normal individuals ≥ 15 years old (54% female) had wide ranges of height (1.43-2.06 m) and BSA (1.27-2.41 m²); aortic root diameter ranged from 2.1 to 4.3 cm. Aortic root diameter was strongly related to BSA or height (both $r=0.48$), age ($r=0.36$) and gender (+2.7 mm in men adjusted for BSA and age) (all $p<0.001$). Multivariable models using age, gender, and either BSA or height predicted aortic diameter strongly (both $R=0.674$, $p < 0.001$) with minimal relation of the residuals to age, primary body size measures or body mass index (all r^2 0.01 to 0.02). Predictive sinus of Valsalva diameters in cm were:

for BSA: $2.423 + (\text{age [yrs]} * 0.009) + (\text{bsa [m}^2\text{]} * .461) - (\text{sex [1=M, 2=F]} * .267)$ SEE = 0.261 cm

for height: $1.519 + (\text{age [yrs]} * 0.010) + (\text{ht [cm]} * .010) - (\text{sex [1=M, 2=F]} * .247)$ SEE = 0.215 cm

Aortic root diameter is larger in male than female adolescents and adults in addition to previously-described effects of body size and age. Regression models incorporating body size, age and gender are applicable to adolescents, and resolve discontinuities in upper normal limits between ranges of age and widening of normal confidence intervals with age. Characteristics of other recent models to derive z-scores for aortic root diameter will be discussed at the symposium.

THE CLINICAL SPECTRUM OF COMPLETE *FBN1* ALLELE DELETIONS

Hilhorst-Hofstee Yvonne¹, Hamel Ben CJ³, Verheij Joke BGM⁵, Rijlaarsdam Marry EB², Mancini Grazia MS⁶, Cobben Jan Maarten⁸, Giroth Cindy¹, Ruivenkamp Claudia AL¹, Hansson Kerstin BM¹, Timmermans Janneke⁴, Moll Henriette A⁷, Breuning Martijn H¹, Pals Gerard⁹

¹Department of Clinical Genetics, ²Department of Pediatric Cardiology, Leiden University Medical Center, Leiden; ³Department of Human Genetics, ⁴Department of Cardiology, Radboud University Nijmegen Medical Centre, Nijmegen; ⁵Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen; ⁶Department of Clinical Genetics, ⁷Department of Pediatrics, Erasmus MC Rotterdam; ⁸Department of Clinical Genetics, Academic Medical Center, Amsterdam; ⁹Department of Clinical Genetics, Center for Connective Tissue Research, VU University Medical Center, Amsterdam, the Netherlands

Background:

Only two reports are known of a complete allele deletion of *FBN1*. The most common mutations found in *FBN1* are missense mutations (56%) mainly substituting or creating a cysteine in a cbEGF domain. Other mutations are frameshift mutations, splice mutations and nonsense mutations.

Objectives: The purpose of this study is to determine the clinical spectrum of deletions of a complete *FBN1* allele

Methods:

We screened 300 patients with clinical features of MFS or a related phenotype by MLPA. All patients had been previously screened by DHPLC and no mutations in *FBN1* were found. In one patient a deletion of the *FBN1* gene was detected by chromosome analysis and array CGH, performed as part of mental retardation screening. In all patients the size of the deletion was determined by SNP array analysis.

Results:

In total 10 patients including a family with five patients were found to have a deletion of one *FBN1* allele. There was a large variation in size of the deletions between the patients. Seven patients fulfilled the Ghent criteria for Marfan syndrome. The other 3 patients were examined at a young age which could explain why they do not yet present the full clinical picture of MFS. Two patients with a large deletion had an extended phenotype. The clinical features of the patients will be presented.

Conclusions:

The results show that complete loss of one *FBN1* allele does not predict a mild phenotype. These findings support the hypothesis that true haploin sufficiency can lead to the classical phenotype of Marfan syndrome.

**Selected for oral presentation from poster submissions*

S. 7

GENOTYPE-PHENOTYPE CORRELATION IN MARFAN SYNDROME: WHAT WE HAVE LEARNED FROM IT?

Faivre Laurence^{1,2}, Collod-Beroud Gwenaëlle^{3,4}, Loeys Bart^{5,6}, Child Anne⁷, Binquet Christine², Gautier Elodie², Callewaert Bert⁵, Arbustini Eloisa⁸, Mayer Karen⁹, Arslan-Krichner Mine¹⁰, Dietz Hal⁶, Halliday Dorothy¹¹, Beroud Christophe³, Bonithon-Kopp Claire², Robinson Peter N¹², Adès Lesly¹³, De Backer Julie⁵, Coucke Paul⁵, Francke Uta¹⁴, De Paepe Anne⁵, Jondeau Guillaume¹⁵, Boileau Catherine¹⁶

1 Centre de Génétique, CHU Dijon, France. 2 Centre d'investigation clinique – épidémiologie clinique/essais cliniques, Dijon, F-21000 France. 3 INSERM, U827, Montpellier, F-34000, France. 4 Univ Montpellier I, Montpellier, F-34000, France. 5 Center for Medical Genetics, Ghent University Hospital, Belgium. 6 Institute of Genetic Medicine and the Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, USA. 7 Department of Cardiological Sciences, St. George's Hospital, London, UK. 8 Molecular Diagnostic Unit, Policlinico San Matteo, Pavia, Italy. 9 Center for Human Genetics and Laboratory Medicine, Martinsried, Germany. 10 Institut für Humangenetik, Hannover, Germany. 11 Department of Biochemistry, University of Oxford, UK. 12 Institut für Medizinische Genetik, Universitätsmedizin Charité, Berlin, Germany. 13 Marfan Research Group, The Children's Hospital at Westmead, Sydney, Australia. 14 Departments of Genetics and Pediatrics, Stanford University Medical Center, USA. 15 Centre de Référence Maladie de Marfan, Hôpital Bichat, APHP, Paris, France. 16 Laboratoire de Génétique moléculaire, Hôpital Ambroise Paré, APHP, Université Versailles-Saint Quentin en Yvelines, Boulogne, France.

Objectives: Mutations in *FBN1* cause Marfan syndrome and have been associated with a wide range of overlapping phenotypes. The factors that modulate phenotypical severity remain unclear.

Methods: The international *FBN1* mutation database (UMD-*FBN1*) has allowed us to carry out the largest collaborative study ever reported in order to investigate the correlation between the *FBN1* genotype and the nature and severity of the clinical phenotype. A range of qualitative and quantitative clinical parameters was compared for different classes of mutation in 1013 probands with a pathogenic *FBN1* mutation. We took advantage of this large series to define the indication for molecular screening of *FBN1*; to learn more about cardiovascular manifestations; to describe the clinical and molecular characteristics of the pediatric cohort (320 children); to further delineate patients with incomplete Marfan syndrome (146 probands); and more recently to apply the new 2010 Ghent criteria.

Results: The main results comprised: 1) a more severe phenotype (younger age at diagnosis, higher probability of ectopia lentis, ascending aortic dilatation, aortic surgery, mitral valve abnormalities, scoliosis, shorter survival) in mutations in exon-24-32, the majority of these results being replicated even when neonatal MFS were excluded; 2) a higher probability of ectopia lentis was found in patients with a missense mutation involving a cysteine when compared to other missense mutations; 3) a more severe skeletal and skin phenotype in patients with an *FBN1* premature truncation than patients with an inframe mutation. The results of the other studies will also be presented.

Conclusions: This large collaborative study have added significant knowledge on Marfan syndrome and type I fibrillinopathies.

ELUCIDATION OF SENSORS AND EFFECTORS IN MATRIX EQUILIBRIUM DERIVES NOVEL THERAPEUTIC STRATEGIES FOR SCLERODERMA

Gerber, Elizabeth E., B.S., Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. Huso, David, D.V.M. Ph.D., Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. Davis, Elaine C., Ph.D, Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada H3A 2B2. Wigley, Fredrick, M.D., Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. Loeys, Bart, M.D. Ph.D., Center for Medical Genetics, Ghent University Hospital, Ghent 9000, Belgium. Dietz, Harry C., M.D. Institute of Genetic Medicine and Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

Objectives: To gain a foothold in a common form of scleroderma, Systemic Sclerosis (SSc), we studied stiff skin syndrome (SSS), a rare form of scleroderma with childhood onset of diffuse skin fibrosis. SSS patients have heterozygous missense mutations in fibrillin-1 in the sole domain that harbors the Arg-Gly-Asp (RGD) sequence that mediates matrix-cell attachments via integrins. SSS skin shows disorganized macroaggregates of fibrillin-1 that fail to contact neighboring cells at the dermal-epidermal junction. SSc skin shows concordant findings. We hypothesize that cells sample matrix using integrins, fibrillin-1 is an informant on the status of the matrix, and loss of integrin-binding to fibrillin-1 results in a failure of communication, context-inappropriate matrix production, and fibrosis.

Methods: We generated mice with a SSS-associated mutation or one that substitutes RGE for RGD, leading to loss of integrin-binding. Tissue and dermal cells from mice, and humans with SSS and SSc were analyzed by histology, RT-PCR, northern, and western blotting.

Results: SSS and FBN1-RGE mice both show progressive scleroderma, with dermal thickening and replacement of subcutaneous fat by collagen. SSc fibroblasts show reduced microRNA-29, an inhibitor of matrix synthesis, and increased miR29 target transcripts; both are corrected upon treatment of cells with an integrin- β 1 activating antibody in a dose-dependent manner. RGE mice show reduced miR29 expression in skin.

Conclusions: Integrins act as sensors and miR29 an effector in regulation of matrix equilibrium. Perturbation of this axis, as in SSS or SSc, initiates profibrotic programs. Therapeutic strategies that activate integrins are being tested in SSS/RGE mice.

S. 9

LOEYS-DIETZ SYNDROME VS MARFAN SYNDROME: BROAD SPECTRA OF AORTIC/NON-AORTIC PHENOTYPES IN JAPANESE PATIENTS

Morisaki, Hiroko, MD, PhD, Department of Bioscience and Genetics, National Cerebral and Cardiovascular Center Research Institute, Suita, Japan

Ogino, Hitoshi, MD, PhD, Department of Cardiovascular Surgery, National Cerebral and Cardiovascular Center, Suita, Japan

Tsutsumi, Yoshiaki, MD, PhD, Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Suita, Japan

Akutsu, Koichi, MD, PhD, Department of Bioscience and Genetics, National Cerebral and Cardiovascular Center Research Institute, Suita, Japan

Ono, Akiko, MS, Department of Bioscience and Genetics, National Cerebral and Cardiovascular Center Research Institute, Suita, Japan

Kono, Atsushi, MD, PhD, Department of Radiology, National Cerebral and Cardiovascular Center, Suita, Japan

Higashi, Masahiro, MD, PhD, Department of Radiology, National Cerebral and Cardiovascular Center, Suita, Japan

Kosho, Tomoki, MD, PhD, Department of Medical Genetics, Shinshu University School of Medicine, Matsumoto, Japan

Mizuno, Seiji, MD, PhD, Department of Pediatrics, Aichi Prefectural Colony Central Hospital, Kasugai, Japan

Morisaki, Takayuki, MD, PhD, Department of Bioscience and Genetics, National Cerebral and Cardiovascular Center Research Institute, Suita, Japan, and Department of Molecular Pathophysiology, Osaka University Graduate School of Pharmaceutical Sciences, Suita, Japan

Objectives: Loeys-Dietz syndrome (LDS) is a systemic connective tissue disorder characterized by vascular and skeletal manifestations caused by mutations in *TGFBR1* or *TGFBR2*. Although characteristic craniofacial and arterial manifestations are helpful for diagnosis of LDS, there are many overlapping features between LDS and Marfan syndrome (MFS). We tried to reveal phenotypic differences between LDS and MFS. We also analyzed the genotype-phenotype correlations in LDS.

Methods: We analyzed the clinical details of 30 Japanese LDS patients with *TGFBR1* mutations (14 patients) and *TGFBR2* mutations (16 patients) and compared them with those of MFS.

Results: Compared with patients genetically diagnosed as MFS, LDS patients were physically less dolichostenomelic and more had ocular hypertelorism. Although Annuloaortic ectasia was observed in most of the LDS patients, 11% of those who had already experienced TAAD were free of AAE. Congenital retinal abnormalities and immunologic disorders were often observed in LDS patients. Dural ectasia was observed in both, but the ectatic pattern was different. Significant differences between patients with *TGFBR1* and those with *TGFBR2* mutations were observed in regard to age at diagnosis, cleft/uvula abnormalities, skeletal involvement, lung involvement, and fulfillment of Ghent MFS diagnostic criteria. LDS patients with *TGFBR2* mutations tended to have more skeletal involvement and be diagnosed at a younger age, often initially as MFS, while those with *TGFBR1* mutations had a greater chance to be diagnosed only when aortic symptoms were manifested.

Conclusions: Patients suspected of LDS should be carefully examined for specific features and be tested for *TGFBR1/TGFBR2* mutations.

S. 10

TGFBR1/2: GENOTYPE-PHENOTYPE CORRELATION

Arbustini Eloisa MD¹, Disabella Eliana BD¹, Favalli Valentina BME¹, Gambarin Fabiana MD¹, Bhattacharyya Shamik MD², Grasso Maurizia PhD¹, Serio Alessandra MD¹, Pasotti Michele MD¹, Catherine Klersy³, Duke Cameron MD⁴, Luca Vricella MD⁴, Harry C Dietz MD².

¹Center for Inherited Cardiovascular Diseases and ³Biometry, IRCSS Foundation Policlinico San Matteo University of Pavia, Italy; ²McKusick-Nathans Institute of Genetic Medicine, ⁴Cardiac Surgery, Johns Hopkins Medical Institutions, Baltimore, Maryland, USA.

Objective Mutations of *Transforming Growth Factor Beta Receptor-1&2 (TGFBR1/2)* genes cause Loeys-Dietz syndromes Type 1 and 2.

A description of vascular phenotypes, follow-up and events in 80 patients.

Method The series consists of 80 patients diagnosed with LDS1 (n=36) and LDS2 (n=44) from 42 families. Proband and relatives underwent multidisciplinary clinical and imaging evaluation, genetic counseling and testing: 36 carried *TGFBR1* and 44 *TGFBR2* mutations. Surgical data are multicentric.

Result Patients with LDS1 and LDS2 showed aortic root aneurysms (97% vs. 91%), dissection at onset (19% vs. 39%), arterial tortuosity (100%, both groups) and non-aortic aneurysms (35% vs. 30%). The mean age at first diagnosis was 12±14 (median=5.5) vs. 39±16 (median=37) years (p<0.0001), respectively. In 24 patients, 17 LDS2 and 7 LDS1, initial diagnosis coincided with acute aortic dissection (19 type A; 5 type B). Nine died shortly after emergency surgery and two a few months later without further interventions. During 57±61 months of follow-up, there were 24 re-interventions in 11 of the 13 survivors with 2 additional deaths at the 3rd and 7th surgery.

Sixteen patients underwent elective aortic surgery: 7 had 15 re-interventions (2/7 for type B dissection). One patient died one year after Bentall for cerebral aneurysm rupture and 1 for renal failure after the 3rd surgery. Overall, 41 patients underwent 71 aortic interventions. Total mortality was 19%. We did not observe gene-related phenotypes and evolution.

Conclusion LDSs are malignant vascular diseases. Timely diagnosis is mandatory as it prevents catastrophic events and allows preventive elective surgery.

S. 11**PEDIATRIC HEART NETWORK (PHN) RANDOMIZED CLINICAL TRIAL OF ATENOLOL VS LOSARTAN IN MARFAN SYNDROME (MFS)**

Ronald V. Lacro, MD (Children's Hospital Boston/Harvard Medical School) and Harry C. Dietz, MD (Johns Hopkins University School of Medicine), for the PHN Investigators

The PHN of the NHLBI/NIH, supported by the National Marfan Foundation, is conducting a randomized clinical trial to compare aortic root growth and other short-term cardiovascular outcomes in subjects with MFS receiving atenolol or losartan. Individuals 6 months to 25 years who meet Ghent criteria for MFS, with a body surface area (BSA)-adjusted aortic root Z score >3.0 are eligible for inclusion. Subjects are randomized in a 1:1 ratio to either atenolol (≤ 4 mg/kg/d to achieve a 20% decrease in mean 24-hr HR) or losartan (≤ 1.4 mg/kg/d); stratified for attainment of maximum height ("adult" vs child) and baseline aortic root Z score (< 4.5 vs ≥ 4.5); and followed for 3 yrs.

The primary outcome measure is the rate of change in aortic root BSA-adjusted Z score. Secondary end points include progression of aortic regurgitation; incidence of aortic dissection, aortic root surgery, and death; progression of mitral regurgitation; left ventricular size and function; measures of central aortic stiffness; skeletal and somatic growth; and incidence of adverse drug reactions. Primary analyses will be performed on an intention-to-treat basis. Three interim analyses are planned.

Additional ancillary studies address genetics/pharmacogenomics, musculoskeletal phenotype, quality of life, and circulating TGF- β levels.

We will enroll 604 subjects. Recruitment, which began in February 2007, continues at 21 sites in the US, Toronto, and Ghent. As of 7/1/2010, 549 subjects were randomized (91% of target).

S. 12

STUDY OF THE EFFICACY OF LOSARTAN ON AORTIC DILATATION IN PATIENTS WITH MARFAN SYNDROME

Guillaume Jondeau, M.D., Hôpital Bichat, France

Background. – Recent studies have demonstrated that blockade of the angiotensin II type 1 receptor with losartan decreases aortic damage in an animal model of Marfan syndrome (a KI mouse model with a pathogenic mutation in the gene coding for fibrillin-1).

Aims. – To demonstrate a beneficial effect of losartan on aortic dilatation when added to optimal therapy in patients with Marfan syndrome.

Methods. – This is a multicentre, randomized, placebo-controlled, double-blind, clinical trial with a 2-year inclusion period and a 3-year follow-up period. Aortic root diameter will be measured using two-dimensional echocardiography. Secondary endpoints will include incidence of aortic dissection, aortic root surgery, death, quality of life, tolerance and compliance with treatments. We aim to enrol a total of 300 patients aged ≥ 10 years who fulfil the Ghent criteria for Marfan syndrome. More than 250 patients have been included up to now. Analyses will be based on intention to treat.

Conclusion. – The results of this clinical trial could lead to profound modification of the management of aortic risk and complications in patients with Marfan syndrome and possibly in patients with thoracic aortic aneurysms of other aetiologies.

S. 13

RATIONALE AND STUDY DESIGN OF A RANDOMIZED, DOUBLE-BLIND TRIAL FOR THE EVALUATION OF THE EFFECT OF LOSARTAN VERSUS PLACEBO ON AORTIC ROOT DILATATION IN MARFAN PATIENTS TREATED WITH B-BLOCKERS.

De Backer Julie, MD, PhD, Center for Medical Genetics and Department of Cardiology, University Hospital Ghent, Belgium

De Nobele Sylvia, MSc, RN, Center for Medical Genetics, University Hospital Ghent, Belgium

Möberg Katarina, BSc, Center for Medical Genetics, University Hospital Ghent, Belgium

Renard Marjolijn, MSc, Center for Medical Genetics, University Hospital Ghent, Belgium

Devos Dan, MD, Center for Medical Imaging, University Hospital Ghent, Belgium

Goetghebeur Els, PhD, Department of Applied Mathematics and Computer Science, Ghent University, Belgium

Segers Patrick, PhD, bioMMeda-Institute Biomedical Technology, Ghent University, Belgium

Trachet Bram, MSc, bioMMeda-Institute Biomedical Technology, Ghent University, Belgium

Vervaet Chris, PhD, Laboratory for Pharmaceutical Technology, Ghent University, Belgium

Coucke Paul, PhD, Center for Medical Genetics, University Hospital Ghent, Belgium

De Paepe Anne, MD, PhD, Center for Medical Genetics, University Hospital Ghent, Belgium

Loeys Bart, MD, PhD, Center for Medical Genetics, University Hospital Ghent, Belgium

Background: The current standard medication for the prevention of aortic root dilatation in Marfan syndrome (MFS) are β -blockers. However, new insights in a mouse model and a small human trial have indicated that losartan, an angiotensin II receptor blocker with TGF- β inhibiting potential attenuates aortic root growth to an even larger extent.

Methods: We are conducting a prospective randomized placebo controlled double blind phase III study aiming to include 174 MFS patients who are already taking β -blockers (age ≥ 10 years and z-score ≥ 2). Patients will be randomised for losartan (50mg if < 50 kg and 100 mg if ≥ 50 kg) or placebo. The primary endpoint is the decrease in aortic root growth rate. Secondary endpoints are aortic dissection and/or surgery, progression of aortic and/or mitral regurgitation, arterial stiffness, left ventricular systolic and diastolic function, quality of life and genetic modifiers. Echocardiography – including TDI and AFI, vascular echo-doppler study and quality of life questionnaire, will be performed at baseline and at follow-up at month 6, 12, 24 and 36. MRI evaluation will be performed at baseline and at end of the trial.

Conclusion: This randomised double blind trial will study new therapeutic strategies for the prevention of serious cardiovascular complications in MFS.

The uniqueness in our trial is related to the fact that the additive effect of losartan and atenolol will be evaluated. Left ventricular function will be studied in detail using a combination of ultrasound and MRI. Adult patients with less severe aortic root dilatation will be included as well as patients who have already undergone surgery, hereby contributing valuable knowledge on how the drugs work on these specific groups of MFS patients. MRI assessment will allow even more accurate measurements of changes in aortic size and the elastic properties of the aorta.

S. 14**MARFAN SYNDROME: THE ITALIAN CLINICAL TRIAL**

Gambarin Fabiana MD¹, Disabella Eliana BD¹, Favalli Valentina BME¹, Grasso Maurizia PhD¹, Serio Alessandra MD¹, Pasotti Michele MD¹, Catherine Klersy MD², Arbustini Eloisa MD¹.

¹Center for Inherited Cardiovascular Diseases and ²Biometry

IRCSS Foundation Policlinico San Matteo

University of Pavia, Italy

Objectives

We will enrol 291 patients diagnosed with Marfan Syndrome and carriers of FBN1 mutations, randomly assigned to Losartan, Nebivolol and both drugs. Primary end-point is the decrease of Aortic Root Diameter (ARD) progression over four years. Secondary end-points: a) arterial stiffness; b) pharmacokinetics of both drugs; c) levels of TGF-beta; d) levels of *FBN1*mRNA (5' and 3'); e) pharmacogenetics: CYP2C9 for Losartan; CYP2D6 for Nebivolol; f) aortic regurgitation, aortic dissection, aortic root surgery, death and progression of mitral regurgitation; LV size and function; g) adverse drug reactions.

Method

The trial is open, randomized, phase-III with a 2-year interim analysis. We enrol patients aged 12 months to 55 years without prior aortic surgery and BSA-adjusted aortic root z score >2.5 (last approved amendment of Local Ethics Committee: z score ≥ 2 : requested by patients/parents and voluntary associations).

Expected results

A significantly lower progression of ARD in Nebivolol plus Losartan arm vs. stand-alone Losartan or Nebivolol; decrease of arterial stiffness higher when treated with both drugs; decrease of serum levels of active TGF-beta in both losartan arms. Other potential end-points results are the improvement of valve function, no death nor delay of surgical timing for the aortic root.

Currently, we have enrolled 190 patients: 90 ≥ 16 years and 100 ≤ 16 years of age.

Conclusions

Controlled clinical trials are especially needed to manage treatment on evidence-based data collected in large patients' population, confirmed in several studies. The enrolment on clinical and genetic ground will provide data for progression of geno-pharmaco-phenotype correlations.

S. 16

A RANDOMIZED, OPEN-LABEL, LOSARTAN THERAPY ON THE PROGRESSION OF AORTIC ROOT DILATION IN PATIENTS WITH MARFAN SYNDROME

Chiu Hsin-Hui, MD, Wang Jou-Kou, MD, PhD, Wu Mei-Hwan, MD, PhD

Department of Pediatrics, and Adult Congenital Heart Center, National Taiwan University Hospital, Taipei, Taiwan

Objective. To assess the efficacy and tolerability of angiotensin II receptor blockade (ARB), Losartan, to prevent progressive dilation of aortic root in patients with Marfan syndrome.

Methods. A randomized, open-label, active control trial was conducted in National Taiwan University Hospital since May 2007. Patients diagnosed as Marfan syndrome with recognized aortic root dilation (Z score >2.0) and under beta-blockade (Atenolol or Propranolol) treatment for more than 3 months were enrolled and randomized into two groups. One group continued previous beta-blockade therapy. The other group received a combination therapy with beta-blockade and Losartan. Primary endpoint is the changes in aortic root growth from baseline to completion of the study.

Results. Until now, twenty-eight patients (M/F: 12/16, 16.5 ± 6.6 years) were enrolled. Subjects were well tolerable to both medications. Preliminary analysis showed the combination therapy with ARB and beta-blockade effectively slowed down the growth rate of aortic root as compared to beta-blockade alone. However, the changes in aortic distensibility and cross-sectional compliance were similar between the two groups.

Conclusion. Based on these findings, we may suggest that combination therapy with ARB and beta-blockade can provide more effective and safe protection to slow down the aortic root diameter than sole beta-blockade in patients with Marfan syndrome.

S. 17

AORTIC IRBESARTAN MARFAN STUDY (AIMS) – UNITED KINGDOM

Anne H Child, FRCP, Cardiac and Vascular Sciences, St George's, University of London, England

John CS Dean, FRCP, Department of Medical Genetics, University of Aberdeen, Scotland.

Michael M Mullen, MRCP, Structural Heart Intervention, The Heart Hospital, London, England

Xu Yu Jin, MRCP, Echocardiology, John Radcliffe Hospital, Oxford, England

Marcus Flather, FRCP, Clinical Trials and Evaluation Unit, Royal Brompton Hospital, London, England
and the AIMS Trial Consortium

A multicentre randomised placebo-controlled double blind trial of irbesartan, an angiotensin receptor blocker, in Marfan syndrome is scheduled to begin later this year in the UK. 490 patients between the ages of 6 and 40 who fulfil the revised Ghent criteria for the diagnosis of Marfan syndrome and who have a dilated aorta (z score > 2), and who are not currently taking an ARB will be recruited from across the UK. Those who have not had fibrillin-1 mutation testing clinically will be tested as part of the study. Subjects will receive irbesartan or placebo in addition to their current medication, and standardised echocardiography will be undertaken over the 6 year course of the study to look for significant differences in the rate of aortic dilatation between the treated and the placebo groups. The final results will be available in 2015. The study is supported by the British Heart Foundation and the Marfan Trust.

BIOMARKERS AS OUTCOME PARAMETERS FOR CLINICAL TRIALSPeter Matt¹, Jennifer E. Van Eyk², Harry C. Dietz²¹University Hospital Basel/Berne, Switzerland, ²Johns Hopkins Medical School, Baltimore, MD, USA

Applying proteomic techniques to studying biomarkers in Marfan syndrome (MFS) allowed us to reveal circulating TGF β as a promising prognostic and therapeutic biomarker in MFS. Circulating TGF β concentrations are elevated in MFS and decrease after administration of losartan, β -blocker therapy, or both. Even if there is little intrinsic prognostic value to a snapshot measurement of circulating TGF β , there remains a high probability of obtaining important information for individual trends observed during the progression of disease and in response to therapy. Circulating TGF β and other selected biomarkers may serve as outcome parameters for clinical trials, e.g. in the Atenolol vs. Losartan Clinical Trial. Those biomarkers should allow to evaluate the safety and effectiveness of medical and/or surgical therapies instead or additionally to the true outcome of interest such as change in aortic root diameter, morbidity and mortality. Selected biomarkers (e.g. circulating TGF β) have the advantage to be gathered in a shorter time frame, are little invasive, less expensive and there is no radiation exposure. Alterations in biomarkers may also be easier to relate causally to an occurred event. An important disadvantage exists if the biomarker is influenced by numerous internal and external factors. Large, prospective screening studies are therefore needed for validation of selected biomarkers. Circulating TGF β may represent a promising outcome parameter for clinical trials related to MFS and associated disorders.

S. 19**NOVEL GENETIC VARIANTS PREDISPOSING TO SPORADIC THORACIC AORTIC DISEASE**

*Dianna M. Milewicz*¹, Dong-chuan Guo¹, Ellen Regalado¹, Siddharth K. Prakash³, Shao-Qing Kuang¹, Joseph S. Coselli^{4,5}, Hazim Safi², Anthony L. Estrera², Suzanne M. Leal³, Scott A. LeMaire^{4,5}, John Belmont³

¹Departments of Internal Medicine and ²Cardiothoracic and Vascular Surgery, University of Texas Health Science Center at Houston, Houston, Texas 77030, USA. ³Department of Molecular and Human Genetics and ⁴Division of Cardiothoracic Surgery, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, Texas 77030, USA. ⁵Cardiovascular Surgery and ⁶Internal Medicine Service, Texas Heart Institute at St. Luke's Episcopal Hospital, Houston, Texas 77030, USA.

We sought to identify the genes that predispose individuals to thoracic aortic aneurysms that progress to acute aortic dissections (TAAD) in patients without a family history, termed sporadic disease (STAAD). Single nucleotide polymorphism (SNP) array data (Illumina Human CNV370-Quad BeadChip) obtained from 800 unrelated Caucasian STAAD patients over the age of 30 years were analyzed for copy number variants (CNVs) and compared with CNVs identified from SNP array data from 4261 Caucasian controls. We identified recurrent, large heterozygous segmental 16p13.1 duplications of variable size in 9 of 800 (1.1%) STAAD patients compared with 4 duplications of 16p13.1 involving the same region in 4261 (0.1%) ethnically matched controls. The findings were replicated in a separate cohort and combining the data from all cohorts (1266 patients and 5609 controls) resulted in a highly significant association between TAAD and duplications of 16p13.1 ($P = 9.0 \times 10^{-7}$, OR = 11.5, 95% CI = 4.1-32). Patients with a 16p13.1 duplication were more likely to harbor a second rare CNV (Fisher's exact $P = 0.017$) and to present with aortic dissections ($P = 0.00058$) than patients without duplications.

In a genome-wide analysis of 418 STAAD cases, we identified an additional 47 CNV regions that were enriched in or unique to TAAD patients compared to population controls. Gene ontology, expression profiling and network analysis showed that genes within TAAD CNVs regulate smooth muscle cell adhesion or contractility and interact with the smooth muscle-specific isoforms of α -actin and β -myosin, which are known to cause familial TAAD when altered. Enrichment of these gene functions in rare CNVs was replicated in independent cohorts with sporadic (STAAD, n=387) and inherited TAAD (FTAAD, n=88). The overall frequency of rare CNVs (23%) was significantly increased in FTAAD compared with STAAD patients (13%, Fisher's exact $p=0.03$). Our findings suggest that rare CNVs disrupting smooth muscle adhesion or contraction contribute to both sporadic and familial disease.

Finally, a genome-wide association study was performed on data from 765 STAAD cases, 1355 WTCCC C58 controls, and 874 NINDS controls. Associations with SNPs in two chromosomal regions met genome-wide significance ($P < 5 \times 10^{-8}$) regardless of which controls were used, and one of these regions replicated in a second STAAD cohort. Details concerning the gene associated with TAAD and the clinical findings in patients with the risk associated haplotypes will be presented.

S. 20**MECHANISTIC INSIGHTS REGARDING FILAMINOPATHIES AND ANEURYSM**

Kim, David, MS, Institute of Genetic Medicine, Johns Hopkins Hospital, Baltimore, MD USA
Patel, Nishant, BA, Institute of Genetic Medicine, Johns Hopkins Hospital, Baltimore, MD USA
Lindsay, Mark, MD PhD, Institute of Genetic Medicine, Johns Hopkins Hospital, Baltimore, MD USA
Goldmuntz, Elizabeth, MD, Division of Cardiology, Children's Hospital of Philadelphia, Philadelphia, PA USA
John, Anitha, MD, Division of Cardiology, Children's Hospital of Philadelphia, Philadelphia, PA USA
Garbarini, Jennifer, MS, Division of Cardiology, Children's Hospital of Philadelphia, Philadelphia, PA USA
Dietz, Harry, MD, Institute of Genetic Medicine, Johns Hopkins Hospital, Baltimore, MD USA

Objectives: We identified a complex mutant allele (R527C/P2423S) of the filamin A gene (FLNA) on the X-chromosome in a male patient with a syndromic presentation of tetralogy of Fallot with ascending aortic aneurysm (ToF/AAA). Subsequent screening of individuals with nonsyndromic ToF/AAA revealed a recurrent missense mutation (A1764T) in 3 unrelated patients. These mutations were not observed in 500 control chromosomes and occurs at the calpain cleavage site of filamin A. Filamins contribute to the organization and stability of the actin cytoskeleton, integrate cellular signaling cascades, and regulate cellular functions including adhesion and motility. In other aneurysm disease states, our lab has shown that increased TGF β activity induces cells within the aortic media to transform into myofibroblasts with many deleterious behaviors including the expression of matrix-degrading enzymes. We hypothesize that cleavage of filamin A by calpain may be required for some TGF β mediated downstream events.

Methods: Filamin A in patient derived cells was measured by western blot. Epithelial to mesenchymal transition (EMT) was assayed in NMuMG epithelial cells treated with TGF β in presence or absence of the calpain inhibitor MDL28170.

Results: Both R527C/P2423S and A1764T alleles were associated with a reduction in filamin A (9.3 and 19.3% of wildtype, respectively). The addition of calpain inhibitor blocked TGF β -induced EMT in a dose-dependent manner (reduced expression of α -smooth muscle actin and vimentin). Calpain inhibition also preserved epithelial cell morphology after TGF β treatment.

Conclusions: These data suggest that reduced filamin A levels and/or accentuated calpain cleavage contributes to the pathogenesis of aortic aneurysm, making calpain inhibitors a potential therapeutic.

S. 21

THE DISEASE PHENOTYPES AND MECHANISMS OF LTBP4 MUTATIONS

Urban, Zsolt, PhD, Department of Human Genetics, University of Pittsburgh, USA
 Huchtagowder, Vishwanathan, PhD, Department of Pediatrics Washington University School of Medicine, St. Louis, MO, USA
 Henger, Silvia, Department of Human Genetics, University of Pittsburgh, USA
 Westman, Rachel, BS, Department of Human Genetics, University of Pittsburgh, USA
 Collenburg, Lena, Department of Human Genetics, University of Pittsburgh, USA
 Schürmann, Nura, Department of Pediatrics, Washington University, St. Louis, MO, USA
 Todorovic, Vesna, BS, Department of Cell Biology, New York University, New York, NY, USA
 Zilberberg, Lior, BS, Department of Cell Biology, New York University, New York, NY, USA
 Choi, Jiwon, MS, Department of Anatomy and Cell Biology, McGill University, Montreal, QC, Canada
 Sens, Carla, BS, Department of Pediatrics, Washington University, St. Louis, MO, USA
 Brown, Chester W, MD, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA
 Clark, Robin D, MD, Department of Pediatrics, Loma Linda School of Medicine, Loma Linda, CA, USA
 Holland, Kristen E, MD, Dept of Dermatology, Medical College of Wisconsin, Milwaukee, WI, USA
 Marble, Michael, MD, Children's Hospital of New Orleans, New Orleans, LA, USA
 Sakai, Lynn Y, PhD, Dept of Biochem and Mol Biol, Shriners Hospital for Children, Portland, OR, USA.
 Branka Dabovic, PhD, Department of Cell Biology, New York University, New York, NY, USA
 Daniel B. Rifkin, PhD, Department of Cell Biology, New York University, New York, NY, USA
 Elaine C. Davis, PhD, Dept of Anatomy and Cell Biology, McGill University, Montreal, QC, Canada

Objectives: To define the mutational and phenotypic spectrum of latent transforming growth factor- β (TGF β) binding protein 4 (*LTBP4*) mutations in cutis laxa and the associated disease mechanisms.

Methods: Ten patients with cutis laxa and severe pulmonary, gastrointestinal and urinary manifestations were included. The coding region of *LTBP4* was screened for mutations by direct DNA sequencing. Skin fibroblasts and tissue samples from patients and controls were analyzed by histology, electron microscopy, immunostaining, immunoblotting and reporter assays to investigate elastic fiber assembly and TGF β signaling. Scratch wound assays were used to investigate cell migration.

Results: Five patients had recessive mutations in *LTBP4*. All 5 had severe respiratory distress, with cystic and atelectatic changes in the lungs, tracheomalacia and diaphragmatic hernia. Visceral malformations included diverticulosis, enlargement, tortuosity and stenosis of the intestinal tract and bladder diverticulosis. Growth delay, low muscle tone, and joint laxity comprised musculoskeletal lesions. Craniofacial features included microretrognathia, flat midface, receding forehead and wide fontanel. Six of 7 *LTBP4* mutations led to premature termination of translation and destabilization of the *LTBP4* mRNA. Impaired synthesis and lack of deposition of LTBP4 into the extracellular matrix caused increased TGF β activity in cultured fibroblasts, increased cell migration and defective elastic fiber assembly. Fibrillin-1 microfibril bundles were absent at the dermo-epidermal junction in patient skin samples and were disorganized in mutant skin fibroblast cultures.

Conclusions: LTBP4 is required for fibrillin-1 microfibril bundle assembly and the regulation of TGF β activation. LTBP4 mutations impair these functions with pleiotropic developmental effects in multiple organ systems.

S. 22**NULL MUTATIONS IN LTBP2 CAUSE PRIMARY CONGENITAL GLAUCOMA**

Ali, Manir PhD.

Section of Ophthalmology and Neuroscience, Leeds Institute of Molecular Medicine, University of Leeds, St. James's University Hospital, Leeds, West Yorkshire, United Kingdom.

Objectives: To investigate the genetic basis of recessively-inherited primary congenital glaucoma in four consanguineous pedigrees from the Punjab province of Pakistan and patients of Gypsy ethnicity.

Methods: Ocular examinations were conducted on each family member to confirm their diagnosis. Genomic DNA was analysed by homozygosity mapping using the 50K Affymetrix SNP array and linkage was confirmed with polymorphic microsatellite markers. Candidate genes were sequenced. Protein immunolocalisation was performed on mouse and bovine eyes. Some of the patients were re-examined for bone mineral density and echocardiography defects.

Results: We established linkage to a region on chromosome 14q. Sequencing the *latent transforming growth factor (TGF)-beta binding protein-2 (LTBP2)* gene identified null mutations in each of the four consanguineous pedigrees from Pakistan and in 38% of the Gypsy PCG patients. We confirmed localisation of LTBP2 in the anterior segment of the eye, at the ciliary body and particularly the ciliary process. LTBP2 is an extracellular matrix protein with multi-domain structure. It has homology to fibrillins, interacts with fibrillin1 (mutations in which cause Marfan syndrome) and is a structural component of fibrillin-rich microfibrils. Upon re-examination, patients with homozygous *LTBP2* mutations had no obvious cardiac or skeletal abnormalities, though some of the patients had ectopia lentis as an ocular feature.

Conclusions: These findings reveal that LTBP2 is essential for normal development of the anterior chamber of the eye, where it may have a structural role in maintaining ciliary muscle tone and supporting the lens.

S. 23**IDENTIFICATION OF *ADAMTS10* AND *ADAMTSL2* MUTATIONS IN THE ACROMELIC DYSPLASIA GROUP**

Le Goff Carine, Cormier-Daire Valérie

Department of genetics, INSERM U781, Paris Descartes University,
Hopital Necker Enfants Malades, Paris, France

The acromelic dysplasia group includes Weill-Marchesani syndrome (WMS), geleophysic dysplasia (GD) and acromicric dysplasia, characterized by short stature, brachydactyly and joint stiffness. WMS is distinct by the presence of microspherophakia and by two modes of inheritance, autosomal recessive (AR) and autosomal dominant (AD). We have identified four mutations in the fibrillin-1 (FBN1) gene in 4 AD WMS families. In the mean time, we have identified 9 distinct mutations in *ADAMTS10* (a disintegrin and metalloprotease with thrombospondin motifs) in 7 AR WMS families. The observation of *FBN1* and *ADAMTS10* mutations in the two forms of WMS suggests that *ADAMTS10* interacts with *FBN1*.

We then focus on GD, which is characterized by a progressive cardiac disease with dilation and thickening of the pulmonary, aortic or mitral valves often leading to an early death. Studying six GD families, we have mapped the disease gene on chromosome 9q34.2 and identified five distinct mutations in A Disintegrin And Metalloproteinase with Thrombospondin repeats- like 2 gene (*ADAMTSL2*). We then identified Latent TGF β Binding Protein 1 (LTBP1) as a partner of *ADAMTSL2* and found an increased level of active TGF β and phosphorylated SMAD2 in GD fibroblasts.

Following this study, we have screened 30 additional GD families and identified *ADAMTSL2* mutations in 13/30 comprising 9 novel mutations. We also found an increased TGF β level in non mutated *ADAMTSL2* fibroblasts.

On-going studies will hopefully lead to the identification of another disease gene and to the understanding of *ADAMTS10* and *ADAMTSL2* function in TGF β pathway.

ISOLATED ECTOPIA LENTIS: REPORT OF A NEW DELETION IN THE *ADAMTSL4* GENE AND EVIDENCE FOR GENETIC HETEROGENEITY OF THE AUTOSOMAL RECESSIVE FORM OF THE DISEASE

Hanna Nadine PhD^{1,2}, Sultan Gilles MD³, Muti Christine MD², Grandchamp Bernard MD, PhD², Gouya Laurent MD, PhD^{1,2}, Funtowicz Sarah², Lacombe Didier MD⁴, Dollfus H el ene MD, PhD⁵, Baudouin Christophe MD, PhD^{3,6}, Jondeau Guillaume MD, PhD², Boileau Catherine PhD^{1,2,7}.

1. Laboratoire de G en tique mol culaire, H opital Ambroise Par , AP-HP, Universit  Versailles-Saint Quentin en Yvelines, Boulogne, France.
2. Centre de R f rence Marfan, H opital Bichat, AP-HP, Paris, France.
3. Service d'Ophtalmologie, H opital Ambroise Par , AP-HP, Boulogne, France.
4. Service de G en tique M dicale, H opital Pellegrin, CHU de Bordeaux, Universit  Bordeaux II, France.
5. Centre de R f rence pour les Affections Rares en G en tique Ophtalmologique (CARGO) et Service de G en tique M dicale, H opitaux Universitaires de Strasbourg, Strasbourg, France.
6. Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris, France.
7. Inserm U781, H opital Necker-Enfants Malades, Paris, France.

Introduction:

Often associated with systemic diseases (such as homocystinuria, Marfan syndrome or Weill-Marchesani syndrome), ectopia lentis (EL) can appear as an isolated condition with autosomal dominant (ADEL, OMIM#1296000) or autosomal recessive (AREL, OMIM#225100) inheritance. ADEL has been associated with mutations within the *FBN1* gene, while only two mutations have been identified to date in the *ADAMTSL4* gene in families with AREL (Ahram *et al.*, 2009; Greene *et al.*, 2010).

Objective:

Evaluate the contribution of *ADAMTSL4* gene mutations to isolated EL.

Methods:

We studied 12 French probands negative for homocystinuria and with no mutation within the *FBN1* gene. Bidirectional sequencing of the 17 coding exons of the *ADAMTSL4* gene was performed. When available, family studies were performed and regional haplotypes were constructed with 9 microsatellite markers flanking the gene at 1q21.3.

Results:

An unreported and identical homozygous frameshift deletion was found in 2 unrelated probands: c.767_786del, p.Gln256ProfsX38. Family analysis showed that the mutation was carried on two different haplotypes, one proband carrying the two haplotypes. In the 10 remaining probands, no mutation was identified in the *ADAMTSL4* gene to explain the EL phenotype. Interestingly, one of these probands belonged to a small French family of Gypsy origin with 4 affected children. None of the affected children were haplo-identical thus demonstrating exclusion of the gene locus. In the same way, linkage was also excluded to the *FBN1* gene.

Conclusion:

In a small sample of EL probands, only 2/12 (16 %) of EL cases were related to a mutation in the coding sequence of the *ADAMTSL4* gene. Furthermore, we report a family with AREL unlinked to either of the known genes associated with EL. This family demonstrates the existence of further genetic heterogeneity in isolated EL.

**Selected for oral presentation from poster submissions*

ROLE OF ADAMTSL4 MUTATIONS IN FBN1 MUTATION NEGATIVE ECTOPIA LENTIS PATIENTS

Child A. H., FRCP, Cardiac and Vascular Sciences, St George's, University of London, London, United Kingdom

Aragon-Martin J.A. PhD, Cardiac and Vascular Sciences, St George's, University of London, London, United Kingdom

Ahnood D., Medical Student, Cardiac and Vascular Sciences, St George's, University of London, London, United Kingdom

Saggar A., MRCP Medical Genetics, St. George's, University of London, United Kingdom.

Nischal K., FRCOphth, Department of Ophthalmology, Hospital for Sick Children, London, UK.

Charteris D., FRCOphth, Vitreoretinal Surgery, Moorfields Eye Hospital, London, United Kingdom.

Arno G., PhD, Cardiac and Vascular Sciences, St George's, University of London, London, United Kingdom

Objectives

Although clinically homogeneous, ectopia lentis (EL) is genetically heterogeneous with both autosomal-dominant (MIM 129600) and autosomal-recessive (MIM 225100) forms. The dominant disorder can be caused by mutations in FBN1, at the milder end of the type-1 fibrillinopathy spectrum. Recently in a consanguineous Jordanian family, recessive EL was mapped to locus 1q21 containing the ADAMTSL4 gene and a nonsense mutation found in exon 11 (c.1785T>G, p.Y595X) of ADAMTSL4. Could we demonstrate ADAMTSL4 mutations in our UK ectopia lentis patients?

Methods

In this study, 12 consecutive Caucasian U.K. probands with EL and demonstrating no, or very mild, heart involvement on echocardiogram were included. Probands did not fulfil the Ghent criteria for Marfan syndrome and were previously found mutation-negative for FBN1. Mutation screening in ADAMTSL4 by direct sequencing of all exons including their intron/exon boundaries was performed.

Results

Homozygous or compound heterozygous mutations were identified in 6/12 (50%) probands. Mutation data are summarised in table 1. Where available, familial screening of these families confirmed the mutation co-segregated with the EL phenotype. None of the ADAMTSL4 mutations described here were identified in 156 normal control chromosomes.

Conclusions

This study is the first confirmation that homozygous mutations in ADAMTSL4 are associated with autosomal recessive EL. The first compound heterozygous mutations are described. The identification of a causative mutation in ADAMTSL4 may allow exclusion of Marfan syndrome in these families and guide genetic counselling and clinical management, of particular relevance in young children affected by EL.

*Selected for oral presentation from poster submissions.

Mutations in ADAMTSL4 in Ectopia Lentis Patients**Table 1**

Case	Age	Exon	Nucleotide	Amino acid	Domain	Zygoty
1	6	6	c.767_786del	p.Gln256ProfsX38	-	Compound heterozygous
		6	c.826_836del	p.Arg276SerfsX21	-	
2	8	6	c.767_786del	p.Gln256ProfsX38	-	Compound heterozygous
		6	c.826_836del	p.Arg276SerfsX21	-	
3	15	6	c.826_836del	p.Arg276SerfsX21	-	Homozygous
4	41	12	c.1960C>T	p.Pro654Ser	-	Homozygous
5	34	12	c.2008C>T	p.Arg670X	ADAM TSR-1	Homozygous
6	4	19	c.3153C>A	p.Tyr1051X	PLAC domain	Compound heterozygous
		19	c.3161A>G	p.Tyr1054Cys	PLAC domain	
		6	c.926G>A	p.Arg309Gln	-	

ADAMTSL6 β RESCUES MICROFIBRIL DISORDER IN MARFAN SYNDROME THROUGH THE PROMOTION OF FIBRILIN-1 ASSEMBLY

Saito Masahiro DDS Ph.D¹, Kurokawa Misaki¹, Ooshima Masamitsu DDS PhD², Tsutsui Ko PhD⁴, Hada Yasunobu^{1,5}, Sekiguchi Kiyotoshi PhD⁴ and Takashi Tsuji PhD^{1,2,3}

¹Faculty of Industrial Science and Technology, Tokyo University of Science, Chiba, Japan ²Research Institute for Science and Technology, Tokyo University of Science, Chiba, Japan, ³Organ Technologies Inc., Tokyo, Japan, ⁴Institute for Protein Research, Osaka University, Osaka, Japan, ⁵Oral Implantology and Regenerative Dental Medicine, Graduate school, Tokyo Medical and Dental University, Tokyo, Japan

Objective

Marfan syndrome (MFS) is a systemic disorder affecting connective tissues caused by insufficient fibrillin-1 microfibril formation and deregulation of TGF- β signaling in various connective tissues. Recent observations provide that TGF- β antagonism is a general therapeutic strategy for MFS, however reconstruction of the microfibril in connective tissues remains to be determined. A disintegrin-like metalloprotease domain with thrombospondin type I motifs like (ADAMTSL) 6 β is a microfibril-associated extracellular matrix protein associated with fibrillin-1 microfibrils through direct interaction with the fibrillin-1 which promotes fibrillin-1 matrix assembly *in vitro* and *in vivo*. Here, we report that ADAMTSL6 β has an essential role in the development and regeneration of connective tissues.

Methods

To investigate the potential for clinical application of ADAMTSL-6 β as a novel MFS therapy, we investigated if ADAMSL6 β expression can rescue fibrillin-1 microfibril formation and regulating of TGF β activation through the promotion of fibrillin-1 microfibril assembly in mgR/mgR mice as a model of MFS microfibril disorder.

Results

ADAMTSL6 β expression rescues microfibril disorder after periodontal ligament (PDL) injury in MFS model mice through the promotion of fibrillin-1 microfibril assembly. In addition, improved fibrillin-1 assembly following administration of ADAMTSL6 β attenuates the over-activation of TGF β signals associated with increased release of active TGF- β from disrupted fibrillin-1 microfibrils within PDLs of MFS model mice.

Conclusion

This study demonstrates the essential contribution of ADAMTSL6 β to fibrillin-1 microfibril formation and suggests a new therapeutic strategy for the treatment of MFS through ADAMTSL6 β -mediated fibrillin-1 microfibril assembly.

**Selected for oral presentation from poster submissions*

A NOVEL GENETIC PATHWAY UNDERLIES WEILL-MARCHESANI SYNDROME

Gerhard Sengle¹, Ko Tsutsui^{1,2,3}, Douglas R. Keene³, Eric J. Carlson¹, Noe L. Charbonneau³, Mary K. Wirtz⁴, John R. Samples⁴, Susan J. Hayflick⁵, Lisa I. Fessler⁶, John H. Fessler⁶, Kiyotoshi Sekiguchi², and Lynn Y. Sakai^{1,3}

¹Department of Biochemistry and Molecular Biology, Oregon Health & Science University

²Division of Protein Chemistry, Institute for Protein Research, Osaka University

³Shriners Hospital for Children, Portland OR

⁴Casey Eye Institute, Oregon Health & Science University

⁵Department of Molecular and Medical Genetics, Oregon Health & Science University

⁶Department of Molecular, Cell and Developmental Biology, UCLA

We have identified a novel 3 domain deletion in FBN1 that results in autosomal dominant Weill-Marchesani syndrome (WMS). While individuals with Marfan syndrome are tall with hypomuscularity and hypermobile joints, individuals with WMS display the “opposite” phenotypes of short stature, hypermuscularity, and stiff joints. *Objectives:* To investigate the underlying molecular mechanisms leading to WMS. *Methods:* Both in vitro and in vivo approaches were used. Routine in vitro interaction studies (surface plasmon resonance; co-immunoprecipitation) were performed. We also analyzed mice (using histological stains, immunoelectron microscopy, microCT, and qPCR) in which our WMS mutation in Fbn1 was knocked in. *Results and Conclusions:* WMS mutant mice survived well in homozygosity and did not display aortic disease, emphasizing again that this 3 domain deletion does not result in Marfan syndrome in humans or mice. WMS mutant mice, in heterozygosity and homozygosity, demonstrated a thick skin phenotype, phenocopying one of the features of human WMS. Our biochemical interaction studies showed that the deleted FBN1 domains serve as a binding site for Adamtslike-2, -3, -6, and papilin and that Adamtslike-3 interacts with ADAMTS10. Based on these results, we hypothesize that Adamtslike proteins form complexes with Adamts enzymes and that Adamtslike proteins bind to fibrillin-1, targeting the complex to microfibrils. Furthermore, in the absence of this binding site, we hypothesize that Adamts10 is not properly targeted and sequestered in the matrix, resulting in WMS.

**Selected for oral presentation from poster submissions*

S. 28

AORTIC DISEASE IN GERMLINE, VASCULAR SMOOTH MUSCLE CELL (VSMC) SPECIFIC AND ENDOTHELIAL CELL (EC) SPECIFIC FBN1 MUTANT MICE

Elise C. Manalo, Noe L. Charbonneau, Eric J. Carlson, Jamie R. Langdon, Gerhard Sengle, Sara F. Tufa, Douglas R. Keene, and Lynn Y. Sakai

Department of Biochemistry and Molecular Biology
And Shriners Hospital for Children
Oregon Health & Science University
Portland, OR 97239

Aortic dilatation and fragmentation of elastic lamellae are common features of disease in the Marfan syndrome.

Objectives: To determine the contributions of vascular smooth muscle cells and endothelial cells to aortic disease.

Methods: A floxed *Fbn1* knock-in mutation and Cre-lox technology were utilized to produce (1) a germline mutant mouse model (GT-8) using mice in which Cre recombinase was knocked into the Rosa26 locus; (2) VSMC-specific mutant mice (GT/+;SM22Cre/+) using mice in which Cre was knocked into the SM22 locus; and (3) EC-specific mutant mice (GT/+;Tie2Cre) using mice expressing a Cre transgene under the control of the Tie2 promoter. Cre recombination resulted in fibrillin-1 molecules that are truncated and tagged with eGFP. All mice were generated on a C57/Bl6 background. Evaluation of aortic disease was performed with mice between 2 months and 10 months of age.

Results and Conclusions: Aortic disease was evident in GT-8/+ mice from 2 months of age, with progressive fragmentation of elastic lamellae resulting in dilatation, aneurysm, and sometimes dissection. Evidence of fragmentation of the elastic lamellae was clear in 6 month VSMC-specific mutant mice. In contrast, EC-specific mutant mice showed no evidence of aortic disease. We conclude that VSMC secrete the largest amounts of fibrillin-1 and therefore contribute in a major way to aortic disease. However, since progression of aortic disease in VSMC-specific mutant mice is not as rapid as in GT-8/+ mice, we also conclude that cells other than SM22 positive cells likely contribute to the severity of aortic disease in GT-8/+ mice.

S. 29

A NEW MOUSE MODEL FOR MARFAN SYNDROME PRESENTS PHENOTYPIC VARIABILITY ASSOCIATED WITH THE GENETIC BACKGROUND, AND OVERALL LEVELS OF *Fbn1* EXPRESSION.

Lima BL, SantosEJC., FernandesGR, PereiraLV.

Laboratório de Genética Molecular, Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo. São Paulo-SP, Brazil.

In 1997, a murine model for Marfan Syndrome - $mg\Delta$ - was created to mimic the dominant-negative effect of fibrillin-1 mutations seen in MFS patients. Surprisingly, heterozygote animals were histologically indistinguishable from wild-type mice. Expression analysis showed that the $mg\Delta$ allele had a 90% lower transcript level compared to the normal *Fbn1* allele. Accordingly, it was postulated that the *neoR* cassette sequence had interfered with the mutant allele expression, consequently restricting the dominant-negative effect of the mutation. Here we report the generation of a novel variant of the $mg\Delta$ mouse model for MFS, in which the same mutant *Fbn1* allele is present, but with *neoR* flanked by lox-P sequences ($mg\Delta^{loxPneo}$), allowing Cre-recombinase-mediated deletion of the resistance cassette. Two complementary 38-bp oligonucleotides with the loxP consensus sequence were synthesized, annealed, and cloned flanking the *neoR* expression cassette, which was then used to replace the original *neoR* cassette of the $mg\Delta$ vector. Unexpectedly, this allele now resulted in heterozygous $mg\Delta^{loxPneo}$ mice presenting some aspects of the MFS phenotype, including aortic, skeletal and respiratory system manifestations, before the removal of *neoR* sequence. Moreover, these phenotypes differ significantly between two different isogenic mouse strains, C57BL/6 and 129/Sv, and also vary within the 129/Sv background. We show a correlation between overall levels of *Fbn1* expression and disease severity in the 129/Sv heterozygotes. Therefore, in addition to modeling the clinical manifestations of MFS disease, the $mg\Delta^{loxPneo}$ mouse model is an experimental system in which both the genetic background and epigenetic contributions to MFS clinical variability can be evaluated.

S. 30

INVESTIGATION OF LOEYS-DIETZ SYNDROME USING AN ALLELIC SERIES OF MUTANT MICE

Loch, David, PhD, Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Gallo, Elena, PhD, Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Dietz, Harry, MD, Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Objectives: Loeys-Dietz syndrome (LDS) is a connective tissue disorder with significant phenotypic overlap with Marfan syndrome (MFS) that, unlike MFS, demonstrates more widespread and aggressive vascular disease. Such pathology is often difficult to manage surgically, highlighting the need for the development of medical therapies through elucidation of disease pathogenesis. LDS is caused by heterozygous missense mutations in the genes encoding transforming growth factor-beta receptors, TGFBR1 and TGFBR2. Unexpectedly, analysis of LDS patient aortas demonstrate paradoxically enhanced TGF β signaling, like MFS.

Methods: To address mechanism, we have created three mutant mouse models of LDS; two knock-in strains with missense mutations in Tgfr1 (M318R) or Tgfr2 (G357W) and a transgenic that ubiquitously over-expresses mutant Tgfr2. Mice heterozygous for null alleles of either Tgfr1 or Tgfr2 have also been analyzed.

Results: Both knock-in strains fully recapitulate the LDS vascular phenotype. G357W transgenic mice also develop vascular disease, with increased severity seen in homozygosity, despite full TGF β signaling capacity in cultured cells, arguing against a dominant-negative mechanism. In contrast, both haploinsufficient mouse strains show normal vasculature and longevity. LDS knock-in mice show increased canonical and noncanonical TGF β signaling in aortic tissue (pSmad2 and pERK1/2 expression respectively), suggesting a gain-of-function mechanism. A pilot study of losartan in LDS mice prevented aortic root dilatation with reduced canonical and noncanonical TGF β signaling, similar to MFS.

Conclusions: This study demonstrates that LDS mice recapitulate the complex pathology of the human disease. Further, therapies developed for MFS may find broader application in disorders of vessel wall homeostasis like LDS.

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ADAMTSL2 BINDS FIBRILLIN-1, ACCELERATES FIBRILLIN-1 MICROFIBRIL BIOGENESIS, AND IS MUTATED IN CANINE MUSLADIN-LUEKE SYNDROME, A HERITABLE CONNECTIVE TISSUE DISORDER WITH EXTENSIVE SKIN FIBROSIS.

Hannah L. Bader¹, Alison L. Ruhe², Lauren W. Wang¹, Aaron K. Wong², Kari F. Walsh², Rebecca A. Packer³, Luis Gabriel¹, Jonathan Mitelman⁴, Alana K. Majors⁵, Kathryn R. Robertson², Dennis P. O'Brien⁶, Karl W. Broman⁷, G. Diane Shelton⁸, Mark W. Neff² and Suneel S. Apte¹

¹ Department of Biomedical Engineering, and ⁵ Department of Pathobiology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, United States of America, ² Veterinary Genetics Laboratory, University of California, Davis, CA, United States of America, ³ Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, United States of America, ⁴ Kingston Road Animal Hospital, Toronto, ON, Canada, ⁶ Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO, United States of America, ⁷ Department of Biostatistics and Medical Informatics, University of Wisconsin, Madison, WI, United States of America, ⁸ Department of Pathology, School of Medicine, University of California San Diego, La Jolla, CA, United States of America

Objectives: ADAMTSL2 mutations cause human geleophysic dysplasia, a TGF β dysregulation syndrome featuring short stature, tight skin, joint contractures, and other characteristic manifestations. We asked if ADAMTSL2 bound to fibrillin-1 and influenced microfibril assembly. We investigated the role of ADAMTSL2 mutations in canine Musladin-Lueke syndrome (MLS).

Methods: Binding of ADAMTSL2 and an N-terminal fibrillin-1 peptide was investigated using Biacore. The effect of exogenous ADAMTSL2 on fibrillin-1 biogenesis in cultures of bovine fetal nuchal ligament cells (bFNLC) was determined by fibrillin-1 immunofluorescence. The locus for MLS was mapped and *ADAMTSL2* was sequenced as a candidate gene in the linked region. Mutant ADAMTSL2 was expressed in mammalian cells to determine effects of the mutation.

Results: Biacore showed that wild-type ADAMTSL2 bound to the N-terminal half of fibrillin-1 (K_D = 60nM). ADAMTSL2 accelerated fibrillin-1 microfibril biogenesis in bFNLC matrix. MLS was caused by a founder mutation in *ADAMTSL2*, p.Arg221Cys. Mutant ADAMTSL2 formed anomalous disulfide-bonded dimers and did not enhance microfibril biogenesis. MLS skin fibroblasts strongly expressed smooth-muscle alpha-actin, and were highly contractile, suggesting a myofibroblast transition.

Conclusions: We propose that ADAMTSL2 may influence TGF β through its interactions with fibrillin-1, and as previously shown, latent TGF β -binding protein 1. The molecular effect of the MLS mutation on ADAMTSL2 is formation of disulfide-bonded dimers and loss of ability to accelerate microfibril biogenesis. Fibrosis in MLS results from myofibroblast transition of dermal fibroblasts.

POSTNATAL FUNCTION OF TRANSFORMING GROWTH FACTOR BETA2 IN CARDIOVASCULAR DISEASE

Azhar, Mohamad, Ph.D., BIO5 Institute, University of Arizona, Tucson, AZ, USA

Nusayr, Eyad, B.S., BIO5 Institute, University of Arizona, Tucson, AZ, USA

Haskett, Darren, B.S., Department of Aerospace & Mechanical Engineering, University of Arizona, Tucson, AZ, USA

Utzinger, Urs, Ph.D., Dept of Biomedical Engineering, University of Arizona, Tucson, AZ, USA

Vande Geest, Jonathan, Ph.D., Department of Aerospace & Mechanical Engineering, University of Arizona, Tucson, AZ, USA

Objectives: Although Transforming Growth factor beta2 (TGF β 2beta2) is required for cardiovascular development, the postnatal function of TGFbeta2 in cardiovascular system remains unknown.

Methods: Characterization of various genetic mouse combinations using in vivo cardiovascular imaging and closed-chest cardiac function (pressure-volume loop) analysis, histological and morphometric examination, and experimental AngII-induction of cardiovascular disease in mice. Microbiaxial optomechanical analysis of unfixed mouse aortas simultaneously determined both the macroscopic biomechanical response (pressure and diameter) and microscopic (matrix fiber content, fiber length, interfiber space, average fiber orientation) properties of the aortic walls.

Results: Echocardiographic examination and closed-chest cardiac function analysis indicated cardiomyopathy and cardiac dysfunction in *Tgfb2* heterozygous animals. Short-term AngII-infusion of *Tgfb2* heterozygous animals worsened the cardiomyopathy and drastically reduced the cardiac function as compared to the saline-infused *Tgfb2* heterozygous mice. More importantly, AngII-infused but not saline-infused mice were suddenly died during the course of their cardiac function examination (invasive procedure requires anesthesia) indicating the inability of AngII-infused *Tgfb2* heterozygous animals to handle any further stress. Interestingly, echocardiographic examination revealed that AngII-infusion also selectively caused significant aortic aneurysm in *Tgfb2* heterozygous mice as compared to saline-infused *Tgfb2* heterozygous animals. Finally, microbiaxial optomechanical analysis indicated significant worsening of both microstructural and biomechanical responses in the *Fibrillin-1/Tgfb2* double heterozygous animals as compared to *Tgfb2* or *Fibrillin-1* heterozygous mutant animals.

Conclusions: Postnatal reduction of TGFbeta2 predisposes mice to a progressive but asymptomatic cardiovascular disease. Further insult such as AngII-infusion or *Fibrillin-1* mutation leads to a symptomatic or active cardiovascular disease in *Tgfb2* heterozygous animals.

*Selected for oral presentation from poster submissions.

CHARACTERIZATION AND TREATMENT OF OSTEOPENIA IN MICE WITH SEVERE MARFAN SYNDROME

Luca Carta, Harikiran Nistala, Sui Lee-Arteaga, Jason Cook, Silvia Smaldone, Aaron Rifkin, and Francesco Ramirez

Introduction:

Reduced bone mineral density (osteopenia) is a poorly characterized manifestation of pediatric and adult patients afflicted with Marfan syndrome (MFS). However, previous studies have not clarified the role of fibrillin-1 mutations in bone loss.

Objective:

The present study investigated the impact of fibrillin-1 deficiency on bone homeostasis in a mouse model of progressively severe MFS (*Fbn1*^{mgR/mgR} mice), in addition to comparing the efficacy of losartan treatment on bone remodeling and aortic disease in these mutant mice.

Method:

In vivo analyses revealed that *Fbn1*^{mgR/mgR} mice are osteopenic and display a greater response to experimentally induced osteolysis. Cell culture experiments correlated these in vivo findings with enhanced osteoblast-supported osteoclastogenesis, which was largely attributed to TGFβ-induced up-regulation of RANKL expression. Based on these observations, we compared the effects of losartan and alendronate on aortic wall degeneration and loss of bone mass in *Fbn1*^{mgR/mgR} mice, as the former drug improves TGFβ-driven aortic aneurysm in *Fbn1*^{C1039G/+} mice and the latter one restricts osteoclast activity. Accordingly, bone and aortic tissue were evaluated in wild-type and *Fbn1*^{mgR/mgR} mice that were treated postnatally with losartan for 8 or 16 weeks or with alendronate for 8 weeks. Losartan treatment mitigated aortic aneurysm progression but not bone loss. Conversely, a significant improvement of bone quality was observed following alendronate treatment without any beneficial effect on vascular disease.

Conclusions:

Increased bone resorption is the main contributor to osteopenia in MFS mice and is largely accounted for by TGFβ-dependent stimulation of osteoblast-driven osteoclastogenesis. Losartan treatment is ineffective in treating bone loss.

*Selected for oral presentation from poster submissions.

CONDITIONAL INACTIVATION OF FIBRILLIN-1 IN AORTIC TISSUE COMPARTMENTS

Jason R. Cook, Sui Lee-Arteaga, Luca Carta, Harikiran Nistala, Maria del Solar and Francesco Ramirez.

Department of Pharmacology and Systems Therapeutics at the Mount Sinai School of Medicine, New York, NY 10021

Introduction: Dissecting thoracic aortic aneurysm (TAA) in Marfan syndrome (MFS) is associated with elevated TGF β activity secondary to structural or quantitative defects in fibrillin-1 (*FBN1*). Perinatal lethality of mice with germ line inactivation of *Fbn1* has underscored the importance of fibrillin microfibrils in aortic growth and homeostasis without, however, identifying the main tissue compartment contributing to TAA.

Objective: Determine the contribution of fibrillin-1 production by vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) in MFS vascular pathology.

Method: The aortic wall is composed of the intima (ECs), media (VSMCs) and adventitial (fibroblasts) layers. A new line of mutant mice that harbor a conditional allele of *Fbn1* was therefore created to allow the selective study of fibrillin-1 in unique tissues. Female mice with the conditional allele (*Fbn1* ^{Δ neo}) were crossed with male transgenic mice harboring a germ line null allele (*Fbn1*^{mgN}) and expressed Cre specifically in either the forming VSMC (*Sm22 α -Cre*) or ECs (*Cdh5-Cre*). The germ line null allele was paired with the conditional allele to increase the probability of tissue specific Cre excision. Tissue specific Cre excision was monitored by crossing the tissue specific mice with RosaLacZ reporter mice.

Results: Inactivation of fibrillin-1 in VSMCs (*Fbn1* ^{Δ neo/mgN}; *Sm22 α -Cre*⁺) replicates the germ line null phenotype with elastic fiber fragmentation, aortic aneurysm, and death due to dissection by P14.

Conclusions: Lack of fibrillin-1 production by VSMCs in the media of the ascending aorta is necessary and sufficient for the development of TAA.

*Selected for oral presentation from poster submissions.

S. 35**SMOOTH MUSCLE CONTRACTILE FUNCTION AND THORACIC AORTIC DISEASE**

Dianna M. Milewicz¹, Dong-chuan Guo¹, Ellen Regalado¹, Christina Papke¹, Jiumei Cao¹, Limin Gong¹, Callie Fogler¹, Hazim Safi², Steve Scherer³, Anthony L. Estrera²

¹Departments of Internal Medicine and ²Cardiothoracic and Vascular Surgery, University of Texas Health Science Center at Houston, Houston, Texas 77030, USA. ³Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas 77030, USA.

Thoracic aortic aneurysms leading to type A dissections (TAAD) can occur in isolation or in association with genetic syndromes. TAAD can also be inherited in an autosomal dominant manner in the absence of syndromic features and associated with decreased penetrance and variable expression, termed familial TAAD. Familial TAAD exhibits significant clinical and genetic heterogeneity. The first genes identified to cause FTAAD were *FBN1*, *TGFBR2*, and *TGFBR1*. The identification and characterization of these genes and mouse models harboring mutations in these genes has suggested that altered TGF- β signaling plays a role in pathogenesis of FTAAD. The more recent discovery that mutations in the vascular smooth muscle cell (SMC) specific β -myosin (*MYH11*) and α -actin (*ACTA2*) can also cause FTAAD has focused attention on the importance of the maintenance of SMC contractile function in preserving aortic structure and preventing TAAD. The identification of loss of function mutations in the kinase that controls SMC contraction, myosin light chain kinase (*MYLK*), in familial TAAD further supports that disruption of SMC contractile function can lead to FTAAD. Analysis of contractile proteins in *TGFBR2* mutant SMCs and myofibroblasts provides a possible link between mutations in TGF-beta receptors and decreased contractile function.

S. 36**EPIGENETIC CONTROL OF AORTIC SMOOTH MUSCLE CELLS IN MARFAN AND NON-MARFAN THORACIC ANEURYSMS**

GOMEZ Delphine, PhD, INSERM U698, Paris, France; KESSLER Ketty, INSERM U698, Paris, France; JONDEAU Guillaume, MD, INSERM U698, Paris, France; MICHEL Jean-Baptiste, MD, PhD, INSERM U698, Paris, France; VRANCKX Roger, PhD, INSERM U698, Paris, France.

Thoracic aortic aneurysm (TAA) development affects both smooth muscle cell (SMC) survival and vascular extracellular matrix integrity. These features are present in all types of TAA: i) monogenic diseases (Marfan syndrome), ii) aneurysms associated with bicuspid aortic valve (BAV) or iii) degenerative forms. All types of TAA exhibit a perturbation of the TGF- β signalling characterized by the activation of Smad2 (TGF- β intracellular mediator).

We investigated the expression of the transcription factor Smad2 in Marfan syndrome (*FBN1*: n=15, *TGF β R2*: n=3), non-Marfan (n=15) aneurysms and control (n=10), using tissue extracts and cultured SMCs. A Smad2 overexpression (mRNA and protein) is observed in aneurysmal tissue and SMCs. Interestingly, this overexpression is SMC-specific and heritable over cell divisions in culture. Overexpression is limited to particular Smad2 mRNA variants suggesting a specific regulation at the promoter level. Cell-specificity and heritability of this overexpression strongly suggest the implication of epigenetic mechanisms. Modifications of the histone code are observed with increase in H3 methylation and acetylation, in aneurysmal tissue compared with control. Same results are obtained in Marfan and non-Marfan samples. Smad2 overexpression is dependant of H3 Histone AcetylTransferases (HAT) activity. Two of these, GCN5 and PCAF, directly participate to hyperacetylation into a co-activator complex including the transcription factor p53. By CHIP, we prove the recruitment of this complex on Smad2 promoter.

In conclusion, we have demonstrated that the Smad2 overexpression is controlled by an epigenetic mechanism, in Marfan and non-Marfan TAA.

S. 37**CANONICAL AND NONCANONICAL TGF β SIGNALING IN MARFAN SYNDROME**

Doyle, Jefferson, MBBChir;¹ Holm, Tammy, MDPHd;¹ Pardo-Habashi, Jennifer, MD;¹
Bedja, Djahida, PhD;² Dietz, Hal, MD.^{1,3}

¹Institute of Genetic Medicine; ²Department of Molecular and Comparative Pathobiology, Johns Hopkins School of Medicine, Baltimore, MD, USA; ³Howard Hughes Medical Institute, Baltimore, MD, USA.

Objective: Excess TGF β signaling drives aortic aneurysm in Marfan syndrome (MFS), while its attenuation by TGF β neutralizing antibody (TGF β NAb) or losartan prevents aneurysm progression. TGF β signals through both canonical (Smad2/3) and noncanonical (MAPK: JNK, ERK and p38) cascades. We determined the relative contributions of these cascades in the aortas of MFS mice.

Methods: Western blot analysis was performed on the ascending aortas of placebo-, TGF β NAb- and losartan-treated C1039G/+ mice, and wild-type (WT) littermates. Haploinsufficiency for Smad4 was introduced on the C1039G/+ background to reduce Smad-dependent signaling. Abrogation of MAPK signaling was achieved using selective pharmacological inhibitors.

Results: Western blot analysis showed pSmad2, pERK1/2 and pMEK1 to be increased in the ascending aortas of C1039G/+ mice compared to WT littermates, while pJNK1 and pp38 were no different. pERK1/2 activation was reduced to WT levels in both TGF β NAb- and losartan-treated C1039G/+ mice, while treatment with RDEA-119, a selective inhibitor of ERK1/2 activation, reduced aortic root growth in C1039G/+ mice to WT rates. Unexpectedly, S4+/-:C1039G/+ mice showed synthetic lethality due to aortic rupture. Both C1039G/+ and S4+/-:C1039G/+ mice show marked and equivalent activation of pSmad2 and pERK1/2, while S4+/-:C1039G/+ mice uniquely showed activation of JNK, and a JNK antagonist fully rescued the synthetic lethality. Furthermore, JNK antagonism reduced aortic root growth in C1039G/+ mice, despite the fact that JNK activation was not increased in these mice.

Conclusion: These data define MAPK signaling as the predominant driver of aortic aneurysm progression in MFS, with ERK and JNK being additive and interchangeable in this process.

MOLECULAR AND STRUCTURAL CHARACTERISATION OF THE INTERACTIONS BETWEEN THE N- AND C-TERMINAL REGIONS OF HUMAN FIBRILLIN-1

Yadin David, Robertson Ian, Stoddart David, Jensen Sacha, Redfield Christina and Handford Penny

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU UK

Objectives: Interactions between the N- and C-terminal regions of fibrillins are known to be important for the assembly of the 10-12 nm microfibrils in the extracellular matrix. Previous work has shown that recombinant N- and C-terminal fragments of fibrillins interact *in vitro* and the C-terminal interaction site localised to cbEGF41-43. We have produced recombinant human fibrillin-1 fragments using our established *Escherichia coli* expression, purification and refolding system to further identify the molecular basis for this interaction.

Methods: Purified tagged-proteins were examined by pull-down assays to define the limited fragments which contain the sites of interaction. NMR spectroscopy was used to investigate structural features of the proteins.

Results: Pull-down assays showed an interaction between fragments comprising the fibrillin unique N-terminal (FUN) and first three epidermal growth factor (EGF)-like domains in the N-terminal region (NE3) and the last three calcium binding EGF-like domains in the C-terminal region (cbEGF41-43). Using NMR spectroscopy, the structure and dynamics of NE3 were characterised, including the fold of the FUN domain. We have identified important structural motifs and flexible regions in NE3 that may be relevant to its interaction with the C-terminus and other molecules. The packing interactions between domains are independent of calcium ions, unlike in many other domain pairs in fibrillin-1. On completion of the final, refined structure of NE3, the structural basis of its interaction with cbEGF41-43 will be investigated.

Conclusion: These data identify the solution structure of the N-terminal domain which extends our knowledge of the structure of fibrillin-1 and provides insight into the molecular pathology of MFS.

S. 39**INVESTIGATION OF THE SIGNALING PATHWAY ALTERED IN A MOUSE MODEL OF ASCENDING AORTIC ANEURYSMS**

Huang Jeanine, Ph.D. and Yanagisawa Hiromi, M.D., Ph.D.
Department of Molecular Biology
University of Texas Southwestern Medical Center, Dallas, Texas, U.S.A.

Objectives: Fibulin-4 is a component of microfibrils essential for the formation of elastic fibers in vivo. We recently reported that a loss of fibulin-4 in vascular smooth muscle cells (SMCs) resulted in development of ascending aortic aneurysms in mice. These mice (termed *Fbln4*^{SMKO}) exhibited defects in terminal differentiation of SMCs and focal proliferative changes of the aortic wall with concomitant upregulation of phosphorylated (p-) ERK1/2. To better understand the underlying mechanism of aneurysm formation, we investigated signaling pathways involved in pathological changes of SMCs and evaluated the efficacy of angiotensin II type I receptor blockade (ARB) on the treatment of aortic aneurysms.

Methods: Aortas were harvested from *Fbln4*^{SMKO} mice treated with losartan (0.6 g/l in drinking water ad libitum) or vehicle from mid-gestation to 3-months old and compared with that of control mice. The level of components of MAPK pathways involved in proliferation of SMCs and the expression of SMC marker genes were examined by Western blot and qPCR. In addition, pSmad2/3 and pSmad1/5/8, both of which are shown to be upregulated in Marfan mouse models, were examined before and after the treatment with losartan.

Results and Conclusions: Losartan prevented formation of ascending aortic aneurysm as judged by a marked reduction of internal perimeter of the ascending aorta, whereas no difference was observed in the descending aorta with or without losartan. Further, we found that p-ERK1/2 levels most closely correlated with the severity of aortic aneurysms. These data suggest a potential usage of ARB in the treatment of congenital ascending aortic aneurysms in human.

EXTRACELLULAR REGULATION OF BMP SIGNALING BY FIBRILLIN MICROFIBRILS

Gerhard Sengle¹, Valerie M. Carlberg¹, Noe L. Charbonneau², Sara F. Tufa², Douglas R. Keene², and Lynn Y. Sakai^{1,2}

¹Department of Biochemistry and Molecular Biology, Oregon Health & Science University

²Shriners Hospital for Children, Portland OR

Genetic evidence in humans and mice as well as biochemical data implicate the microfibrillar network in the regulation of TGF- β and BMPs in the extracellular space. We have shown that members of the TGF- β family (BMP-4, -5, -7, -10 and GDF-5) form growth factor complexes in association with their prodomains, which target the complexes to the extracellular microfibril network. We have also shown that, unlike TGF- β , the BMP-7 prodomain/growth factor complex is not latent. Our goal is to test whether interaction with fibrillin confers latency and is required in vivo for appropriate regulation of BMP signaling.

Methods: Both in vitro and in vivo approaches were used. Fbn1 mutant mice (GT-8) were analyzed.

Results and conclusions: Biochemical data indicated that fibrillin-1 can confer latency to the BMP-7 prodomain/growth complex. In vivo analyses showed that C-terminal truncation of fibrillin-1 in mice leads to increased fragmentation of elastic fibers in skin and aorta in postnatal life accompanied with increased BMP signaling in these tissues. Together, these data show for the first time that fibrillin is required to limit BMP activity. We hypothesize that this control over BMP activity is exerted through proper targeting of BMP complexes and also through sequestration of BMP activity by fibrillin. BMP signaling may be activated by proteases as cells respond to their mutant microfibril matrix environment.

REGULATORY ROLE OF FIBRILLIN IN BONE HOMEOSTASIS

Komarova Svetlana V.,¹ Tiedemann Kerstin,¹ Reinhardt Dieter P.^{1,2}

McGill University, ¹Faculty of Dentistry and ²Faculty of Medicine, Montreal, Qc, Canada

Osteopenia and skeletal deformations in Marfan syndrome (MFS) and congenital contractural arachnodactyly are consistent with disrupted bone homeostasis. Changes in bone structure and composition are regulated by the activities of osteoblasts and osteoclasts. Fibrillin-1 and -2 are expressed throughout the skeleton, including long bones, ribs, vertebral bodies and cartilage. Here, we address the role of fibrillins in osteoblast and osteoclast differentiation and function.

Treatment of mouse bone marrow cells with ascorbic acid results in induction of osteoblast markers, alkaline phosphatase, osteopontin and Runx2. We have found that expression of fibrillin-1 is up-regulated throughout osteoblast differentiation, whereas fibrillin-2 is very strongly and transiently induced during the first week of culture. Fibrillin-1, but not fibrillin-2, was assembled into microfibrils in the extracellular matrix of the differentiated osteoblasts. Using established osteoclast differentiation and activity models with primary bone marrow-derived precursors and monocytic RAW 264.7 cells, we found that the soluble N-terminal (but not the C-terminal) half of fibrillin-1 inhibits osteoclastogenesis. In contrast, when recombinant fibrillin-1 fragments were coated on calcium phosphate plates, they did not affect osteoclast formation or resorptive activity. Using a panel of recombinant sub-fragments of the fibrillin-1 N-terminal half, we localized the inhibitory activity of fibrillin-1 on osteoclast differentiation to the N-terminal region of the protein. The same region acted as a specific inhibitor of cathepsin K and matrix metalloproteinase 9 expression by differentiated osteoclasts. In contrast, fibrillin-1 or its fragments did not affect differentiation of osteoblasts. We have found that osteoclast-specific protease cathepsin K is capable of cleaving fibrillin-1 *in vitro*. The observed cleavage sites are located at domain boundaries resulting in liberation of N-terminal fragments very similar to the N-terminal fibrillin-1 fragment exerting the inhibitory activity on osteoclast differentiation. Furthermore, fibrillin-1 fragments with neonatal MFS mutations were more susceptible for cleavage by cathepsin K than fragments with classical mutations. We obtained further data, indicating that the N-terminal region of fibrillin-1 exerts its anti-osteoclastogenic activity through sequestering "Receptor activator of nuclear factor kappaB ligand" (RANKL) and by inhibiting apoptosis of osteoclast precursors. These data demonstrate that fibrillin-1 can directly inhibit osteoclast formation and suggest that MFS mutations affect osteoclast-inhibiting properties of fibrillin-1 leading to osteoclast activation and osteopenia.

*Selected for oral presentation from poster submissions.

S. 42**INTRODUCTION TO HEART VALVE DEVELOPMENT**Katherine E. Yutzey

The Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229

Cardiovascular manifestations of Marfan syndrome include anomalies in the aortic and mitral valves of the heart. In recent years, significant advances have been made in the definition of regulatory pathways and morphogenetic mechanisms that control normal and abnormal cardiac valve development. Here, we introduce the cellular and molecular mechanisms underlying the early development of valve progenitors and the establishment of normal valve structure and function. Valve formation during embryonic development is initiated with the formation of endocardial cushions in the atrioventricular canal and outflow tract of the primitive heart. The endocardial cushions then elongate and differentiate to form individual leaflets of the atrioventricular and semilunar valves. The mature heart valves are made up of highly organized stratified extracellular matrix with elastin-, proteoglycan- and collagen-rich layers that confer distinct biomechanical properties to the leaflets and supporting structures. Loss of fibrillin-1 or altered TGF-beta signaling, associated with Marfan syndrome, affect the overall structure and function of the aortic and mitral valves. Regulatory hierarchies consisting of intersecting signaling pathways, transcription factors, and downstream structural genes are conserved during vertebrate valvulogenesis. Therefore animal model systems provide valuable insights into valve development and disease mechanisms. Overall, there is increasing evidence that the regulatory mechanisms governing normal heart valve development also contribute to human valve pathology, including manifestations of Marfan syndrome.

S. 44**DEVELOPMENTAL MECHANISMS OF ADULT HEART VALVE DISEASES (VHD)**

Markwald, Roger PhD and Norris, Russell PhD, Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston SC. (USA)

Introduction: VHD is a common medical condition that increases with age and can be associated with Marfan syndrome. Despite improvements in treatment options, mortality and morbidity rates remain high. New strategies are needed which include stimulating endogenous repair pathways. Valvulogenesis is a complex process involving temporal “waves” of progenitor cells derived, in part, by the process of endocardium-mesenchyme transformation (EMT) to form prevalvular “cushions”. Embryonic cushions bear resemblance to myxomatous valves associated with degenerative forms of VHD. The genes regulating post-EMT processes whereby undifferentiated, myxomatous-like cushions are remodeled and compacted into normal, mature, collagenous leaflets are less understood. Based on expression studies, knockout mice and genetic screening, periostin (PN) and filamin A (FLNA) have been implicated as candidates for regulating remodeling during early postnatal life.

Methods: 3D collagen gel compaction assays employing null mice progenitor cells, silencing vectors and phosphokinase assays were used to determine the roles of PN and FLNA in valve maturation.

Results: Periostin through integrin signaling pathways promoted phosphorylation of FLNA, fibroblastic differentiation, collagen fibrillogenesis and compaction. Progenitor cells lacking periostin or silenced for FLNA or an FLNA binding protein, transglutaminase 2, had significantly reduced potential for collagen compaction.

Conclusions: These findings suggest that developmental mutations in periostin, a secreted protein or transducers of PN signaling (e.g. FLNA) can “generate”, over time, dystrophic adult valves *Supported by HL 33756 and a Leducq Foundation Transatlantic Network Grant (MITRAL).*

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DEVELOPMENTAL UNDERPINNINGS OF ACQUIRED AORTIC ANEURYSM IN MARFAN SYNDROMEMark E. Lindsay^{1,2} Ibrahim J. Domian⁴, Kenneth R. Chien⁴, and Harry C. Dietz^{1,3}

1) Institute of Genetic Medicine; 2) Division of Pediatric Cardiology, Department of Pediatrics; 3) HHMI, Johns Hopkins Hospital, Baltimore, MD; 4) Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA

Marfan Syndrome (MFS), caused by deficiency of fibrillin-1, shows early death due to aortic aneurysm. Increased TGF β signaling contributes to aneurysm progression. Anatomically, predisposition to aortic enlargement in MFS occurs primarily at the base of the aorta, at the level of the sinuses of Valsalva. This anatomic segment of the aorta is unique in that it has a cardiogenic origin, deriving in large part from cells of the Second Heart Field (SHF). To gain further insight into the basis for this predisposition, we have undertaken detailed phenotypic analysis of *Fbn1*^{C1039G} homozygote mice that show profound fibrillin-1 deficiency. These animals demonstrate failed morphogenesis in structures populated by the SHF including right ventricular cardiomyopathy, aortic-mitral valve discontinuity, and proximal great arterial hypercellularity with longitudinal overgrowth. To investigate the performance of SHF cells in the context of severe fibrillin-1 deficiency, we have generated ES cell lines harboring fluorescent reporter alleles that allow for the specific isolation of the SHF population. *Fbn1*^{C1039G} homozygote cell lines show a reproducible expansion of the SHF progenitor population during *in vitro* differentiation assays versus wild type cells. Consistent with our *in vitro* data, lineage tracing analyses using newborn *Fbn1*^{C1039G} heterozygous and homozygous animals demonstrate *in vivo* expansion of this cell lineage in the proximal aorta in terms of both cell number and transmural distribution. We are currently using our ES based assay as a discovery platform to distinguish the causative pathway for pathologic proliferative signaling within the developing SHF in MFS. These data could have direct therapeutic implications to pharmacologic management of severe MFS. Additionally they may help explain the unique anatomic susceptibility to proximal aortic aneurysm in MFS.

S. 47

CALCIUM CHANNEL BLOCKERS EXACERBATE AORTIC DISEASE AND CAUSE PREMATURE LETHALITY IN MARFAN SYNDROME

Doyle, Jefferson, MBBChir;¹ Lindsay, Mark, MD/PhD;¹ Pardo-Habashi, Jennifer, MD;¹ Bedja, Djahida, PhD;² Dietz, Hal, MD.^{1,3}

¹Institute of Genetic Medicine; ²Department of Molecular and Comparative Pathobiology, Johns Hopkins School of Medicine, Baltimore, MD, USA; ³Howard Hughes Medical Institute, Baltimore, MD, USA.

Objective: We previously showed that excessive TGF β -dependent activation of ERK signaling drives aneurysm progression in MFS. TGF β neutralizing antibody, selective inhibitors of ERK activation, or the AT1R blocker losartan abrogate aneurysm growth in MFS (C1039G/+) mice by antagonizing TGF β -dependent ERK activation. Pending results of clinical trials of losartan, calcium channel blockers (CCBs, e.g. amlodipine) are currently used as second-line therapy for MFS in patients intolerant of beta-blockers.

Methods: Wild-type (WT) and C1039G/+ mice were treated with amlodipine or placebo from 2 months of age. Echocardiography was performed at 2 months, and every 2 months thereafter.

Results: Placebo-treated C1039G/+ mice had larger aortic roots at 4 months and more rapid growth from 2 to 4 months, compared to WT littermates. Amlodipine-treated C1039G/+ mice showed no improvement in either parameter, compared to placebo-treated mice. There was no difference in more distal ascending aortic (AscAo) diameter at 4 months, or growth from 2 to 4 months, between C1039G/+ and WT mice. In contrast, both AscAo size and growth were increased in amlodipine-treated WT and C1039G/+ mice, with a greatly amplified effect in C1039G/+ mice. Amlodipine-treated C1039G/+ mice uniquely showed early mortality, with 50% death from aortic dissection within 3 months of treatment. Accelerated AscAo growth and dissection correlated with increased ERK activation. In a retrospective analysis, 2 of 6 MFS patients treated with CCBs showed aortic dissection in childhood, one of whom dissected at unusually small aortic dimensions.

S. 48

DOXYCYCLINE AND LOSARTAN COMBINATION TREATMENT FURTHER DELAYS ANEURYSM RUPTURE IN A MOUSE MODEL OF MARFAN SYNDROME COMPARED TO SINGLE DRUG TREATMENT

B. Timothy Baxter, M.D., University of Nebraska Medical Center, Omaha, NE, USA

Objectives: Thoracic aneurysms are the main cardiovascular complication of Marfan Syndrome (MFS) resulting in premature death. Our previous study showed that doxycycline, a nonspecific MMP inhibitor, significantly delays aneurysm rupture in a mouse model of MFS, mgR/mgR. Several studies showed that losartan, a angiotensin II type 1 receptor (AT1) blocker prevented aortic aneurysm in a mouse model of MFS, *Fbn1*^{C1039G/+}. The objective of the study was to determine whether doxycycline and losartan can work synergistically to attenuate matrix degradation and aortic diameter growth and prolong the lifespan of mgR/mgR mice.

Methods: The study used mgR/mgR mice that died spontaneously from rupture of the thoracic aorta between 2-4 months of age. Mice were given doxycycline(0.5g/L), or losartan(0.6g/L), or combined doxycycline(0.5g/L) and losartan(0.6g/L) in the drinking water beginning at postnatal day 1. Mice were divided into 2 groups. One group of mice was followed until death or for 7 months to determine lifespan and monitor aortic diameter growth rate with echocardiograms. In the second group of mice, the ascending thoracic aortas were collected for histological analysis and zymography to examine MMP-2 and MMP-9 levels at 8 weeks.

Results: both doxycycline and losartan prolong the lifespan of mgR/mgR mice compared to untreated mice (73.1 ± 4.57 days) (n=30). More importantly, doxycycline and losartan combination treatment significantly reduced aortic diameter growth rate and prolong the survival of mgR/mgR mice (208.9 ± 7.43 days) (n=9) compared to doxycycline (136.4 ± 19.96 days) (n=30) or losartan (130.6 ± 17.3 days) (n=9) treatment alone. Combination treatment had more protective effect on elastic fiber degradation and further reduction of MMP-2 and MMP-9 expression compared to doxycycline or losartan alone treated mice.

Conclusions: this study demonstrates that combination treatment of doxycycline and losartan provide better therapeutic strategy for MFS and has the potential to prevent the major life-threatening manifestation of this disorder.

COMPARISON OF PRAVASTATIN, LOSARTAN AND DOXYCYCLINE FOR ATTENUATION OF AORTIC ROOT DILATION IN A MURINE MODEL OF MARFAN SYNDROME

McLoughlin Darren, MRCS, Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland
 McGuinness Jonathan, PhD, MRCS, Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Byrne John Stephen, MD, MRCS, Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Terzo Elosia, MVB, Department of Veterinary Radiology, University College Dublin, Dublin, Ireland

Huuskonen Vehilmiine, MVB, Department of Veterinary Radiology, University College Dublin, Dublin, Ireland

McAllister Hester, MRCVS, Department of Veterinary Radiology, University College Dublin, Dublin, Ireland

Black Alex, MSc, Department of Anatomy, National University of Ireland Galway, Galway, Ireland

Kearney Sinead, BSc, Department of Anatomy, National University of Ireland Galway, Galway, Ireland

Hill Arnold D.K., MCh, FRCS, Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Redmond J.Mark, MD, FRCS, Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Background:

Statins inhibit biosynthesis of isoprenoids, essential for prenylation of proteins such as metalloproteinases. We hypothesised that pravastatin could reduce the transportation and secretion of metalloproteinases to prevent aneurysm formation. Using a murine model of Marfan Syndrome, we compared the effect of treatment and mechanism of action with Pravastatin, Losartan, and Doxycycline on aortic root dilation, combined with ultrastructural analysis of vascular smooth muscle (VSMC) cells using electron microscopy.

Methods:

The effects of treatment in a mouse model of MFS with Pravastatin 0.5g/L, Losartan 0.6 g/L, and Doxycycline 0.24g/L on the end-points of aortic root diameter, and aortic pathology (using both light and electron microscopy) were assessed using untreated Marfan mice and normal mice as controls.

Results:

The aortic root diameter of untreated Marfan mice are significantly increased in comparison to normal mice (1.61±0.012 mm vs. 2.52±0.04 mm, P<0.05). Losartan produced a significant reduction in aortic root dilation (2.21±0.04 mm, P<0.05), as did Pravastatin (2.20±0.03 mm, P<0.05) compared to untreated marfan mice. Doxycycline treatment had no beneficial effect at 8 months (2.43±0.04 mm, P=0.1). Ultrastructural analysis of VSMC showed greater reduction of rough endoplasmic reticulum with Losartan compared to Pravastatin, while cisternal volume within these cells was equally reduced with both Pravastatin and Losartan. This suggests that Pravastatin acts by inhibiting excessive TGF-beta signalling mechanism at a post-transcriptional level.

Conclusions:

We have now shown that Pravastatin is equally as effective as Losartan in reducing aortic root dilatation. Our findings suggest that marfan aortic pathology may be targeted through different pathways.

*Selected for oral presentation from poster submissions.

S. 50

ANGIOTENSIN II INFUSION PROMOTES THE FORMATION OF ASCENDING AORTIC ANEURYSMS

Alan Daugherty, Alan Ph.D., D.Sc. Cassis, Lisa A, Ph.D. Rateri, Debra L, B.Sc.

Saha Cardiovascular Research Center and Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY, USA

Objectives: Human studies and mouse models have provided evidence for AngII-based mechanisms as an underlying cause of ascending aorta aneurysms (AAs). In agreement with this premise, we observed recently that AngII infusion leads to vascular pathology that is highly localized to this region in both hyper- and normolipidemic mice.

Methods: Infusion of pressor and non pressor rates of AngII.

Results: The pathology in ascending aortas is characterized by progressive luminal dilation. The media in this region is markedly thickened with expansion of the distance between elastin layers that increases from the lumen to the adventitial aspect of the media. The characteristics of this aortic pathology are distinct from the aneurysms formed by AngII infusion in the abdominal aorta. Whole body AT1a receptor deficiency ablated AngII-induced ascending AAs. This information was used to define the cell type involved in development of ascending AAs. To determine the role of AT1a receptors on leukocytes, LDL receptor $-/-$ x AT1a receptor $-/-$ mice were irradiated and repopulated with bone marrow-derived cells isolated from either AT1a receptor $+/+$ or $-/-$ mice. Deficiency of AT1a receptors in bone marrow-derived cells had no effect on AngII-induced ascending AAs. To determine the role of AT1a receptors on vascular wall cells, we developed AT1a receptor floxed mice with depletion on smooth muscle or endothelial cells using Cre driven by SM22 or Tek, respectively. Surprisingly, AT1a receptor deletion in smooth muscle cells had no effect on ascending AAs. In contrast, endothelial-specific depletion attenuated this pathology.

Conclusion: An undefined endothelial mechanism contributes to ascending AAs.

S. 51**VASCULAR SMOOTH MUSCLE EXPRESSION OF S100A12 INDUCES AORTIC ANEURYSM FORMATION**

¹Hofmann Bowman, Marion, M.D, Ph.D., ¹Wilk, Jeannine, M.A., ¹Heydemann, Alhlke, Ph.D., ¹Kim, Gene, M.D., ¹Rehman, Jalees, M.D, ¹Lodato, Joseph. M.D., ²Raman, J., M.D., Ph.D., ¹McNally, Elizabeth M. M.D., Ph.D.

¹Department of Medicine, Section of Cardiology, The University of Chicago

²Department of Cardiac Surgery, The University of Chicago

Objectives: S100A12 is a small calcium binding protein found in humans that is a signal transduction ligand of the Receptor for Advance Glycation Endproducts (RAGE). RAGE has been extensively implicated in inflammatory states such as atherosclerosis, but the role of S100A12 as a ligand is less clear. Mice do not have an S100A12 ortholog. We wished to determine if expression of human S100A12 in murine aortic vascular smooth muscle was sufficient to induce aneurysm formation in vivo.

Methods: Mice were engineered to express human S100A12 in smooth muscle under control under the control of the SM22 α . We also examined human aortic samples extracted at the time of aneurysm report for S100A12 expression.

Results: Transgenic mice displayed pathologic vascular remodeling with aberrant thickening of the aortic media, disarray of elastic fibers as well as increased collagen deposition. Beginning at 10 weeks of age, mice displayed progressive dilatation of the aorta. In primary aortic smooth muscle cells, we found that S100A12-mediated vascular remodeling was associated with increased IL-6 production, activation of TGF β pathways and increased metabolic activity with enhanced oxidative stress. We also found enhanced S100A12 expression in human aortic aneurysmal disease.

Conclusions: Increased S100A12 expression in vascular smooth muscle can directly activate several pathogenic pathways and modulate oxidative stress, inflammation and vascular remodeling in vivo and may serve as a target for future cardiovascular therapies

INCREASED T-HELPER 1 CELL RESPONSE IN MARFAN SYNDROME IS MODIFIED BY LOSARTAN

Radonic, Teodora¹, de Witte, Piet², Lutter Rene³, Baars Marieke, H.⁴, Hilhorst-Hofstee Yvonne⁵, van Tintelen Peter J.⁶, Hamel Ben C.J.⁷, Mulder Barbara J.M.⁹, Groenink Maarten¹⁰, Zwiderman Aeilko H.⁸

¹ MD, Dept. of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center Amsterdam, Netherlands

² MD, Dept. of Cardiology AMC Amsterdam and Interuniversity Cardiology Institute of the Netherlands

³ PhD, Dept. of Pulmonology and Experimental Immunology, Academic Medical Center Amsterdam, Netherlands

⁴ MD PhD, Dept. of Clinical Genetics, Academic Medical Center Amsterdam, Netherlands

⁵ MD, Dept. of Human Genetics, Leiden University Medical Center, Leiden, Netherlands

⁶ MD PhD, Dept. of Clinical Genetics, Groningen University Medical Center, Groningen, Netherlands

⁷ MD PhD, Dept. of Clinical Genetics, St. Radboud University Medical Center, Nijmegen, Netherlands

⁸ PhD, Dept. of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center Amsterdam, Netherlands

⁹ MD PhD, Dept. of Cardiology AMC Amsterdam and Interuniversity Cardiology Institute of the Netherlands

¹⁰ MD PhD, Dept. of Cardiology AMC Amsterdam and Interuniversity Cardiology Institute of the Netherlands

Objectives

We investigated gene expression in Marfan patients in order to define genes and pathways that change after losartan treatment and modify the aortic dilatation.

Methods

Punch skin biopsies were obtained in participants of the COMPARE trial before therapy (baseline), after 4 weeks and one year of losartan therapy. In 88 samples RNA was isolated and Whole Transcriptome Gene Expression (WTGE) was measured using Human Exon 1.0 ST Arrays (Affymetrix). Results were validated using rtPCR. Baseline WTGE measurements were correlated with aortic dilatation rate and aortic distensibility change over 12 years measured with MRI. Results were validated in another 13 patients. We measured levels of 48 cytokines in 160 blood samples of these patients.

Results

Analysis of gene expression after 4 weeks of losartan therapy revealed 20 differently expressed genes ($\Delta=0.54$, $p<10^{-7}$), 2 of which in the TGF- β pathway: CIDEA and ENG. Losartan therapy changed splicing of ACSM3 and ADCY6 ($\Delta=0.3$, $p<10^{-7}$).

When correlated with the aortic dilatation rate, baseline expression of 2 genes was significant: HLA-DRB5 and HLA-DRB1 ($r=0.46$, $r=0.42$; $p<10^{-7}$), suggestive of immune response involvement. Prevailing cytokine profile was Th1 T-cell response. Remarkably, losartan significantly lowered levels of Th1 chemokines CTACK and SCGF- β ($p<0.05$) and up-regulated levels of IL-9 and MIG ($p<0.05$).

Conclusion

MFS is associated with increased Th-1 immune response. As 4 weeks of losartan therapy attenuates TH1 chemokine levels this may lead to reduced Th-1 responses with time. The involvement of TGF- β in these pathways is currently being analyzed.

*Selected for oral presentation from poster submissions.

FATE OF THE DISTAL AORTA IN PATIENTS WITH MARFAN SYNDROME**Coselli, Joseph S., M.D.**

The Texas Heart Institute at St. Luke's Episcopal Hospital and the Division of Cardiothoracic Surgery,
Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, Texas, USA

Although the aortic root is the most common location for aortic aneurysms or dissection in patients with Marfan syndrome, these lesions may develop in the distal aorta, as well. Dissection of the distal aorta may be residual, remaining after a proximal repair of an acute DeBakey type I aortic dissection, or it may arise de novo. Aneurysms may develop over time in a previously dissected aortic segment or they may develop spontaneously in previously healthy aortic tissue. At present, uncomplicated distal aortic dissection is medically managed; operative repair is indicated only when symptoms or significant aortic aneurysmal expansion occurs. Meticulous imaging surveillance and strict control of blood pressure are standard in patients with Marfan syndrome and help to determine whether and when surgical intervention will be necessary. Currently, the size threshold for distal aortic repair is a 6-cm aortic diameter.

In our contemporary series of 463 thoracoabdominal aortic aneurysm (TAAA) repairs, 57 patients (12%) were suspected of having a connective tissue disorder, and 24 (5%) were confirmed as meeting the original Ghent criteria for Marfan syndrome. Of these 24 patients, 23 (96%) also had chronic aortic dissection; only 1 patient underwent TAAA repair because of a degenerative aortic aneurysm. Although the majority of repairs in this patient subset were extensive (ie, extent II), there were no operative deaths or cases of paraplegia. However, 1 patient had a stroke (4%), and 2 patients required renal dialysis at discharge (8%). Overall, TAAA outcomes tend to be better for patients with Marfan syndrome than for our typical TAAA patients, because the Marfan patients are generally much younger and healthier.

LONGTERM RESULTS AFTER AORTIC ROOT REPLACEMENT IN PATIENTS WITH MARFAN SYNDROME

Bernhardt, AMJ, MD¹, Treede, H, MD¹, Rybczynski, M, MD², Sheikzadeh, S, MD², Meinertz, T, MD², Kodolitsch, Y, MD², Reichenspurner, H, PhD¹

¹ University Heart Center Hamburg/ Germany, Department of Cardiovascular Surgery

² University Heart Center Hamburg/ Germany, Department of General and Interventional Cardiology

Objectives: Composite valve grafting (CVG) is still the standard for prophylactic aortic root replacement in patients with Marfan-syndrome (MFS). Although the reimplantation technique according to David (AVR) has shown favourable durability results in mid-term studies, its use is still being debated in patients with MFS.

Methods: We retrospectively evaluated the results of aortic root replacement of patients with MFS who underwent surgery between January 1995 and January 2010. AVR was used in 58 patients and CVG in 30 patients. Mean follow-up was 8.1 years \pm 2.2 years.

Results: Thirty day mortality in both groups was 0%. 10.0% in the CVG group required re-sternotomy for postoperative bleeding versus 3.7% in the AVR group ($p=0.4$). Within the follow-up 10% died in the CVG group vs 3.7% in the AVR group ($p=0.275$). Reoperation of the reimplanted valve was required in 2 patients (3.7%). 3 patients (10.0%) who underwent CVG had an endocarditis and 2 patients (6.7%) had a stroke during follow-up whereas no endocarditis and stroke was observed after AVR. Kaplan-Meier-analysis showed an event-free survival after 12 years of 92.3% after AVR and 76.6% after CVG ($p=0.04$). Transthoracic echocardiography at last visit showed aortic regurgitation of less than second degree in 87.9% of patients who underwent AVR.

Conclusion: AVR was associated with excellent survival and a low rate of complications despite longer ECC and cross-clamp times. Reimplanted valves showed good results in transthoracic echocardiography even after years. Event-free survival is significantly better after AVR and favors this technique in patients with MFS.

**Selected for oral presentation from poster submissions*

AORTIC VALVE-SPARING IN MARFAN SYNDROME: MIDTERM RESULTS WITH DAVID OPERATION (STANFORD MODIFICATION)

Forteza, Alberto MD, Bellot, Raquel MD, Sánchez, Violeta MD, Sanz, Paz MD, Gracia, Teresa MD, Centeno, Jorge MD, Cortina, Jose MD
Marfan Center. Hospital Universitario 12 de Octubre. Madrid. Spain

Background

We reviewed our experience with aortic valve-sparing operations in Marfan syndrome during last 6 years.

Methods

Between March 2004 and may 2010, 115 patients with aortic root aneurysms underwent valve-sparing operations. Of these, 48 were diagnosed with Marfan syndrome, according to the Ghent diagnostic criteria. Mean age was 29 ± 9 years (range, 11 to 66 years). Moderate/severe aortic regurgitation was present in 13%, and the mean diameter of the Valsalva sinuses was 51 ± 3 mm (range, 42 to 70 mm). The Stanford modification was performed in the last 39 patients. Mean follow-up was 30 ± 15 months (range, 1 to 75 months).

Results

There were no in-hospital deaths and no major adverse outcomes. One patient required implantation of a mechanical prosthesis during the same procedure because of moderate aortic regurgitation. One late death occurred. No patients required reoperation. In the last follow-up, 32 patients did not have aortic regurgitation, 14 had grade I, and 1 had grade II. No thromboembolic complications have been documented, and 97% of the patients are free from anticoagulation. There were no differences between Valsalva graft implantation and Stanford modification but this technique facilitates exposure and gives the surgeon flexibility to readjust annulus, sinotubular junction and the height of the commissures without any additional limitations.

Conclusions

Short-term and midterm results with the reimplantation technique for aortic root aneurysms in Marfan patients are excellent. This technique should be the treatment of choice for these patients in experienced Centers.

*Selected for oral presentation from poster submissions

MIDTERM RESULTS OF VALVE-SPARING AORTIC ROOT REPLACEMENT FOR ANNULO-AORTIC ECTASIA

Nawata Kan, MD,PhD, The Department of Cardiothoracic Surgery, The University of Tokyo Hospital, Tokyo, Japan

Morota Tetsuro, MD,PhD, The Department of Cardiothoracic Surgery, The University of Tokyo Hospital, Tokyo, Japan

Taketani Tsuyoshi, MD,PhD, The Department of Cardiothoracic Surgery, The University of Tokyo Hospital, Tokyo, Japan

Ono Minoru, MD,PhD, The Department of Cardiothoracic Surgery, The University of Tokyo Hospital, Tokyo, Japan

Takamoto Shinichi, MD,PhD, President, Mitsui Memorial Hospital, Tokyo, Japan

OBJECTIVES

Valve-sparing aortic root replacement (VSARR) has been gaining acceptance as an ideal procedure to treat annulo-aortic ectasia (AAE) in patients with or without Marfan syndrome (MFS). This study examines the midterm results of VSARR.

METHODS

Medical records were retrospectively examined in 68 consecutive cases of VSARR for AAE from 1998 to 2010. Among them were 52 MFS patients (76%).

RESULTS

Three types of VSARR were performed: 'remodeling procedure' for 4 patients, 'reimplantation procedure with cylindrical graft' ('David-I') for 19 and 'reimplantation procedure with reconstruction of Valsalva sinuses' ('David-V') for 45. The average age was 34 (5-68) years old and 43 patients (63%) were male. The average preoperative size of Valsalva sinus was 55 (40-83) mm and the preoperative aortic insufficiency (AI) grade was 1.8 \pm 1.3. Follow-up length was 52 \pm 34 (median 43) months and was significantly shorter in David-V (35 \pm 21 months).

There were no hospital deaths but 7 remote deaths including 6 MFS patients. Rhythm death was most likely. The AI grade at hospital discharge was significantly lower (0.7 \pm 0.7), although progression of AI couldn't be fully avoided in each procedure, and 4 patients underwent aortic valve replacement (AVR). However, no patient after David-V has needed AVR. AI \leq 3 free rate analyzed by Kaplan-Meier method showed a better tendency in David-V, compared with that in David-I.

CONCLUSIONS

VSARR seemed effective to treat AAE even in patients with MFS. David-V reimplantation would be the most profitable procedure for longer durability of the preserved native aortic cusps, although 8% of patients with MFS required AVR. Longer follow-up study of larger number of cases is mandatory to clarify the limits of VSARR.

*Selected for oral presentation from poster submissions.

S. 57**ASSESSMENT OF DURAL ECTASIA IN MARFAN SYNDROME**

Lundby, Rigmor, MD^{1,3}, Rand-Hendriksen, Svend, MD, PhD^{1,2}, Skaar, Sigrun MD³
Pripp Are H.⁴, Lilleås F G MD⁵, Smith, Hans-Jørgen, MD, PhD^{1,3}, Hald, John K. MD, PhD³

1University of Oslo 2 Sunnaas Rehabilitation Hospital, Nesoddtangen,
3 Oslo University Hospital, Rikshospitalet, 4 Section of Biostatistics, Research Services Department, Oslo
University Hospital, Rikshospitalet 5 Curato, Oslo , Norway.

Introduction

Dural ectasia (DE) is one of the major criteria of Marfan Syndrome (MFS) in the Ghent nosology. An incidence of DE in MFS of 63 - 92% has been reported. No gold standard for the diagnosis of DE has emerged.

Materials and Methods: Our aim was to establish the prevalence of DE in an adult population fulfilling the Ghent criteria for MFS and to assess definitions of DE.

105 adults with suspected MFS, and 101 sex- and age-matched controls were included. Based on MRI, lumbosacral AP vertebral body diameters (VBD) and dural sac diameters (DSD) were measured. Dural sac ratios (DSR = DSD/VBD) at levels L3 - S1 were calculated. Anterior meningoceles, herniations of nerve root sleeves, and scalloping were characterized.

Results: Three patient groups were identified: 1) MFS independent of DE (n = 73), 2) MFS dependent on DE (n = 14), and 3) suspected MFS, not fulfilling Ghent (n =18). DE was found in 86% of group 1. At levels L4-S1, mean DSRs were significantly higher in group 1 than in group 3 and controls (P < .001). Herniations of the nerve root sleeves were present in 73% in group 1 versus 1% in controls. Anterior meningoceles were found in 37% and 14% in groups 1 and 2, respectively, but not in group 3 or controls.

Conclusions:

The diagnosis of DE should be based on the presence of at least one of the following criteria: anterior meningoceles or nerve root sleeve herniations, DSD at S1 or below larger than DSD at L4, and DSR at S1 >0.59.

S. 58

OCULAR SIGNS AND SYMPTOMS OF MARFAN SYNDROME

LEROY Bart Peter, MD, PhD^{1,2}

WALRAEDT Sophie, MD¹

SCHAUWVLIEGHE Ann-Sofie, MD¹

DELBEKE Patricia, MD¹

CLAERHOUT Ilse, MD, PhD¹

KESTELYN Philippe, MD, PhD¹

¹Department of Ophthalmology & ²Center for Medical Genetics, Ghent University Hospital & Ghent University, Ghent, Belgium

Objectives:

To describe the ocular features of Marfan syndrome (MS) patients known in the Ghent Ophthalmic Genetic Clinic, and to provide frequencies thereof.

Methods:

In total, 71 patients (31 females & 41 males) with MS were examined (total of 142 eyes). Available data on lens (sub)luxation, myopia, axial length and corneal refractive power were evaluated. All patients were evaluated with slit-lamp biomicroscopy in extreme downgaze after maximal dilatation. Contrary to the Ghent Nosology cut-off of more than 3 diopters, any degree of myopia was considered as myopia. In contrast, axial length was interpreted against a cut-off of 25,76mm or more for men, and 25,16mm for women (mean plus 2 SDs). Mean corneal refractive power was considered decreased if equal or lower than 40,69D for men and 40,89D for women (mean minus 2SDs).

Results:

Some degree of lens (sub)luxation was seen in at least one eye of 51/67 patients (76%) or 98/142 eyes (69%).

In total 32 patients of 53 (60%) (63 of 106 eyes) were myopic.

Axial lengths were increased in 5 of 25 patients (20%) (7 of 50 eyes). These were all female.

A flat cornea was noted in 8 of 25 patients (32%) (14 of 50 eyes).

Conclusions:

About three quarters of patients have some degree of lens subluxation in at least one eye. A majority of MS patients has some degree of myopia. The limited data available on axial lengths and corneal flattening suggest that these are present in less than a third of patients.

S. 60

PAIN MANAGEMENT FOR MARFAN SYNDROME

Kost-Byerly, Sabine, M.D., Associate Professor, Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University, Baltimore, MD, USA

While pain in patients with Marfan syndrome appears to be common and varied study assessing prevalence, etiology, presentation and effective therapy has been limited.

A evaluation in an outpatient genetic clinic in Baltimore in 1995 suggested that the prevalence of musculoskeletal pain may be high with 70% of the children and 96% of the adults reporting at least one and 40% in both groups having multiple musculoskeletal symptoms, such as arthralgias and back pain. Joint hypermobility has been associated with pain in other connective tissue disorders though there may not be a direct correlation between increased mobility and severity of pain. Any joint can be affected, those in extremities as well as, costochondral joints, TMJ or SI joints. Protrusio acetabuli can be associated with pain though the severity of the defect does not correlate with clinical symptoms. Dural ectasia has been identified as an etiology for chronic back pain, pelvic pain and radicular neuropathic pain. Dural leaks of spinal fluid may contribute to recurrent low pressure headaches. Chronic pain will affect daily function and quality of life which both have been found to be lower in patients with Marfan than in comparable populations. An association between chronic pain, orthostatic intolerance, and fatigue needs to be further evaluated. There is limited evidence for effective therapy at this point. Pharmacological therapies primarily include adjunctive analgesics but it is recommended to add multi-disciplinary treatments based on the biopsychosocial model of chronic pain.

S. 61

HEALTH RELATED QUALITY OF LIFE IN MARFAN SYNDROME. A CROSS SECTIONAL STUDY OF SF=36 IN 84 ADULTS WITH A VERIFIED DIAGNOSIS

Rand-Hendriksen, Svend, MD, TRS National Resource Centre for Rare disorders, Sunnaas Rehabilitation Hospital, Nesoddtangen and Faculty of Medicine, University of Oslo, Norway.

Johansen, Heidi, Msc, TRS National Resource Centre for Rare disorders, Sunnaas Rehabilitation Hospital, Nesoddtangen, Norway.

Semb, Svein Ove, MD, PhD, Center for Eye Research, Faculty of Medicine, University of Oslo and Department of Ophthalmology, Oslo University Hospital, Norway.

Geiran, Odd R., MD, PhD, Faculty of medicine, University of Oslo and Department of Thoracic and Cardiovascular Surgery, Oslo University Hospital, Norway.

Stanghelle, Johan K., MD, PhD, Faculty of medicine, University of Oslo and Sunnaas Rehabilitation Hospital, Nesoddtangen, Norway.

Finset, Arnstein, PhD, Institute for Basic Medical Sciences, Faculty of Medicine, University of Oslo, Norway.

Objectives: To explore health related quality of life, measured with Short Form 36 (SF-36), in adults with verified Marfan syndrome, and to compare with the health related quality of life in the general population, other groups with chronic health problems and with the results in other studies on Marfan syndrome, respectively. We also wanted to study correlations between the scores on the subscales of SF-36 and the presence of biomedical criteria and symptoms of Marfan syndrome.

Methods: Cross-sectional study. SF-36 was investigated in 84 adults with verified Marfan syndrome.

Results: The study group had reduced scores on all eight subscales of SF-36 compared to the results in the general population, but similar scores as reported in other groups with chronic diseases.

Compared to earlier SF-36 results in Marfan syndrome, we found lower scores for social function, vitality, general health, bodily pain and role physical.

No correlations of substantial explanatory values were found between the SF-36 subscales and gender, BMI, ascending aortic surgery, use of β -blockers, visual acuity, joint hypermobility, fulfilment of the five major Ghent criteria, and number of major criteria fulfilled. Potential explanations are discussed.

Conclusion: Persons with Marfan syndrome report reduced scores on health related quality of life measured with SF-36, comparable to those with other chronic diseases and disabilities. The reduction in health related quality of life does not seem to be related to biomedical criteria or specific organ manifestations in Marfan syndrome.

S. 62

COMPREHENDING THE OTHER SIDE: PATIENT AND FAMILY PERSPECTIVES

Guttmacher, Brigid C. MA, LPC

Washington, DC USA

From the moment of diagnosis (if not before) the unending process of coping with the array of effects a rare disease has on patients and their families begins. How do they navigate these constant challenges?

It is important to recognize and be informed about the transition process we all go through when faced with major change in our lives. Recognizing that this is a normal, necessary reaction and understanding the range of coping styles can provide valuable insight when working with patients and families.

When faced with a rare disease, the process is the same, differing by degree of intensity. We will discuss the impact of anticipating new treatments, vulnerability to failure and the strain of multiple requests for participating in clinical trials.

ABSTRACTS FROM POSTER PRESENTATIONS



GENE TEST PRIORITY IN MARFAN SYNDROME SCREENING CLINIC

Child, Anne FRCP, CVS Department, St George's, University of London, UK
Aragon-Martin, Jose PhD, CVS Department, St George's, University of London, UK
Willoughby, Catherine PhD, South West Thames Regional Genetics, St George's Hospital, London, UK
Hasso, Sultana BSc, CVS Department, St George's, University of London, UK
Hughes, Kate, CVS Department, St George's, University of London, UK
Boileau, Catherine PhD, Laboratoire de Biochimie et de Genetique Moleculaire, Hopital Ambroise Pare, Boulogne, France
Arno, Gavin PhD, CVS Department, St George's, University of London, UK

Background

FBN1 mutation screening of 508 marfanoid clinic patients has detected causative mutations in 91% affected by classical MFS, 64 % with predominant ectopia lentis (EL), as low as 27% of the heterogeneous 'incomplete Marfan' study group, and in 0% of ascending aortic aneurysm probands.

Aim

In the significant proportion of probands with no demonstrable *FBN1* mutation, this study aimed to identify genetic determinants.

Methods

FBN1 cDNA sequencing identified possible complicated gene rearrangements/deletions in 3/11 probands. MLPA screening has identified large deletions in 4/182 (2.2%) *FBN1* mutation negative patients. Mutations in *TGFBR2* were identified in 2/53 (4%) patients with ascending aortic aneurysm and no *FBN1* mutation. Homozygous or compound heterozygous mutations in *ADAMTSL4* have been identified in 6/12 probands with predominant EL. Screening of *FBN2* has identified a mutation in 1/10 probands with suspected congenital contractual arachnodactyly.

Conclusions

Suggested practical screening protocol for Marfanoid patients is therefore (in order) *FBN1* with dHPLC and MLPA; *TGFBR1/2*; *FBN1*mRNA; *FBN2*. In dominant EL families, *FBN1*. In possibly autosomal recessive EL families, *ADAMTSL4*.

MARFAN SYNDROME AND ASSOCIATED DISORDERS (MSAD) DATABASES: 15 YEARS OF EXPERIENCE.

COLLOD-BEROUD, Gwenaëlle^{1,2}, PhD, FAIVRE, Laurence³, MD, PhD, STHENEUR, Chantal⁵, MD, HANNA, Nadine⁶, PharmD, PhD, HAMROUN, Dalil⁷, PhD, JONDEAU, Guillaume^{8,9,10}, MD, PhD, BOILEAU, Catherine^{6,8,11}, PharmD, PhD, BEROUD, Christophe^{1,2,7}, PharmD, PhD.

1: INSERM U827, Montpellier, France ; **2:** Université Montpellier 1, Montpellier, France ; **3:** CHU de Dijon, Centre de Génétique, Dijon, France ; **4:** AP-HP, CHU Ambroise Paré, Service de Pédiatrie, Boulogne, France ; **5:** AP-HP, CHU Ambroise Paré, Service de Pédiatrie, Boulogne, France ; **6:** AP-HP, CHU Ambroise Paré, Laboratoire de Biochimie, d'Hormonologie et de Génétique moléculaire, Boulogne, France ; **7:** CHU de Montpellier, Hôpital Arnaud de Villeneuve, Laboratoire de génétique Moléculaire, Montpellier, France ; **8:** AP-HP, Hôpital Bichat, Consultation Multidisciplinaire Marfan, Paris, France ; **9:** AP-HP, Hôpital Bichat, Service de cardiologie, Paris, France ; **10:** INSERM U698, Paris, France. **11:** INSERM U781, Paris, France.

In an effort to standardize the information regarding mutations, we had developed in 1995 (*FBN1* database) and more recently (*TGFBR1* and *2*, *FBN2* databases) computerized Locus Specific DataBases (LSDB) containing information about mutations of these 4 genes involved in MSAD. These LSDB have been helpful in identifying genotype/phenotype correlations. With the growing number of identified variations, being able to distinguish neutral sequence variations from those responsible for the phenotype is now of major interest in clinical diagnosis. Since in vitro validation of mutations is not always possible, indirect arguments must be accumulated to define if a missense variation is causative. The collection of these data is often both time-consuming and costly. The availability of LSDB now provides valuable information to help in the decision process (Was this mutation already described? Was the associated phenotype similar?). Nevertheless, although much effort is dedicated to the collection of mutations in these LSDBs, many families harbor private mutations for which no data are yet available. To further differentiate neutral variants from pathogenic nucleotide substitutions, we have developed two new prediction tools, UMD-predictor[®] for missense mutations and HSF[®] for mutations affecting splicing signals. We demonstrated that UMD-predictor[®] is the most efficient prediction algorithm in the context of MSAD with a positive predictive value of 99.4%, a sensitivity of 95.4 % and a specificity of 92.2 %. This tool could therefore be used as a help in the clinical diagnosis process.

TOWARDS THE DISSECTION OF MARFANOID SYNDROMES WITH MENTAL RETARDATION

Callier Patrick (1), Lambert Sandy (1), Aral Bernard (1), Thauvin-Robinet Christel (1), Jondeau Guillaume (2), Boileau Catherine (2), Faivre Laurence (1) and the network of the French reference centres for rare diseases

(1) Centre de Référence Maladies Rares Anomalies du développement et syndromes malformatifs, CHU Dijon, France

(2) Centre de référence Maladie de Marfan, Hôpital Bichat, APHP, France

Background: The term «marfanoid phenotype» is used to describe patients with skeletal signs suggestive of Marfan syndrome. The association of a marfanoid phenotype and mental retardation (MR) has been reported in the literature: Lujan-Fryns syndrome in X-linked pedigrees with rare mutations in the *ZDHHC9*, *UPF3B* and *MED12* genes; Shprintzen-Goldberg syndrome in patients with craniosynostosis, with rare mutations in the *FBN1* and *TGFBR2* genes; chromosomal imbalances.

Objectives: The association of a marfanoid phenotype and MR raises a number of problems with regard to diagnosis and genetic counselling. The implication of each gene is unknown as well as the risk of aortic involvement.

Methods: 55 males and 18 females were recruited. A 244K specially designed array-CGH (Agilent®) and sequencing of *MED12*, *ZDHHC9*, *UPF3B*, *FBN1*, *FBN2*, *TGFBR1*, and *TGFBR2* is in progress.

Preliminary results: 51 patients could be classified as Lujan-Fryns: 5 non recurrent chromosomal rearrangements were found. 17 patients could be classified as mental retardation with other extra-skeletal features of the MFS spectrum: 1 pathogenic *FBN1* mutation, 1 large genomic deletion including *FBN1*, and 1 other chromosomal rearrangements were found. 5 patients could be classified as Shprintzen-Goldberg: 1 *TGFBR1* mutation was found.

Conclusions: The preliminary results of this study suggest that the *MED12*, *ZDHHC9*, *UPF3B* genes are not major disease genes of such phenotypes and that submicroscopic rearrangements are the most prevalent anomalies. *FBN1* mutations or rearrangements were found in a subset of patients and should led to aortic evaluation in patients with marfanoid habitus and mental retardation.

THE CLINICAL SPECTRUM OF COMPLETE *FBN1* ALLELE DELETIONS

Hilhorst-Hofstee Yvonne¹, Hamel Ben CJ³, Verheij Joke BGM⁵, Rijlaarsdam Marry EB², Mancini Grazia MS⁶, Cobben Jan Maarten⁸, Giroth Cindy¹, Ruivenkamp Claudia AL¹, Hansson Kerstin BM¹, Timmermans Janneke⁴, Moll Henriette A⁷, Breuning Martijn H¹, Pals Gerard⁹

¹Department of Clinical Genetics, ²Department of Pediatric Cardiology, Leiden University Medical Center, Leiden; ³Department of Human Genetics, ⁴Department of Cardiology, Radboud University Nijmegen Medical Centre, Nijmegen; ⁵Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen; ⁶Department of Clinical Genetics, ⁷Department of Pediatrics, Erasmus MC Rotterdam; ⁸Department of Clinical Genetics, Academic Medical Center, Amsterdam; ⁹Department of Clinical Genetics, Center for Connective Tissue Research, VU University Medical Center, Amsterdam, the Netherlands

Background:

Only two reports are known of a complete allele deletion of *FBN1*. The most common mutations found in *FBN1* are missense mutations (56%) mainly substituting or creating a cysteine in a cbEGF domain. Other mutations are frameshift mutations, splice mutations and nonsense mutations.

Objectives: The purpose of this study is to determine the clinical spectrum of deletions of a complete *FBN1* allele

Methods:

We screened 300 patients with clinical features of MFS or a related phenotype by MLPA. All patients had been previously screened by DHPLC and no mutations in *FBN1* were found. In one patient a deletion of the *FBN1* gene was detected by chromosome analysis and array CGH, performed as part of mental retardation screening. In all patients the size of the deletion was determined by SNP array analysis.

Results:

In total 10 patients including a family with five patients were found to have a deletion of one *FBN1* allele. There was a large variation in size of the deletions between the patients. Seven patients fulfilled the Ghent criteria for Marfan syndrome. The other 3 patients were examined at a young age which could explain why they do not yet present the full clinical picture of MFS. Two patients with a large deletion had an extended phenotype. The clinical features of the patients will be presented.

Conclusions:

The results show that complete loss of one *FBN1* allele does not predict a mild phenotype. These findings support the hypothesis that true haploin sufficiency can lead to the classical phenotype of Marfan syndrome.

QUANTITATIVE SEQUENCE ANALYSIS OF *FBN1* PREMATURE TERMINATION CODONS PROVIDES EVIDENCE FOR INCOMPLETE NMD IN LEUKOCYTES

Magyar István, MSc, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland; **Colman Dvora**, MSc, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland; **Arnold Eliane**, MSc, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland, Division of Metabolism and Molecular Pediatrics, University Children's Hospital, Zurich, Switzerland; **Baumgartner Daniela**, MD, Department of Pediatric Cardiology, Innsbruck Medical University, Innsbruck, Austria; **Bottani Armand**, MD, Division of Medical Genetics, Geneva University Hospitals, Geneva, Switzerland; **Fokstuen Siv**, MD, Division of Medical Genetics, Geneva University Hospitals, Geneva, Switzerland; **Addor Marie-Claude**, MD, Service of Medical Genetics, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; **Berger Wolfgang**, PhD, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland; **Carrel Thierry**, MD, Clinic for Cardiovascular Surgery, University Hospital, Berne, Switzerland; **Steinmann Beat**, MD, Division of Metabolism and Molecular Pediatrics, University Children's Hospital, Zurich, Switzerland; **Mátyás Gábor**, PhD, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland

In order to assess the pathogenic effects of mutations, we improved, evaluated, and used Sanger sequencing for quantification of SNP variants in transcripts and gDNA samples. This improved assay resulted in highly reproducible relative allele frequencies (e.g. for a heterozygous gDNA $50.0 \pm 1.4\%$, $P=0.05$, and for a missense mutation-bearing transcript $46.9 \pm 3.7\%$, $P=0.05$) with a lower detection limit of 3-9%. It provided excellent accuracy (e.g. for a duplicated gDNA $66.6 \pm 2.2\%$, $P=0.05$) and linear correlation between expected and observed relative allele frequencies. This sequencing assay, which can also be used for the quantification of CNVs, methylations, mosaicisms, and DNA pools, enabled us to analyze transcripts of the *FBN1* gene in fibroblasts and blood samples of patients with suspected Marfan syndrome not only qualitatively but also quantitatively. We present a total of 19 novel and 18 known *FBN1* sequence variants leading to a premature termination codon (PTC), 26 of which we analyzed by quantitative sequencing both at gDNA and cDNA levels. The relative amounts of PTC-containing *FBN1* transcripts in fresh and PAXgene-stabilized blood samples were significantly higher ($33.0 \pm 3.9\%$ to $80.0 \pm 7.2\%$, $P=0.05$) than those detected in affected fibroblasts with inhibition of nonsense-mediated mRNA decay (NMD) ($11.0 \pm 2.1\%$ to $25.0 \pm 1.8\%$, $P=0.05$), while in fibroblasts without NMD inhibition no mutant alleles could be detected. These results provide evidence for incomplete NMD in leukocytes and have particular importance for RNA-based analyses not only in *FBN1* but also in other genes.

THE SPECTRUM OF CLINICAL MANIFESTATIONS IN MARFAN SYNDROME

Rybczynski, Meike, MD, Centre of Cardiology an Cardiovascular Surgery, University Hospital Eppendorf, Hamburg, Germany

Sheikhzahdeh, Sara, MD, Centre of Cardiology an Cardiovascular Surgery, University Hospital Eppendorf, Hamburg, Germany

Rehder, Uwe, PhD, Department of Trauma-, Hand- and Reconstructive Surgery, University Hospital Eppendorf, Hamburg, Germany

Fuisting, Bettina, MD, Clinic of Ophthalmology, University Hospital Eppendorf, Hamburg, Germany

Detter, Christian, MD, Centre of Cardiology an Cardiovascular Surgery, University Hospital Eppendorf, Hamburg, Germany

Robinson, Peter, MD, Institute of Medical Genetics, Charite University Hospital, Humboldt University, Berlin

Arslan-Kirchner, Mine, MD, Institute of Human Genetics, Medizinische Hochschule Hannover, Hannover, Germany

v. Kodolitsch, Yskert, MD, Centre of Cardiology an Cardiovascular Surgery, University Hospital Eppendorf, Hamburg, Germany

Objectives:

To make or exclude the diagnosis of Marfan syndrome (MFS), assessment of a catalog of major and minor clinical criteria is required. However, the prevalence and diagnostic value of these criteria are unknown in persons in whom MFS has been diagnosed or ruled out.

Methods and Results: We prospectively evaluated 279 consecutive patients with suspected MFS (144men and 135 women at a mean age of 34 ± 13 years) for presence of 27 distinct clinical criteria considered characteristic of MFS. The most frequent reasons to refer individuals for suspected MFS were skeletal features (31%), family history of MFS or aortic complications (29%), aortic dissection or aneurysm (19%), and eye manifestations (9%). Using established criteria, we confirmed MFS in 138 individuals (group 1), and diagnosed other connective tissue diseases, both with vascular involvement in 30 (group 2) and without vascular involvement in 39 individuals (group 3), and excluded any distinct disease in 72 (group 4). Clinical manifestations of MFS were present in all four patient groups and there was no single criterion that exhibited positive and negative likelihood ratios that were per se sufficient to confirm or rule out MFS.

Conclusion: Our study reveals a comparatively low diagnostic power of all 27 of the most typical clinical manifestations of MFS. Most notably, clinical features of MFS emerged to be common among individuals with excluded MFS who exhibited numerous alternative diseases with still poorly defined long term prognosis and strategies for clinical management.

THE DIAGNOSTIC VALUE OF THE FACIAL FEATURES OF MARFAN SYNDROME

Ting, Beverlie, M.D., Johns Hopkins University, Baltimore, MD, USA.
Mathur, Deepti, MB;BS, San Fernando General Hospital, Trinidad and Tobago.
Loeys, Bart, M.D., Ph.D., Ghent University, Ghent, Belgium.
Dietz, Hal, M.D., Johns Hopkins University, Baltimore, MD, USA.
Sponseller, Paul, M.D. Johns Hopkins University, Baltimore, MD, USA.

OBJECTIVES:

Facial features are sometimes influential in forming impressions regarding genetic diagnoses. This study examines the clinical utility (sensitivity, specificity, and accuracy) of using known facial features of Marfan syndrome (MFS) – dolichocephaly, malar hypoplasia, enophthalmos, micrognathia/retrognathia, and down-slanting palpebral fissures – for screening and diagnosis.

METHODS:

Anteroposterior and lateral photographs were taken of n=76 MFS patients (ages 1.5-55 years, average 18.3 years) and their age- and gender-matched controls (n=76). The photographs were randomized and compiled into an online survey. Three physicians experienced with the MFS rated each photograph in terms of the degree to which they believed each of the features to be present and whether they had a strong suspicion for MFS.

A smaller selection of photographs were then used to survey how well non-expert orthopaedic surgeons perform. We examined whether a brief instructional sheet could improve the accuracy of diagnosis.

RESULTS:

Each facial feature had a higher prevalence among MFS patients compared to controls. Using facial features alone, experienced physicians correctly discriminated between Marfan patients and controls with 73% accuracy. Facial features have a 54% sensitivity and a 91% specificity for MFS, with a positive predictive value of 86% and a negative predictive value of 67%. For each additional facial characteristic identified as being present, the odds of having MFS more than doubled (OR=2.4). Among non-expert physicians, the brief instructional sheet did not lead to any significant diagnostic improvement.

CONCLUSIONS:

Facial features are more specific than they are sensitive. Therefore, recognition of facial features of MFS can be used as an initial screening tool. However, facial features do not have a high sensitivity for MFS.

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IS IT REALLY MARFAN SYNDROME? DIFFERENTIAL DIAGNOSES AT THE BRISBANE MARFAN CLINIC.

West, Malcolm, J BSc MBBS PhD FRACP, School of Medicine, The University of Queensland, Prince Charles Hospital, Brisbane, Queensland, Australia.

West Jennifer A, RN, School of Medicine, The University of Queensland, Prince Charles Hospital, Brisbane, Queensland, Australia.

Summers Kim M, BSc PhD PG Dip Human Biol, School of Medicine, The University of Queensland, Prince Charles Hospital, Brisbane, Queensland, Australia and The Roslin Institute, University of Edinburgh, Edinburgh, UK.

Objectives.

We established a Multidisciplinary Diagnostic MFS Clinic in August 1995 in Brisbane Australia. As the major cardiology and cardiovascular surgery provider in the state of Queensland (population 4.5 million) and the referral centre for surgical management of thoracic aortic aneurysm, Prince Charles Hospital was a natural base for such a clinic. We have analysed the presenting features and final diagnosis of all index cases.

Methods and Results.

The records of the 59 clinics since 1995 were reviewed. A total of 432 index cases were examined. The majority of individuals were referred because of skeletal signs or aortic dilatation. 92 index cases (21%) were given a diagnosis of Marfan syndrome and eight mutations in the *FBN1* gene were found. For the remaining 340 index cases there was a range of diagnoses. 28 (8%) were found to have familial aortic aneurysm without Marfanoid features. 18 (5%) had a non-specific connective tissue condition, 16 (5%) had bicuspid aortic valve and 11 (3%) had homocystinuria. 201 (59%) index cases were diagnosed with a condition which overlapped in phenotype with Marfan syndrome.

Conclusions.

Referrals to our Marfan clinic reflect increasing recognition of the signs and symptoms of Marfan syndrome, which was only supported in one fifth after clinical assessment. DNA diagnosis may become a significant tool for confirmation and management of the condition but access remains a problem. A broad range of related conditions may present initially as Marfan syndrome and it is important to educate referring practitioners on differential prognosis and treatment.

MUSCULOSKELETAL FINDINGS OF LOEYS-DIETZ SYNDROME

Paul D. Sponseller, MD; Gurkan Erkula MD, Laura C. Paulsen; Gretchen L. Oswald, MS; Bart L. Loeys, MD, PhD; and Harry C. Dietz, MD

Objective:

To characterize the clinical and imaging findings of the skeleton in Loeys-Dietz syndrome to aid in diagnosis and treatment.

Methods:

We retrospectively analyzed the clinical, and imaging data of sixty-five patients with Loeys-Dietz syndrome seen at one institution from May 2007 through December 2008. Skeletal findings were studied, and results of treatment analyzed when available

Results:

The patients had a mean age of 22 years (range, 2 to 76 years); 55.4% (36 of 65) were less than 18 years old. Previous diagnoses included Marfan syndrome (sixteen patients) and Ehlers-Danlos syndrome (two patients). Spinal and foot abnormalities were the most significant skeletal findings. Eleven patients had talipes equinovarus, and 19 patients had cervical anomalies and instability (defect of the anterior C1 arch, six; lateral cervical translation, five; defect of posterior C1 arch, four; cervical malrotation, three; C1-C2 subluxation, one; and C2-C3 subluxation, one). Scoliosis was present in 30 patients (mean Cobb angle, $30^{\circ} \pm 18^{\circ}$). Two patients had spondylolisthesis, and two-thirds had dural ectasia (twenty-two of thirty-three imaged). 35 patients had pectus excavatum and eight had pectus carinatum. Combined thumb and wrist signs were present in one-fourth of patients. Mild acetabular protrusion was present in one-third of patients. Fourteen patients had had previous orthopaedic procedures: scoliosis surgery, cervical stabilization, clubfoot correction, knee arthroscopy, and hip arthroplasty.

Conclusions:

Skeletal findings are variable but patients with Loeys-Dietz syndrome most commonly present to the orthopaedist with cervical malformations, spinal and foot deformities, as well as findings in the craniofacial and cutaneous systems. Heightened awareness may aid early diagnosis.

THE NATURAL HISTORY OF DURAL ECTASIA IN MARFAN SYNDROME

Mesfin, Addisu, MD, Department of Orthopaedic Surgery, Johns Hopkins University, Baltimore MD, USA
Ahn, Nicholas MD, Department of Orthopaedic Surgery, Case Western Reserve University, Cleveland, OH USA
Carrino, John, MD, MPH, Department of Radiology, Johns Hopkins University, Baltimore MD, USA
Sponseller, Paul, MD, MBA, Department of Orthopaedic Surgery, Johns Hopkins University, Baltimore MD, USA

Introduction

Dural ectasia, or widening of the dural sac, in the lumbosacral spine is a common feature of Marfan syndrome. Dural ectasia has been associated with low back pain as well as increased surgical complications. The natural history of dural ectasia in Marfan syndrome is not known.

Methods

Twenty Marfan patients with dural ectasia who underwent MRI and CT of the lumbosacral spine and completed the Oswestry Disability Index(ODI) in 1998-1999 were prospectively followed. In 2009 new ODI and MRI of the lumbosacral spine were obtained. Our exclusion criteria were surgery of the lumbosacral spine or loss to follow up. MRI based volumetric measurements (GE Advantage Workstation) of the dura from L5-S2 were obtained to determine if the dural ectasia size increased over time.

Results

Fifteen (75%) of the original twenty patients were available for follow up. All fifteen completed the ODI questionnaire. Mean age was 49.6 (41.1-61.2). Mean follow up was 10.5 yrs (9.8-11). Mean ODI score (higher = worsening symptoms) in 1998-99 was 25.8, SD 19.7 (0-46.67) and mean ODI score in 2009 was 22.2, SD: 21.3 (0 – 62.2). No statistical significance was noted ($p = 0.46$). MRI dural volumetric measurements were available for 7 patients from 2009 and 7 from 1998-99. Mean dural volume in 1998-99 was 73.4cm^3 , SD 38.1 (22-138.9) and in 2009 was 79cm^3 , SD 43.7 (16.7-140.3). No statistical significance was noted ($p= 0.37$).

Conclusion

The natural history of dural ectasia in Marfan syndrome does not demonstrate an increase in ODI scores and does not demonstrate a significant increase in dural ectasia size.

LONGTERM RESULTS AFTER AORTIC ROOT REPLACEMENT IN PATIENTS WITH MARFAN SYNDROME

Bernhardt, AMJ, MD¹, Treede, H, MD¹, Rybczynski, M, MD², Sheikzadeh, S, MD², Meinertz, T, MD², Kodolitsch, Y, MD², Reichenspurner, H, PhD¹

¹ University Heart Center Hamburg/ Germany, Department of Cardiovascular Surgery

² University Heart Center Hamburg/ Germany, Department of General and Interventional Cardiology

Objectives: Composite valve grafting (CVG) is still the standard for prophylactic aortic root replacement in patients with Marfan-syndrome (MFS). Although the reimplantation technique according to David (AVR) has shown favourable durability results in mid-term studies, its use is still being debated in patients with MFS.

Methods: We retrospectively evaluated the results of aortic root replacement of patients with MFS who underwent surgery between January 1995 and January 2010. AVR was used in 58 patients and CVG in 30 patients. Mean follow-up was 8.1 years \pm 2.2 years.

Results: Thirty day mortality in both groups was 0%. 10.0% in the CVG group required re sternotomy for postoperative bleeding versus 3.7% in the AVR group ($p=0.4$). Within the follow-up 10% died in the CVG group vs 3.7% in the AVR group ($p=0.275$). Reoperation of the reimplanted valve was required in 2 patients (3.7%). 3 patients (10.0%) who underwent CVG had an endocarditis and 2 patients (6.7%) had a stroke during follow-up whereas no endocarditis and stroke was observed after AVR. Kaplan-Meier-analysis showed an event-free survival after 12 years of 92.3% after AVR and 76.6% after CVG ($p=0.04$). Transthoracic echocardiography at last visit showed aortic regurgitation of less than second degree in 87.9% of patients who underwent AVR.

Conclusion: AVR was associated with excellent survival and a low rate of complications despite longer ECC and cross-clamp times. Reimplanted valves showed good results in transthoracic echocardiography even after years. Event-free survival is significantly better after AVR and favors this technique in patients with MFS.

MITRAL VALVE SURGERY IN PATIENTS WITH MARFAN SYNDROME

Bernhardt AMJ^{1*}, MD, Treede H¹, MD, Rybczynski M², MD, Sheikzadeh S², MD, Meinertz T², MD, von Kodolitsch Y², MD, Reichenspurner H¹, MD, PhD

¹ University Heart Center Hamburg/ Germany, Department of Cardiovascular Surgery

² University Heart Center Hamburg/ Germany, Department of General and Interventional Cardiology

Objectives:

Mitral valve regurgitation is a common manifestation in patients with Marfan syndrome (MFS) and is age-dependent. It shares some features of myxomatous mitral valve disease. The surgical treatment is still being debated and not well characterized in MFS.

Methods:

We retrospectively evaluated the results of mitral valve repair and replacement of patients with MFS who underwent surgery between January 1995 and December 2009. MFS was diagnosed by Ghent criteria. Mitral valve surgery was performed in 10 patients, 8 patients had mitral valve repair and 2 patients mitral valve replacement. Mean follow-up was 45.5 months (range 10 – 153). Mitral repair was performed in 3 patients.

Results:

Thirty day mortality in both groups was 0%. One patient died 6 years after mitral valve repair due to endocarditis. Transthoracic echocardiography at last visit showed mild mitral regurgitation in only one patient and mitral regurgitation was absent in the other patients.

Conclusion:

Mitral valve repair was associated with excellent survival and a low rate of complications. Repaired valves showed good results in transthoracic echocardiography even years later. Mitral repairs are feasible even in deformed thoraces, lowering the risk for future aortic surgery. Due to good long-term results, mitral valve repair may also be justified in asymptomatic Marfan patients.

MINIMIZING PARAPLEGIA IN PATIENTS WITH MARFAN SYNDROME UNDERGOING DESCENDING AND THORACOABDOMINAL AORTIC ANEURYSM REPAIR

Bischoff MS, MD, Scheumann J, MS, Griep RB, MD, and DiLuozzo G, MD

Department of Cardiothoracic Surgery, Mount Sinai School of Medicine, New York, NY 10029, USA

Objectives:

Too many patients with Marfan syndrome (MS) still suffer acute aortic dissection and subsequently require distal aortic repair, which is accompanied by a significant incidence of spinal cord injury (SCI). The collateral network concept of spinal cord perfusion developed over the past 15 years allows a rational approach to spinal cord protection despite the need to sacrifice intercostals and/or lumbar arteries during aortic resection.

Methods:

From 1994 to 2009, a total of 623 descending and thoracoabdominal aortic aneurysm repairs were performed at our institution: 23 were in patients with MS (17 patients; 11 male, 37±12 years). Spinal cord protection strategy was based on an understanding of the plasticity of the collateral network. 16/17 patients had undergone previous aortic surgery: ascending and/or arch replacement in 15, and descending aorta repair in 1.

Results:

There were no 30 day or hospital deaths. An average of 7.6 ± 4.0 intercostal and/or lumbar arteries were sacrificed. All patients had normal neurological function postoperatively, but one 55 year old patient who had been on chronic dialysis developed paraplegia one month postoperatively.

Conclusions:

Despite the dramatic improvement in prognosis following elective replacement of the ascending aorta, fear of paraplegia may discourage MS patients from undertaking elective repair of a life threatening dissected and enlarging distal aorta. The risk of SCI can be minimized by recognizing that even extensive sacrifice of intercostal/lumbar vessels can be adequately compensated by the collateral arterial network if scrupulous attention is paid during and immediately following surgery to provide optimal conditions for spinal cord perfusion.

EVALUATION OF LEFT VENTRICULAR FUNCTION IN 200 PATIENTS WITH MARFAN SYNDROME USING CONVENTIONAL DOPPLER-ECHOCARDIOGRAPHY TISSUE DOPPLER AND 2D STRAIN IMAGING

D. Detaint¹, A. Touati¹, F. Arnoult¹, M. Gautier¹, C. Bouffard¹, Y. Korinenko¹, G. Delorme¹, G. Jondeau¹ - (1) CNR Marfan et apparentés, AP-HP - Hospital Bichat-Claude Bernard, Paris, France

Background: Early impairment of left ventricular (LV) systolic and diastolic function in Marfan syndrome (MFS) has been suggested but remains uncertain.

Methods : 554 patients consecutively screened in our outpatient clinic devoted to MFS were considered for the study. Diagnosis was confirmed in 257 (according to Ghent criteria), ruled out in 122 and uncertain in 175. MFS patients with previous surgery (50), ongoing pregnancy (4), or significant mitral and aortic valve disease (3) were excluded. Hence the clinical and echocardiographic characteristics of 122 Healthy (controls) and 200 MFS patients were compared.

Results : MFS and controls were comparable for age and gender (mean age 31±14 years, male 45%), but, as expected MFS had larger aortic dimensions (at Valsalva level: 39±7 vs 30±4mm, p<0.001). MFS had also larger LV dimensions: larger volumes in diastole (EDV:118±33 vs 104±32mL, p<0.01) and in systole (ESV: 40±18 vs 34±11mL, p<0.001), and larger diameters in diastole (EDd: 50±7 vs 47±5 mm, p=0.001 and in systole (ESd 31±6 vs 29±4 mm, p=0.004). Adjustment for BSA, lessened differences between groups but differences remained significant for all normalized LV dimensions (all p<0.01). In contrast, no difference between groups was observed in fractional shortening, or ejection fraction using both Teicholz and Simpson rules (for all p=NS). Assessment of diastolic function with E/A and deceleration time showed no difference between groups (for all p=NS). Using tissue Doppler, MFS had lower early diastolic (Ea: 10±4 vs. 14±4cm/s, p<0.001), late diastolic (Aa: 7.5±2 vs. 8.3±2cm/s, p=0.02) and systolic velocities (Sm:8.9±3 vs 11.2±4 cm/s, p<0.001). These differences in early diastolic and systolic velocities remained significant after adjustment for age, heart rate and beta-blocker therapy (both p<0.01 in multivariate analyses) whereas difference in late diastolic velocities lost significance. In contrast, 2D strain was similar in the 2 groups for both global and regional longitudinal systolic function.

Conclusion : MFS patients have normal systolic and diastolic LV function despite larger BSA-normalized LV dimensions. Only mild impairment of LV longitudinal function can be evidenced by comprehensive echocardiographic evaluation, suggesting a structural role for fibrillin 1 in the human heart.

PROTEINURIA AND MICROALBUMINURIA AS PREDICTORS OF RENAL DYSFUNCTION IN A COHORT OF FOURTY EIGHT MARFAN SYNDROME PATIENTS

Forteza, Alberto MD, Bellot, Raquel MD, Morales, Enrique¹ PhD, MD, Sánchez, Violeta MD, Sanz, Paz MD, Gracia, Teresa MD, Evangelista, Arturo², PhD, MD, Cortina, Jose MD
Marfan Center. Hospital Universitario 12 de Octubre. Madrid. Spain.

¹ Nephrology Department. Hospital Universitario 12 de Octubre. Madrid. Spain.

² Marfan Center. Hospital Universitario Vall'dHebron. Barcelona. Spain.

Proteinuria and microalbuminuria are considered predictors of renal dysfunction but also as cardiovascular risk indicators.

Objectives:

To assess the prevalence of proteinuria and microalbuminuria as predictors of renal dysfunction in Marfan syndrome (MS). In spite of a wide organ affectation, there have been very few reports of renal involvement in MS.

Methods:

Forty-eight patients with MS according Ghent criteria have been evaluated to assess renal function. Mean age was $32,3 \pm 8,7$ and 54% were male. Urine samples of 24 hours were analyzed in each patient to determine proteinuria and microalbuminuria. Additional measures like serum creatinine, creatinine clearance, glomerular filtration, hematuria, presence of ANAs or immunoglobulin A levels were also considered. We established positive microalbuminuria values higher than 30 mg/24 h, and presence of significant proteinuria higher than 0,20 g/24 h.

Results:

Significant proteinuria was found in 25% of patients (mean $0,23 \pm 0,03$) and two more patients (4%) presented isolated microalbuminuria. Immunoglobulin A levels were increased in 8 patients (16,7%). Serum creatinine range was between 0,5 and 1,3 (mean $0,83 \pm 0,19$). None had decreased values of creatinine clearance (mean $128,45 \pm 43,38$) or glomerular filtration (mean $104,17 \pm 23,99$).

Conclusions:

Prevalence of proteinuria and microalbuminuria in MS patients are higher than in general population. Although renal function was not affected it could be an early renal dysfunction predictor. Immunoglobulin A levels were higher than expected, but its relevance is unknown.

AORTIC VALVE-SPARING IN MARFAN SYNDROME: MIDTERM RESULTS WITH DAVID OPERATION (STANFORD MODIFICATION)

Forteza, Alberto MD, Bellot, Raquel MD, Sánchez, Violeta MD, Sanz, Paz MD, Gracia, Teresa MD, Centeno, Jorge MD, Cortina, Jose MD

Marfan Center. Hospital Universitario 12 de Octubre. Madrid. Spain

Background.

We reviewed our experience with aortic valve-sparing operations in Marfan syndrome during last 6 years.

Methods.

Between March 2004 and may 2010, 115 patients with aortic root aneurysms underwent valve-sparing operations. Of these, 48 were diagnosed with Marfan syndrome, according to the Ghent diagnostic criteria. Mean age was 29 ± 9 years (range, 11 to 66 years). Moderate/severe aortic regurgitation was present in 13%, and the mean diameter of the Valsalva sinuses was 51 ± 3 mm (range, 42 to 70 mm). The Stanford modification was performed in the last 39 patients. Mean follow-up was 30 ± 15 months (range, 1 to 75 months).

Results.

There were no in-hospital deaths and no major adverse outcomes. One patient required implantation of a mechanical prosthesis during the same procedure because of moderate aortic regurgitation. One late death occurred. No patients required reoperation. In the last follow-up, 32 patients did not have aortic regurgitation, 14 had grade I, and 1 had grade II. No thromboembolic complications have been documented, and 97% of the patients are free from anticoagulation. There were no differences between Valsalva graft implantation and Stanford modification but this technique facilitates exposure and gives the surgeon flexibility to readjust annulus, sinotubular junction and the height of the commissures without any additional limitations.

Conclusions.

Short-term and midterm results with the reimplantation technique for aortic root aneurysms in Marfan patients are excellent. This technique should be the treatment of choice for these patients in experienced Centers.

MIDTERM RESULTS OF VALVE-SPARING AORTIC ROOT REPLACEMENT FOR ANNULO-AORTIC ECTASIA

Nawata Kan, MD,PhD, The Department of Cardiothoracic Surgery, The University of Tokyo Hospital, Tokyo, Japan

Morota Tetsuro, MD,PhD, The Department of Cardiothoracic Surgery, The University of Tokyo Hospital, Tokyo, Japan

Taketani Tsuyoshi, MD,PhD, The Department of Cardiothoracic Surgery, The University of Tokyo Hospital, Tokyo, Japan

Ono Minoru, MD,PhD, The Department of Cardiothoracic Surgery, The University of Tokyo Hospital, Tokyo, Japan

Takamoto Shinichi, MD,PhD, President, Mitsui Memorial Hospital, Tokyo, Japan

OBJECTIVES

Valve-sparing aortic root replacement (VSARR) has been gaining acceptance as an ideal procedure to treat annulo-aortic ectasia (AAE) in patients with or without Marfan syndrome (MFS). This study examines the midterm results of VSARR.

METHODS

Medical records were retrospectively examined in 68 consecutive cases of VSARR for AAE from 1998 to 2010. Among them were 52 MFS patients (76%).

RESULTS

Three types of VSARR were performed: 'remodeling procedure' for 4 patients, 'reimplantation procedure with cylindrical graft' ('David-I') for 19 and 'reimplantation procedure with reconstruction of Valsalva sinuses' ('David-V') for 45. The average age was 34 (5-68) years old and 43 patients (63%) were male. The average preoperative size of Valsalva sinus was 55 (40-83) mm and the preoperative aortic insufficiency (AI) grade was 1.8+/-1.3. Follow-up length was 52+/-34 (median 43) months and was significantly shorter in David-V (35+/-21 months).

There were no hospital deaths but 7 remote deaths including 6 MFS patients. Rhythm death was most likely. The AI grade at hospital discharge was significantly lower (0.7+/-0.7), although progression of AI couldn't be fully avoided in each procedure, and 4 patients underwent aortic valve replacement (AVR). However, no patient after David-V has needed AVR. AI-free rate analyzed by Kaplan-Meier method showed a better tendency in David-V, compared with that in David-I.

CONCLUSIONS

VSARR seemed effective to treat AAE even in patients with MFS. David-V reimplantation would be the most profitable procedure for longer durability of the preserved native aortic cusps, although 8% of patients with MFS required AVR. Longer follow-up study of larger number of cases is mandatory to clarify the limits of VSARR.

NURSING AND RESEARCH WORKING TOGETHER

Radojewski, Liz, RN, Cardiology, SickKids, Toronto, Ontario, Canada

Slater, Nancy, RN MN, Cardiology, SickKids, Toronto, Ontario, Canada

Colman, Jack, MD, Cardiology, University Health Network, Toronto, Ontario, Canada

Bradley, Tim, MD, Cardiology, SickKids, Toronto, Ontario, Canada

Marfan syndrome is a congenital disorder of connective tissue primarily involving the heart, eyes and skeleton. The incidence of Marfan syndrome is approximately 2-3/10,000. Based on Canada's current population of approximately 33 million, there are estimated 6,500 – 10,000 Canadians affected. The need for a specialized clinic to care for and address the needs of patients and families with Marfan and other connective tissue disorders was recognized at the Hospital for Sick Children and Toronto General Hospital in Toronto, Ontario Canada. The role of the clinic nurse within these clinics has been developed to improve patient care, quality of life and education as well as to maintain and promote further clinic growth with the goal of developing a multidisciplinary clinic. The close proximity of the two clinics has allowed the clinic nurse and paediatric cardiologist to provide care in both clinics and to facilitate the smooth transition of paediatric patients to the adult congenital cardiac clinic.

The NIH Pediatric Heart Network Atenolol v/s Losartan Trial has presented some challenges for both the clinic nurse and research nurse coordinator. These nurses have been able to coordinate their efforts to meet their individual responsibilities and work together with mutual respect for each other's scope of practice to serve the patient and support this research initiative.

Both nurses have worked together to help with coordination of clinical and research responsibilities to lessen the burden on patients and families including, sharing of information, measurements, and health update at research visits. This has also resulted in improved patient and family satisfaction and easier transition back to clinical care.

MECHANICAL PROPERTIES OF ASCENDING AORTA WITH MARFAN AND LOEYS-DIETZ SYNDROMES

Authors: Rojo, Francisco J¹ (PhD, MSc Eng); Forteza, Alberto² (PhD, MD); Guinea, Gustavo V¹ (PhD, MSc Eng); Cortina, Jose M² (PhD, MD)

¹ Departamento de Ciencia de Materiales, E.T.S.I. de Caminos, Canales y Puertos, Universidad Politécnica de Madrid. c/ Prof. Aranguren s/n, 28040 Madrid, Spain.

² Marfan Centre, Hospital Universitario Doce de Octubre. Avd. Cordoba s/n, 28041 Madrid, Spain.

Objectives: The development of mechanical models and the assessment of arterial integrity would be improved if the mechanical properties of aortic wall, both healthy and pathological, are well understood. This work studies the mechanical behavior and rupture conditions of ascending aorta from patients with Marfan and Loeys-Dietz syndromes comparing them with those of healthy donors.

Methods: Ascending aorta and Valsalva sinus segments were harvested from eight patients with Marfan syndrome and two patients with Loeys-Dietz syndrome undergoing surgery. Ascending aortic specimens were also harvested from 19 healthy donors. Uniaxial tensile tests were carried out *in vitro* under physiological conditions within 24 hours from excision, and the stress vs. strain curves were obtained. *In vivo* stresses, deduced from arterial diameter and thickness, were computed and compared with the mechanical parameters of the aortic wall.

Results: Tensile strength, stretch at failure and stiffness of the arterial wall were obtained for both healthy and diseased arteries. Compliance of stress strain-curves was analyzed by determining the transition point between the compliant and the stiff regions of the curve.

Conclusions: When compared with healthy specimens of corresponding age, Marfan and Loeys-Dietz syndromes seem not to have a significant effect on the mechanical performance of the aortic wall. Aortic stiffness strongly depends on the parietal stress level, being largely increased for dilated vessels where the wall stresses are high. The physiological operation of dilated vessels lays in the stiffer part of their response curve, losing part of its damping function and reducing the safety factor.

FREQUENCY OF SLEEP APNEA IN ADULTS WITH MARFAN SYNDROME

Rybczynski, Meike, MD, Department of Cardiology, University Hospital Hamburg Eppendorf, Hamburg, Germany

Sheikhzadeh, Sara, MD, Department of Cardiology, University Hospital Hamburg Eppendorf, Hamburg, Germany

Treede, Hendrik, MD, Department of Cardiovascular Surgery, University Hospital Hamburg Eppendorf, Hamburg, Germany

Robinson, Peter, MD, Department of Medical Genetics, University Hospital Charite, Berlin, Germany

v. Kodolitsch, Yskert, MD, Department of Cardiology, University Hospital Hamburg Eppendorf, Hamburg, Germany

Objectives:

Obstructive and central sleep apnea are treatable disorders, which contribute to cardiovascular morbidity in older adults. Younger adults with Marfan Syndrome may also be at risk for sleep apnea, but the relationship between cardiovascular complications and sleep apnea is unknown.

Methods: We used MiniScreen8® portable monitoring devices for polygraphy in 68 consecutive adults with marfan syndrome (33 men, 35 woman aged 41 ± 14 years) to investigate both frequency of sleep apnea and its relation to cardiovascular morbidity.

Results: Apnea-hypopnea index (AHI) was 6-15/h in 14 individuals (mild sleep apnea; 21%), and $>15/h$ in 7 individuals (moderate or severe sleep apnea; 10%). Among established risk factors for sleep apnea, only higher age (Spearman $\rho = .35$, $P = .004$) and body mass index (BMI; $\rho = .26$, $P = .03$) were associated with increased AHI. Of all cases with apnea 12 ± 27 were obstructive, 11 ± 25 central and 3 ± 9 mixed. AHI was associated with reduced left ventricular ejection fraction (LVEF; $\rho = -.33$, $P = .01$), increased N-terminal pro-brain natriuretic peptide (NTproBNP) levels ($\rho = .35$, $P = 0.004$), enlarged descending aortic diameters ($\rho = .44$, $P = .001$), atrial fibrillation ($\phi = .43$, $P = .002$), and mitral valve surgery ($\phi = .34$, $P = .02$).

Of these, LVEF, NTproBNP-levels, atrial fibrillation, and mitral valve surgery were associated with AHI independently of age and BMI. We found similar association with oxygen desaturation index.

Conclusions: Sleep apnea exhibits increased frequency in the Marfan syndrome and is not predicted by classical risk factors. Both obstructive and central sleep apnea may relate to cardiovascular disease variables.

PATENT FORAMEN OVALE AND MARFAN SYNDROME: A NEW ASSOCIATION WITH DIFFERENCES BETWEEN CHILDREN AND ADULTS

Sánchez V¹, MD, Forteza A², MD, Lombera F¹, MD, Riva M¹, MD, Delgado JF¹, MD, Cortina JM², MD. ¹Cardiology Department and ²Cardiac Surgery Department. Hospital Doce de Octubre, Madrid, Spain. Patent foramen ovale (PFO) has been associated with aortic root dilatation and mitral valve prolapse (MVP), common features in Marfan Syndrome (MS), but there are no data about prevalence of PFO in MS.

Aim: To assess the prevalence of PFO in MFS patients and to determine if this feature is associated with other cardiovascular manifestations in 2 age cohorts of patients with MS.

Methods: 155 MS patients were evaluated by echocardiography: group I (≤ 16 years, $n = 30$), and group II (age > 16 years, $n = 110$). Aortic root dilatation was defined according to body surface area and age. For PFO study, imaging was performed in the apical four chambers view, with injection of 10 ml of agitated saline. The existence of PFO was determined by the presence of bubbles in the left heart within five cardiac cycles.

Results: A high prevalence of PFO was found: 83% in group I and 56% in group II. 33% of children and 16% of adult presented important shunt. In the multivariable analysis only atrial septum aneurysm was associated with PFO (OR 10.398, 95% CI = 2.33-46.42). No differences were found in the prevalence of aortic root dilatation or MVP.

Conclusions: Prevalence of PFO in MS was significantly higher than in general population, especially in children group. We suggest PFO could be part of the spectrum of disorders of connective tissue associated with MS and should be included in the echocardiographic study because of its clinical and surgical implications.

BIVENTRICULAR PERFORMANCE IN PATIENTS WITH MARFAN SYNDROME IN PATIENTS WITHOUT SIGNIFICANT VALVULAR DISEASE: COMPARISON TO NORMAL SUBJECTS AND LONGITUDINAL FOLLOW-UP

Scholte, Arthur, MD, PhD	Department of Cardiology, Leiden University Medical Center, the Netherlands
Scherptong, Roderick, MD	Department of Cardiology, Leiden University Medical Center, the Netherlands
Delgado, Victoria, MD	Department of Cardiology, Leiden University Medical Center, the Netherlands
Vliegen, Hubert, MD, PhD	Department of Cardiology, Leiden University Medical Center, the Netherlands
Van der Wall, Ernst, MD, PhD	Department of Cardiology, Leiden University Medical Center, the Netherlands
Hilhorst-Hofstee, Yvonne, MD	Department of Clinical Genetics, Leiden University Medical Center, the Netherlands
Bax, Jeroen, MD, PhD	Department of Cardiology, Leiden University Medical Center, the Netherlands

Objective:

The presence and progressive nature of primary myocardial involvement in patients with Marfan syndrome is subject of debate. The aim of the current study was to evaluate the clinical relevance of left ventricular (LV) and right ventricular (RV) strain, in adult patients with Marfan syndrome without significant valvular disease.

Methods and Results:

Marfan patients (n=50) were followed prospectively. Every two years during follow-up echocardiography, that included speckle tracking imaging, was performed. The baseline strain values of the Marfan population were compared to the values of normal controls and the follow-up evaluations were used to assess changes in ventricular strain. The association between the incidence of adverse events (heart failure, (supra) ventricular arrhythmias and proximal aorta surgery) and baseline strain values, was investigated.

As compared to controls, patients with Marfan syndrome had significantly lower peak longitudinal LV strain ($-18.9 \pm 2.3\%$ vs. $-20.1 \pm 1.9\%$, $P < 0.01$) and RV strain ($-26.9 \pm 5.2\%$ vs. $-29.3 \pm 4.25\%$, $P < 0.01$). Absolute changes in LV longitudinal, radial and circumferential strain and RV longitudinal strain were $0.1 \pm 2.8\%$, $1.12 \pm 7.6\%$, $0.3 \pm 3.7\%$ and $0.9 \pm 5.5\%$, respectively, which was not statistically significant. Cox regression demonstrated that reduced LV or RV strain was not associated with the incidence of adverse events (supraventricular arrhythmias, n=3; proximal aorta surgery, n=4).

Conclusion:

Adult patients with Marfan syndrome have significantly lower ventricular strain values as compared to healthy controls; however no relevant changes in left and right ventricular function occur during mid-term follow-up. Although strain is mildly reduced in Marfan patients, this does not affect outcome negatively.

AGE-RELATED REGIONAL CHANGES OF AORTIC COMPLIANCE IN THE MARFAN SYNDROME: ASSESSMENT WITH VELOCITY-ENCODED MRI

Westenberg Jos JM PhD¹, Scholte Arthur JHA PhD MD², van der Geest Rob J MSc¹, Radonic Teodora MD³, Groenink Maarten PhD MD³, Mulder Barbara JM PhD MD³, Reiber Johan HC PhD¹, de Roos Albert PhD MD¹

¹ Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands

² Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands

³ Department of Cardiology, Academic Medical Center, Amsterdam, The Netherlands

Objective:

To describe age-related changes in global and regional aortic compliance using velocity-encoded (VE) MRI in Marfan syndrome (MFS) as compared to healthy volunteers.

Methods:

Twenty-five MFS patients (age range: 18-63 years, mean age 36±14 years, 13 men) and 25 age/gender-matched healthy volunteers were examined with VE-MRI. Compliance was expressed as pulse wave velocity (PWV) and distensibility. PWV was assessed in the aortic arch (AA), descending (DA) and total aorta. Distensibility was assessed in the ascending aorta. PWV and distensibility were compared between patients and healthy volunteers using paired t-tests. The relation between age and PWV and distensibility was determined by stepwise multiple linear regressions with forward entry analysis and Pearson's correlation.

Results:

PWV in MFS patients was significantly higher in the AA, DA and total aorta compared to healthy volunteers (5.7±1.5m/s, 6.4±2.4m/s and 5.9±1.7m/s vs. 4.8±0.9m/s, 5.0±1.2m/s and 4.9±1.1m/s, all $p < 0.004$). Secondly, distensibility was significantly lower in patients ($4.5 \pm 2.6 \cdot 10^{-3} \text{ mmHg}^{-1}$ vs. $6.7 \pm 4.3 \cdot 10^{-3} \text{ mmHg}^{-1}$, $p = 0.002$).

All PWV-values correlated significantly with age (Pearson R between 0.45 and 0.94), for both patients as well as for healthy volunteers. In the AA, the yearly PWV-increase was significantly higher in patients (mean increase 7±2 cm/s/year) than in healthy volunteers (mean increase 3±1 cm/s/year, $p = 0.03$), but not for DA and total aorta.

Conclusions:

Velocity-encoded MRI detects age-related changes of aortic wall compliance in patients with Marfan syndrome. Aortic arch compliance decreases more pronounced with age in Marfan patients, suggesting more severe wall disease in the ascending aorta.

AUGMENTATION INDEX PREDICTS CARDIOVASCULAR DISEASE IN ADULTS WITH MARFAN-LIKE FEATURES

Sheikhzadeh, Sara, MD, Centre of Cardiology an Cardiovascular Surgery, University Hospital Eppendorf, Hamburg, Germany

Mortensen, Kai, MD, Centre of Cardiology, University Hospital Lübeck, Lübeck, Germany

Rybczynski, Meike, MD, Centre of Cardiology an Cardiovascular Surgery, University Hospital Eppendorf, Hamburg, Germany

v. Kodolitsch, Yskert, MD, Centre of Cardiology an Cardiovascular Surgery, University Hospital Eppendorf, Hamburg, Germany

Objectives:

Non-invasive applanation tonometry (APT) can be used to assess aortic stiffness. The method is established to predict cardiovascular disease in arterial hypertension and other conditions, but it has not been tested in patients with Marfan-like (MFL) features including Marfan syndrome (MFS), with a high risk for aortic aneurysm.

Methods and results: We performed APT to assess aortic stiffness in 155 consecutive persons with MFL features, in whom we previously confirmed MFS in 97 individuals (group 1) and established other connective tissue diseases, both with vascular involvement in 21 (group 2) and without vascular involvement in 9 (group 3) and excluded any distinct disease in 28 persons (group 4). Linear regression analysis identified age ($P < 0.0001$), gender ($P = 0.007$), heritable aortic syndrome ($P < 0.0001$), previous conduit operation ($P = 0.009$), current β -blocker ($P = 0.007$) or ACE inhibitors medication ($P = 0.02$) and cardiovascular severity score according to Gray and Davies ($P = 0.0001$) as determinants of augmentation index ($Alx@HR75$), but excluded an impact of skeletal and ocular severity scores. Multivariable regression analysis confirmed age, gender and cardiovascular severity scores as independent predictors of increased $Alx@75HR$. In contrast to peripheral vascular parameters, linear regression analysis corroborated association of cardiovascular severity with other central stiffness parameters including central systolic blood pressure ($P = 0.04$), central pulse pressure ($P < 0.0001$) and pulse wave velocity ($P = 0.02$).

Conclusions:

We provide evidence that APT assesses aortic stiffness parameters that relate to cardiovascular disease severity in patient with MFL features. We believe that APT should play a future role to improve risk stratification in the clinical setting of MFL features.

IMPROVED SCREENING FOR AORTIC ROOT DILATION BY TRANSTHORACIC ECHOCARDIOGRAPHY

Shiran, Hadas MD, Haddad, Francois MD, and Liang, David MD, PhD, Department of Cardiovascular Medicine, Stanford University Hospital, Stanford, CA, USA

Intro: Aortic root dilation is a potent risk factor for aortic complications. TTE reliably assesses aortic root size, which is currently best correlated with BSA, but only weakly in older patients ($r=0.4$). Accurately detecting aortic dilation can help optimize surgical intervention and minimize complications.

Objective: To use internal cardiac structures, specifically LVOT size, to screen for aortic root dilation.

Methods: Images from the Stanford echocardiography database were reviewed and measures of the aortic root, at the level of the sinuses of Valsalva, and the LVOT, were performed on parasternal long axis view. Studied were 76 normal controls and 74 patients with known cardiovascular disease selected at random. Patients with poor image quality, history of aortic root/valve repair or replacement, malformed aortic valves, cardiac transplant, and significant aortic regurgitation were excluded.

Results: There were 79 women and 71 men, with mean age of 49 ± 18 years, height 169 ± 10 cm, and weight 74 ± 23 kg. The mean aortic root diameter was 3.26 ± 0.31 cm and LVOT 2.15 ± 0.24 cm. Overall, there was excellent correlation between aortic root and LVOT diameters with $r=0.82$, $p<0.0001$ (Figure 1). For men, $r=0.69$, and for women, $r=0.74$. For the controls, $r=0.77$, and $r=0.9$ for the patients. The correlation of aortic root diameter was 0.58 with BSA, 0.32 with BMI, 0.55 with height, and 0.49 with weight ($p<0.0001$ for all).

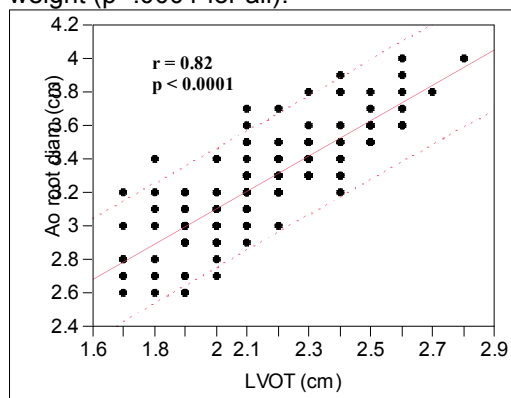


Figure 1: Bivariate fit model, 95% confidence limits

Conclusion: LVOT size on TTE is an excellent parameter for screening of aortic root dilation in adults and is more accurate than the current practice of utilizing BSA.

PROTRUSIO ACETABULI AND TOTAL HIP ARTHROPLASTY (THA) IN PATIENTS WITH MARFAN SYNDROME

Thakkar, Savyasachi MD¹; Foran, Jared MD²; Mears, Simon MD-PhD¹; Sponseller, Paul MD¹

¹ Johns Hopkins Hospital and Johns Hopkins Bayview Medical Center,
Department of Orthopaedic Surgery,
Baltimore, Maryland, USA

² Rush University Medical Center,
Department of Orthopaedic Surgery,
Chicago, Illinois, USA

Introduction:

Our objectives were to: (1) identify radiographic features of Marfan patients needing THA and (2) study THA results in this group.

Methods:

29 Marfan patients (38 hips) having THA were enrolled. Pre-operative radiographs were studied for: (1) center-edge angle (CEA) of Wiberg, (2) acetabular-ilioischial (AK) distance and (3) osteoarthritis. Protrusio was defined by either CEA or AK distance. Radiographs were compared with a Marfan database of non-operative patients. Patient records and post-operative radiographs were studied. Patients completed a hip-rating questionnaire to assess post-operative function.

Results:

Protrusio was more prevalent in the THA population than the Marfan database ($p < 0.05$). The mean age at index THA was 44.6 ± 17 years. More severe protrusio was associated with THA at younger ages ($p < 0.05$). Osteoarthritis was also common. 61% prostheses were uncemented, 29% were cemented and 10% were hybrids. Only 10.5% of hips received acetabular reinforcement with bone graft. The mean follow-up time was 127 months (range 29 to 384 months). The mean time to revision was 161 months, with 78% implant survival until 384 months. 10.5% of acetabular and 2.6% of femoral components were loose prior to revision; dislocation frequency was high (10.5%), and two hips (5.3%) were infected. The mean questionnaire score was 82 ± 13 points indicating good hip function, similar to the published mean (88.1 ± 12.6) in non-Marfan patients at 1 year after THA.

Conclusions:

Protrusio acetabuli and osteoarthritis are commonly observed in Marfan patients requiring THA. We have shown THA in Marfan syndrome to have a higher risk of dislocation.

AN ACTA2 GENETIC VARIANT IN A FAMILY PRESENTING WITH A THORACIC AORTIC DISSECTION AND A CAROTID ANEURYSM

Linnea M. Baudhuin, Ph.D., Katrina Kotzer, M.S., Susan Lagerstedt, B.S., Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

Objectives: Intracranial aneurysms (IA) and aortic aneurysms (AA) can occur within the same families and common genetic mechanisms between IAs and AAs have been suggested. Mutations in the *ACTA2* gene are the most predominant known genetic cause of familial thoracic aortic aneurysm and dissection (FTAAD). Our objective was to determine whether or not the *ACTA2* gene was associated with the presentation of IA and AA in the same family.

Methods: The index family included the proband: a 46 year old male who died suddenly from a thoracic aortic dissection; his three unaffected children, ages 16, 18, and 20; and his sister (age 45) who had a right internal carotid aneurysm. All of the coding exons and flanking regions of *ACTA2* were sequenced by bi-directional, automated fluorescent sequencing.

Results: We identified the presence of an *ACTA2* mutation, namely c.1126T>C (p.Cys376Arg) in exon 9 in the proband, his sister, and two of his children. This variant was not present in 260 control chromosomes and the cysteine (Cys) to arginine (Arg) mutation is predicted to be deleterious.

Conclusions: An *ACTA2* mutation may be a novel cause of AA and IA in the same family. Whether or not *ACTA2* mutations are involved in a larger scope of cases of IA warrants further investigation.

ROLE OF ADAMTSL4 MUTATIONS IN FBN1 MUTATION NEGATIVE ECTOPIA LENTIS PATIENTS

Child A. H., FRCP, Cardiac and Vascular Sciences, St George's, University of London, London, United Kingdom

Aragon-Martin J.A. PhD, Cardiac and Vascular Sciences, St George's, University of London, London, United Kingdom

Ahnood D., Medical Student, Cardiac and Vascular Sciences, St George's, University of London, London, United Kingdom

Saggar A., MRCP Medical Genetics, St. George's, University of London, United Kingdom.

Nischal K., FRCOphth, Department of Ophthalmology, Hospital for Sick Children, London, UK.

Charteris D., FRCOphth, Vitreoretinal Surgery, Moorfields Eye Hospital, London, United Kingdom.

Arno G., PhD, Cardiac and Vascular Sciences, St George's, University of London, London, United Kingdom

Objectives

Although clinically homogeneous, ectopia lentis (EL) is genetically heterogeneous with both autosomal-dominant (MIM 129600) and autosomal-recessive (MIM 225100) forms. The dominant disorder can be caused by mutations in FBN1, at the milder end of the type-1 fibrillinopathy spectrum. Recently in a consanguineous Jordanian family, recessive EL was mapped to locus 1q21 containing the ADAMTSL4 gene and a nonsense mutation found in exon 11 (c.1785T>G, p.Y595X) of ADAMTSL4. Could we demonstrate ADAMTSL4 mutations in our UK ectopia lentis patients?

Methods

In this study, 12 consecutive Caucasian U.K. probands with EL and demonstrating no, or very mild, heart involvement on echocardiogram were included. Probands did not fulfil the Ghent criteria for Marfan syndrome and were previously found mutation-negative for FBN1. Mutation screening in ADAMTSL4 by direct sequencing of all exons including their intron/exon boundaries was performed.

Results

Homozygous or compound heterozygous mutations were identified in 6/12 (50%) probands. Mutation data are summarised in table 1. Where available, familial screening of these families confirmed the mutation co-segregated with the EL phenotype. None of the ADAMTSL4 mutations described here were identified in 156 normal control chromosomes.

Conclusions

This study is the first confirmation that homozygous mutations in ADAMTSL4 are associated with autosomal recessive EL. The first compound heterozygous mutations are described. The identification of a causative mutation in ADAMTSL4 may allow exclusion of Marfan syndrome in these families and guide genetic counselling and clinical management, of particular relevance in young children affected by EL.

Mutations in ADAMTSL4 in Ectopia Lentis Patients**Table 1**

Case	Age	Exon	Nucleotide	Amino acid	Domain	Zygotity
1		6	c.767_786del	p.Gln256ProfsX38	-	Compound heterozygous
		6	c.826_836del	p.Arg276SerfsX21	-	
2	8	6	c.767_786del	p.Gln256ProfsX38	-	Compound heterozygous
		6	c.826_836del	p.Arg276SerfsX21	-	
3	15	6	c.826_836del	p.Arg276SerfsX21	-	Homozygous
4	41	12	c.1960C>T	p.Pro654Ser	-	Homozygous
5	34	12	c.2008C>T	p.Arg670X	ADAM TSR-1	Homozygous
6	4	19	c.3153C>A	p.Tyr1051X	PLAC domain	Compound heterozygous
		19	c.3161A>G	p.Tyr1054Cys	PLAC domain	
		6	c.926G>A	p.Arg309Gln	-	

ISOLATED ECTOPIA LENTIS: REPORT OF A NEW DELETION IN THE *ADAMTSL4* GENE AND EVIDENCE FOR GENETIC HETEROGENEITY OF THE AUTOSOMAL RECESSIVE FORM OF THE DISEASE

Hanna Nadine PhD^{1,2}, Sultan Gilles MD³, Muti Christine MD², Grandchamp Bernard MD, PhD², Gouya Laurent MD, PhD^{1,2}, Funtowicz Sarah², Lacombe Didier MD⁴, Dollfus Hélène MD, PhD⁵, Baudouin Christophe MD, PhD^{3,6}, Jondeau Guillaume MD, PhD², Boileau Catherine PhD^{1,2,7}.

1. Laboratoire de Génétique moléculaire, Hôpital Ambroise Paré, AP-HP, Université Versailles-Saint Quentin en Yvelines, Boulogne, France.
2. Centre de Référence Marfan, Hôpital Bichat, AP-HP, Paris, France.
3. Service d'Ophtalmologie, Hôpital Ambroise Paré, AP-HP, Boulogne, France.
4. Service de Génétique Médicale, Hôpital Pellegrin, CHU de Bordeaux, Université Bordeaux II, France.
5. Centre de Référence pour les Affections Rares en Génétique Ophtalmologique (CARGO) et Service de Génétique Médicale, Hôpitaux Universitaires de Strasbourg, Strasbourg, France.
6. Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris, France.
7. Inserm U781, Hôpital Necker-Enfants Malades, Paris, France.

Introduction:

Often associated with systemic diseases (such as homocystinuria, Marfan syndrome or Weill-Marchesani syndrome), ectopia lentis (EL) can appear as an isolated condition with autosomal dominant (ADEL, OMIM#1296000) or autosomal recessive (AREL, OMIM#225100) inheritance. ADEL has been associated with mutations within the *FBN1* gene, while only two mutations have been identified to date in the *ADAMTSL4* gene in families with AREL (Ahram *et al.*, 2009; Greene *et al.*, 2010).

Objective:

Evaluate the contribution of *ADAMTSL4* gene mutations to isolated EL.

Methods:

We studied 12 French probands negative for homocystinuria and with no mutation within the *FBN1* gene. Bidirectional sequencing of the 17 coding exons of the *ADAMTSL4* gene was performed. When available, family studies were performed and regional haplotypes were constructed with 9 microsatellite markers flanking the gene at 1q21.3.

Results:

An unreported and identical homozygous frameshift deletion was found in 2 unrelated probands: c.767_786del, p.Gln256ProfsX38. Family analysis showed that the mutation was carried on two different haplotypes, one proband carrying the two haplotypes. In the 10 remaining probands, no mutation was identified in the *ADAMTSL4* gene to explain the EL phenotype. Interestingly, one of these probands belonged to a small French family of Gypsy origin with 4 affected children. None of the affected children were haplo-identical thus demonstrating exclusion of the gene locus. In the same way, linkage was also excluded to the *FBN1* gene.

Conclusion:

In a small sample of EL probands, only 2/12 (16 %) of EL cases were related to a mutation in the coding sequence of the *ADAMTSL4* gene. Furthermore, we report a family with AREL unlinked to either of the known genes associated with EL. This family demonstrates the existence of further genetic heterogeneity in isolated EL.

HEMIZYGOUS DELETION COMPRISING COL3A1 AND COL5A2 CAUSES AORTIC DISSECTION

Meienberg Janine, MSc, Division of Medical Molecular Genetics, Institute of Medical Genetics, University of Zurich, Zurich, Switzerland; Rohrbach Marianne, MD, PhD, Division of Metabolism, University Children's Hospital, Zurich, Switzerland; Neuenschwander Stefan, PhD, Functional Genomics Center Zurich, ETH and University of Zurich, Zurich, Switzerland; Spanaus Katharina, MD, Institute for Clinical Chemistry, University Hospital, Zurich, Switzerland; Giunta Cecilia, PhD, Division of Metabolism, University Children's Hospital, Zurich, Switzerland; Alonso Sira, MSc, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland; Arnold Eliane, MSc, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland; Henggeler Caroline, MSc, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland; Perez Regina, MSc, Division of Medical Molecular Genetics, Institute of Medical Genetics, University of Zurich, Zurich, Switzerland; Regenass Stephan, MD, Division of Clinical Immunology, University Hospital, Zurich, Switzerland; Azzarello-Burri Silvia, MD, Division of Medical Genetics, Institute of Medical Genetics, University of Zurich, Zurich, Switzerland; Carrel Thierry, MD, Clinic for Cardiovascular Surgery, University Hospital, Berne, Switzerland; Berger Wolfgang, PhD, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland; Steinmann Beat, MD, Division of Metabolism, University Children's Hospital, Zurich, Switzerland; Mátyás Gábor, PhD, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland

Aortic dilatation/dissection (AD) can occur spontaneously, non-syndromic or in association with genetic syndromes, such as Marfan syndrome (MFS) caused by *FBN1* mutations, Loeys-Dietz syndrome caused by *TGFBR1* or *TGFBR2* mutations, and vascular Ehlers-Danlos syndrome (EDS IV) caused by *COL3A1* mutations. Although mutations in *FBN1*, *TGFBR1*, and *TGFBR2* account for the majority of AD cases referred to us, we have encountered negative genetic testing results in a large group of patients, suggesting the involvement of other genes, e.g. *COL3A1*, *ACTA2* or *MYH11*. In this study, we have assessed the impact of *COL3A1* mutations in patients with suspected MFS in whom mutation screening in *FBN1*, *TGFBR1* and *TGFBR2* revealed no disease-causing sequence variation. MLPA analysis of 100 unrelated patients identified hemizygous deletion of the entire *COL3A1* gene in one patient with abdominal AD. Subsequent microarray analyses and sequencing of breakpoints revealed the deletion size of 3,408,306bp. This deletion affects not only *COL3A1* but also *COL5A2*, mutations in which have been associated with the classical type of EDS. Our data suggest that the AD phenotype is most likely caused mainly by the true haploinsufficiency of *COL3A1* and the haploinsufficiency of *COL5A2* may have merely a modifier effect. Furthermore, gDNA sequencing revealed *COL3A1* sequence variants in some of our patients. Our data not only emphasize the importance of screening for *COL3A1* mutations in comprehensive genetic testing of AD patients with suspected MFS not fulfilling the Ghent criteria, but also extend the molecular etiology of EDS IV by providing evidence for true haploinsufficiency of *COL3A1*.

**ASCENDING ANEURYSMS WITH A BICUSPID AORTIC VALVE
- WHAT CAN WE LEARN FROM MARFAN SYNDROME?**

Mohamd SA^a, Radtke A^a, Nimzyk R^b, Bullerdiek J^b, Belge G^b, Sievers HH^a

^a*Department of Cardiac Surgery, University of Schleswig-Holstein Campus Luebeck, Luebeck, Germany,*

^b*Center for Humangenetics, University of Bremen, Bremen, Germany*

POSTER

Background: Aneurysm is a common disease among elderly people and usually in patients with bicuspid aortic valve (BAV). There are similarities in the histology of aneurysmal tissue of the aorta in Marfan syndrome patients and in patients with BAV with areas of more or less features of cystic medial necrosis. Since so far no non-surgical therapy against aortic aneurysm is available the need for preventative measures is evident.

Material and Methods: We looked for targets as well as triggers of apoptotic signaling in primary smooth muscle cells isolated from areas of aneurysm (convexal) versus cells from the concaval aortic site in nine BAV patients with aneurysm (mean age 58.7±14.8 years). The damage of the extracellular matrix (ECM) was monitored in 41 patients with thoracic aortic aneurysms (aortic diameter 53,7±5,6 mm/ 31m, 10f).

Results: Cells of the concave aortic site survived significantly more compared with the convex site (63.1±6.3 vs 51.9±9.2% vital cells, $p = 0.008$). A significant amount of MMP-2 (118,5±61,2 vs 96,0±47,6 pg/ml, $p = 0,04$), MMP-8 (7,1 vs 2,8 pg/ml, $p = 0,001$), MMP-9 (18,3 vs 8,9 pg/ml, $p = 0,008$), TIMP-2, TIMP-3 and TIMP-4 was detected in aneurysmal aorta compared to non-aneurysmal areas in multiplex system.

Conclusions: Our preliminary data demonstrate apoptosis as an early event in aneurysm. At a later stage, activation of MMPs plays a major role in the destabilization of the ECM.

A NOVEL GENETIC PATHWAY UNDERLIES WEILL-MARCHESANI SYNDROME

Gerhard Sengle¹, Ko Tsutsui^{1,2,3}, Douglas R. Keene³, Eric J. Carlson¹, Noe L. Charbonneau³, Mary K. Wirtz⁴, John R. Samples⁴, Susan J. Hayflick⁵, Lisa I. Fessler⁶, John H. Fessler⁶, Kiyotoshi Sekiguchi², and Lynn Y. Sakai^{1,3}

¹Department of Biochemistry and Molecular Biology, Oregon Health & Science University

²Division of Protein Chemistry, Institute for Protein Research, Osaka University

³Shriners Hospital for Children, Portland OR

⁴Casey Eye Institute, Oregon Health & Science University

⁵Department of Molecular and Medical Genetics, Oregon Health & Science University

⁶Department of Molecular, Cell and Developmental Biology, UCLA

We have identified a novel 3 domain deletion in FBN1 that results in autosomal dominant Weill-Marchesani syndrome (WMS). While individuals with Marfan syndrome are tall with hypomuscularity and hypermobile joints, individuals with WMS display the “opposite” phenotypes of short stature, hypermuscularity, and stiff joints. *Objectives:* To investigate the underlying molecular mechanisms leading to WMS. *Methods:* Both in vitro and in vivo approaches were used. Routine in vitro interaction studies (surface plasmon resonance; co-immunoprecipitation) were performed. We also analyzed mice (using histological stains, immunoelectron microscopy, microCT, and qPCR) in which our WMS mutation in Fbn1 was knocked in. *Results and Conclusions:* WMS mutant mice survived well in homozygosity and did not display aortic disease, emphasizing again that this 3 domain deletion does not result in Marfan syndrome in humans or mice. WMS mutant mice, in heterozygosity and homozygosity, demonstrated a thick skin phenotype, phenocopying one of the features of human WMS. Our biochemical interaction studies showed that the deleted FBN1 domains serve as a binding site for Adamtslike-2, -3, -6, and papilin and that Adamtslike-3 interacts with ADAMTS10. Based on these results, we hypothesize that Adamtslike proteins form complexes with Adamts enzymes and that Adamtslike proteins bind to fibrillin-1, targeting the complex to microfibrils. Furthermore, in the absence of this binding site, we hypothesize that Adamts10 is not properly targeted and sequestered in the matrix, resulting in WMS.

POSTNATAL FUNCTION OF TRANSFORMING GROWTH FACTOR BETA2 IN CARDIOVASCULAR DISEASE

Azhar, Mohamad, Ph.D., BIO5 Institute, University of Arizona, Tucson, AZ, USA

Nusayr, Eyad, B.S., BIO5 Institute, University of Arizona, Tucson, AZ, USA

Haskett, Darren, B.S., Department of Aerospace & Mechanical Engineering, University of Arizona, Tucson, AZ, USA

Utzing, Urs, Ph.D., Dept of Biomedical Engineering, University of Arizona, Tucson, AZ, USA

Vande Geest, Jonathan, Ph.D., Department of Aerospace & Mechanical Engineering, University of Arizona, Tucson, AZ, USA

Objectives: Although Transforming Growth factor beta2 (TGF β 2beta2) is required for cardiovascular development, the postnatal function of TGFbeta2 in cardiovascular system remains unknown.

Methods: Characterization of various genetic mouse combinations using in vivo cardiovascular imaging and closed-chest cardiac function (pressure-volume loop) analysis, histological and morphometric examination, and experimental AngII-induction of cardiovascular disease in mice. Microbiaxial optomechanical analysis of unfixed mouse aortas simultaneously determined both the macroscopic biomechanical response (pressure and diameter) and microscopic (matrix fiber content, fiber length, interfiber space, average fiber orientation) properties of the aortic walls.

Results: Echocardiographic examination and closed-chest cardiac function analysis indicated cardiomyopathy and cardiac dysfunction in *Tgfb2* heterozygous animals. Short-term AngII-infusion of *Tgfb2* heterozygous animals worsened the cardiomyopathy and drastically reduced the cardiac function as compared to the saline-infused *Tgfb2* heterozygous mice. More importantly, AngII-infused but not saline-infused mice were suddenly died during the course of their cardiac function examination (invasive procedure requires anesthesia) indicating the inability of AngII-infused *Tgfb2* heterozygous animals to handle any further stress. Interestingly, echocardiographic examination revealed that AngII-infusion also selectively caused significant aortic aneurysm in *Tgfb2* heterozygous mice as compared to saline-infused *Tgfb2* heterozygous animals. Finally, microbiaxial optomechanical analysis indicated significant worsening of both microstructural and biomechanical responses in the *Fibrillin-1/Tgfb2* double heterozygous animals as compared to *Tgfb2* or *Fibrillin-1* heterozygous mutant animals.

Conclusions: Postnatal reduction of TGFbeta2 predisposes mice to a progressive but asymptomatic cardiovascular disease. Further insult such as AngII-infusion or *Fibrillin-1* mutation leads to a symptomatic or active cardiovascular disease in *Tgfb2* heterozygous animals.

ROLE OF TRANSFORMING GROWTH FACTOR BETA3 IN ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY/DYSPLASIA TYPE 1 (AVRD1)

Azhar, Mohamad, Ph.D., BIO5 Institute, University of Arizona, Tucson, AZ, USA

Nusayr, Eyad, B.S., BIO5 Institute, University of Arizona, Tucson, AZ, USA

Connie Gard, B.S., BIO5 Institute, University of Arizona, Tucson, AZ, USA

Objectives: ARVC/D is characterized by right ventricular aneurysm and dilatation and fibrosis of the myocardium that predispose to sudden death in young individuals and athletes. TGFB3 was identified as the disease gene involved in the ARVC/D1. However, it is currently unknown how mutations in *TGFB3* cause ARVC/D.

Methods: Histological, morphometric, immunohistochemical and molecular approaches. In vivo cardiovascular imaging and closed-chest cardiac function (pressure-volume loop) analysis, and experimental AngII-infusion to induce cardiovascular disease.

Results: The data showed right ventricular aneurysm in *Tgfb3*^{+/-}*Tgfb2*^{-/-} embryos (E14.5). *Tgfb3*^{-/-}*Tgfb2*^{+/-} embryos were normal but *Tgfb3*^{-/-}*Tgfb2*^{-/-} embryos exhibited right ventricular dilatation and complete detachment of epicardium from the surface of the myocardium. Interestingly, *Tgfb2*^{-/-} mice developed dilated cardiomyopathy at birth. TGFβ signaling (pSMAD2 and CTGF) was reduced in the ventricular myocardium of *Tgfb2*^{-/-} mice at E18.5. *Tgfb2*^{-/-} embryos died at birth and they displayed cardiac dilatation and cardiomyopathy. Echocardiography showed that *Tgfb2*^{+/-} animals developed progressive but asymptomatic cardiomyopathy. AngII-infusion and subsequent anesthesia (avertin) resulted in the worsening of the cardiomyopathy and sudden cardiac death of adult *Tgfb2*^{-/-} mice. Finally, analysis of *Tgfb2/Tgfb3* double heterozygous animals showed extensive fibrosis as compared to the wild-type control mice.

Conclusions: Reduced *Tgfb3* leads to a higher susceptibility to ARVC/D and sudden cardiac death. Although no mutations are yet found in *Tgfb2* in humans, our data have revealed a complementary role for TGFβ2 in the pathogenesis of ARVC/D.

CHARACTERIZATION AND TREATMENT OF OSTEOPENIA IN MICE WITH SEVERE MARFAN SYNDROME

Luca Carta, Harikiran Nistala, Sui Lee-Arteaga, Jason Cook, Silvia Smaldone, Aaron Rifkin, and Francesco Ramirez.

Department of Pharmacology and Systems Therapeutics at the Mount Sinai School of Medicine, New York, NY 10021

Introduction:

Reduced bone mineral density (osteopenia) is a poorly characterized manifestation of pediatric and adult patients afflicted with Marfan syndrome (MFS). However, previous studies have not clarified the role of fibrillin-1 mutations in bone loss.

Objective:

The present study investigated the impact of fibrillin-1 deficiency on bone homeostasis in a mouse model of progressively severe MFS (*Fbn1*^{mgR/mgR} mice), in addition to comparing the efficacy of losartan treatment on bone remodeling and aortic disease in these mutant mice.

Method:

In vivo analyses revealed that *Fbn1*^{mgR/mgR} mice are osteopenic and display a greater response to experimentally induced osteolysis. Cell culture experiments correlated these in vivo findings with enhanced osteoblast-supported osteoclastogenesis, which was largely attributed to TGFβ-induced up-regulation of RANKL expression. Based on these observations, we compared the effects of losartan and alendronate on aortic wall degeneration and loss of bone mass in *Fbn1*^{mgR/mgR} mice, as the former drug improves TGFβ-driven aortic aneurysm in *Fbn1*^{C1039G/+} mice and the latter one restricts osteoclast activity. Accordingly, bone and aortic tissue were evaluated in wild-type and *Fbn1*^{mgR/mgR} mice that were treated postnatally with losartan for 8 or 16 weeks or with alendronate for 8 weeks. Losartan treatment mitigated aortic aneurysm progression but not bone loss. Conversely, a significant improvement of bone quality was observed following alendronate treatment without any beneficial effect on vascular disease.

Conclusions:

Increased bone resorption is the main contributor to osteopenia in MFS mice and is largely accounted for by TGFβ-dependent stimulation of osteoblast-driven osteoclastogenesis. Losartan treatment is ineffective in treating bone loss

IN VIVO DELETION OF THE FIRST HYBRID DOMAIN IN FIBRILLIN-1

Noe L. Charbonneau¹, Gerhard Sengle², Sara F. Tufa¹, Francesco Ramirez³, Douglas R. Keene¹, and Lynn Y. Sakai^{1,2}

¹Department of Biochemistry and Molecular Biology, Oregon Health & Science University

²Shriners Hospital for Children, Portland, OR 97239

And, ³Mt. Sinai School of Medicine, New York, NY 10029

The first hybrid domain in fibrillin-1 was implicated in intermolecular disulfide bond formation and microfibril assembly (Reinhardt et al., *J. Biol. Chem.*, 2000) and in interactions with LTBP-1 and LTBP-4 (Isogai et al., *J. Biol. Chem.*, 2003; Ono et al., *J. Biol. Chem.*, 2009). Marfan syndrome is caused by mutations in FBN1, and in mice, mutations in Fbn1 lead to activation of TGF β signaling (Neptune et al., *Nature Genet.*, 2003; Habashi et al., *Science*, 2006). **Objectives:** To determine whether in vivo deletion of the first hybrid domain results in activation of TGF β signaling and in disease phenotypes similar to those found in Marfan syndrome. **Methods:** Hybrid 1 (H1) was conditionally deleted (Δ) in all cells by gene targeting and Cre-mediated recombination in mice. To simplify analyses, H1 Δ mice were bred onto an Fbn2 null background. The aortic root (from P0 through 10 months of age) was examined for fragmentation of the elastic lamellae. Histology and immunolocalization of LTBP-1, LTBP-4, and the fibulins were analyzed. MicroCT and qPCR were performed. **Results and Conclusions:** H1 Δ mice are the first Fbn1 mutant mice to survive well in homozygosity. The H1 Δ mutation does not interfere with microfibril assembly (Charbonneau et al., *J. Biol. Chem.*, 2010). Deletion of the first hybrid domain on an Fbn2 null background results in loss of LTBP-1 and LTBP-4 immunolocalization and developmental defects in the aortic root. Double homozygous mice are very small. Results suggest that inappropriate sequestration of the large latent complex may result in loss of TGF β signaling.

CONDITIONAL INACTIVATION OF FIBRILLIN-1 IN AORTIC TISSUE COMPARTMENTS

Jason R. Cook, Sui Lee-Arteaga, Luca Carta, Harikiran Nistala, Maria del Solar and Francesco Ramirez.

Department of Pharmacology and Systems Therapeutics at the Mount Sinai School of Medicine, New York, NY 10021

Introduction: Dissecting thoracic aortic aneurysm (TAA) in Marfan syndrome (MFS) is associated with elevated TGF β activity secondary to structural or quantitative defects in fibrillin-1 (*FBN1*). Perinatal lethality of mice with germ line inactivation of *Fbn1* has underscored the importance of fibrillin microfibrils in aortic growth and homeostasis without, however, identifying the main tissue compartment contributing to TAA.

Objective: Determine the contribution of fibrillin-1 production by vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) in MFS vascular pathology.

Method: The aortic wall is composed of the intima (ECs), media (VSMCs) and adventitial (fibroblasts) layers. A new line of mutant mice that harbor a conditional allele of *Fbn1* was therefore created to allow the selective study of fibrillin-1 in unique tissues. Female mice with the conditional allele (*Fbn1* ^{Δ neo}) were crossed with male transgenic mice harboring a germ line null allele (*Fbn1*^{mgN}) and expressed Cre specifically in either the forming VSMC (*Sm22 α -Cre*) or ECs (*Cdh5-Cre*). The germ line null allele was paired with the conditional allele to increase the probability of tissue specific Cre excision. Tissue specific Cre excision was monitored by crossing the tissue specific mice with RosaLacZ reporter mice.

Results: Inactivation of fibrillin-1 in VSMCs (*Fbn1* ^{Δ neo/mgN}; *Sm22 α -Cre*⁺) replicates the germ line null phenotype with elastic fiber fragmentation, aortic aneurysm, and death due to dissection by P14.

Conclusions: Lack of fibrillin-1 production by VSMCs in the media of the ascending aorta is necessary and sufficient for the development of TAA.

DYNAMIC REGULATION OF THE FIBRILLIN-LIKE FBN-1 PROTEIN

Meli, Vijaykumar, Ph.D. and Frand, Alison, Ph.D.

Department of Biological Chemistry, David Geffen School of Medicine
University of California, Los Angeles, United States

The molting cycle of *C. elegans* provides an exemplary opportunity to study the dynamic production and destruction of fibrillin matrices in a model organism amenable to functional genomic and cell biological analysis. The *fbn-1* gene specifies the homolog of human *fibrillin-1* and *-2*, the major components of extracellular fibrils defective in Marfan Syndrome (MFS) and related disorders of connective tissue. FBN-1/fibrillin is essential for molting, a rapid and reiterated developmental process that involves the systemic regeneration of collagen-rich extracellular matrices and related attachments to the underlying epidermis. Similar to the pathology of MFS, the phenotype of *fbn-1* mutants relates to structural defects in the exoskeleton; aberrant signaling by TGF- β -like hormones; and the action of particular matrix metalloproteases including NAS-37. An *fbn-1* reporter gene is transiently expressed in epidermal cells and syncytia whenever the exoskeleton is remade. The conserved nuclear hormone receptor NHR-23 triggers this periodic expression, indicating that steroid hormones regulate the synthesis of FBN-1. Our current research tracks the dynamic assembly and disassembly of FBN-1/fibrillin fibrils during the process of molting in real time, using 3-D confocal fluorescence microscopy. Moreover, we are systematically identifying and characterizing additional genes that promote the formation of FBN-1 fibrils among candidates isolated in previous, full genome RNAi screens for molting defects. We expect to discover additional conserved factors that interact genetically or biochemically with FBN-1/fibrillin. The clinical outcome of MFS and related disorders may depend on heritable variations in the human counterparts of these factors.

AORTIC DISEASE IN A NEW MOUSE MODEL OF MARFAN SYNDROME

Elise C. Manalo¹, Jamie R. Langdon¹, Sara F. Tufa², Eric J. Carlson¹,
Noe L. Charbonneau², Douglas R. Keene², and Lynn Y. Sakai^{1,2}

¹Department of Biochemistry and Molecular Biology, Oregon Health & Science University
And ²Shriners Hospital for Children, Portland, OR 97239

Heterozygous GT-8 mice (“GFP-tagged and Truncated Fbn1 mutants from founder 8”) display progressive fragmentation of elastic lamellae in the aortic root (Charbonneau et al., *J. Biol. Chem.*, in press, 2010). By 8 months of age, aortic elastic lamellae are severely fragmented in this mouse model. **Objectives:** To determine whether doxycycline or losartan treatments can rescue aortic dilatation and fragmentation of elastic lamellae. **Methods:** Treatments were initiated when mice were 2 months old. Established protocols were used (Xiong et al., *J. Vasc. Surg.*, 2008; Habashi et al., *Science*, 2006). Mice were sacrificed and examined at 8 months of age (6 months on treatment protocols), 6 months of age (4 months on treatments) and 4 months of age (2 months on treatments). There were 6 groups of mice at each timepoint: untreated wildtype and heterozygous GT-8 littermates; wildtype and heterozygous GT-8 littermates treated with doxycycline; wildtype and heterozygous GT-8 littermates treated with losartan. Aortic roots from all groups were examined for fragmentation of elastic lamellae. In addition, plasma from all groups was collected and tested for the presence of fibrillin-1 and fibrillin-2 fragments, using a sandwich ELISA. Other tissues were also evaluated for improvement by these treatments. **Results and Conclusions:** Both treatments substantially ameliorated aortic root dilatation. Outcomes are currently being analyzed to determine whether these treatment protocols can reveal informative differences in molecular or cellular mechanisms that are differentially targeted.

ADAMTSL6 β RESCUES MICROFIBRIL DISORDER IN MARFAN SYNDROME THROUGH THE PROMOTION OF FIBRILIN-1 ASSEMBLY

Saito Masahiro DDS Ph.D¹, Kurokawa Misaki¹, Ooshima Masamitsu DDS PhD², Tsutsui Ko PhD⁴, Hada Yasunobu^{1,5}, Sekiguchi Kiyotoshi PhD⁴ and Takashi Tsuji PhD^{1,2,3}

¹Faculty of Industrial Science and Technology, Tokyo University of Science, Chiba, Japan ²Research Institute for Science and Technology, Tokyo University of Science, Chiba, Japan, ³Organ Technologies Inc., Tokyo, Japan, ⁴Institute for Protein Research, Osaka University, Osaka, Japan, ⁵Oral Implantology and Regenerative Dental Medicine, Graduate school, Tokyo Medical and Dental University, Tokyo, Japan

Objective

Marfan syndrome (MFS) is a systemic disorder affecting connective tissues caused by insufficient fibrillin-1 microfibril formation and deregulation of TGF- β signaling in various connective tissues. Recent observations provide that TGF- β antagonism is a general therapeutic strategy for MFS, however reconstruction of the microfibril in connective tissues remains to be determined. A disintegrin-like metalloprotease domain with thrombospondin type I motifs like (ADAMTSL) 6 β is a microfibril-associated extracellular matrix protein associated with fibrillin-1 microfibrils through direct interaction with the fibrillin-1 which promotes fibrillin-1 matrix assembly *in vitro* and *in vivo*. Here, we report that ADAMTSL6 β has an essential role in the development and regeneration of connective tissues.

Methods

To investigate the potential for clinical application of ADAMTSL-6 β as a novel MFS therapy, we investigated if ADAMSL6 β expression can rescue fibrillin-1 microfibril formation and regulating of TGF β activation through the promotion of fibrillin-1 microfibril assembly in mgR/mgR mice as a model of MFS microfibril disorder.

Results

ADAMTSL6 β expression rescues microfibril disorder after periodontal ligament (PDL) injury in MFS model mice through the promotion of fibrillin-1 microfibril assembly. In addition, improved fibrillin-1 assembly following administration of ADAMTSL6 β attenuates the over-activation of TGF- β signals associated with increased release of active TGF- β from disrupted fibrillin-1 microfibrils within PDLs of MFS model mice.

Conclusion

This study demonstrates the essential contribution of ADAMTSL6 β to fibrillin-1 microfibril formation and suggests a new therapeutic strategy for the treatment of MFS through ADAMTSL6 β -mediated fibrillin-1 microfibril assembly.

TRANSGENIC MICE OVEREXPRESSING ADAMTSL-6 IN CARTILAGE EXHIBIT DWARISM AND CRANIOFACIAL ABNORMALITIES

Ko Tsutsui^{1,2,3}, Eric J. Carlson², Douglas R. Keene³, Lynn Y. Sakai^{2,3}, and Kiyotoshi Sekiguchi¹

¹Division of Protein Chemistry, Institute for Protein Research, Osaka University, Osaka, Japan

²Department of Biochemistry and Molecular Biology, Oregon Health & Science University

³Shriners Hospital for Children, Portland, OR 97239

Objectives: Marfan syndrome is an autosomal dominant disease caused by mutations in FBN1, the human gene for fibrillin-1. Our recent results indicated that ADAMTSL-6 promotes fibrillin-1 microfibril formation in vitro (Tsutsui et al., *J. Biol. Chem.*, 2010). To investigate the in vivo effects of ADAMTSL-6 in the skeletal system, we generated and analyzed transgenic mice, in which exogenous Adamtsl6 cDNA was over-expressed in the cartilage.

Methods: Skeletal morphologies were analyzed by soft X-ray, histochemical, and immunohistological observations. Depositions of fibrillin-containing microfibrils were observed by electron microscopy.

Results: Adamtsl6 transgenic mice exhibited dwarfism and craniofacial abnormalities associated with disorganized growth plates of the skull base bones. The developing rib cartilage was surrounded with apparently thinner perichondrium and showed insufficient formations of territorial cartilage matrices. Since ADAMTSL-6 is a microfibril-associated protein and is capable of promoting fibrillin-1 microfibril formation in vitro, fibrillin-1 deposition was analyzed in detail. Immunohistochemical observation indicated accumulated depositions of fibrillin-1 in the perichondrium. Electron microscopy revealed that microfibrils form dense aggregates in the cartilage.

Conclusions: Our results uncovered that ADAMTSL-6 is a potential regulator of microfibril formation. Although it is unclear why abnormal aggregation of microfibrils in the Adamtsl6 transgenic mice results in immature bone formation, the short long bone phenotype is similar to one symptom in Weill-Marchesani syndrome, another autosomal dominant disease caused by FBN1 mutations. To elucidate functional correlations between ADAMTSL-6 and fibrillins, we are breeding our transgenic mice into Fbn1 and Fbn2 mutant mouse lines.

TISSUE AND DEVELOPMENTAL-SPECIFIC VARIABILITY IN FBN1 ISOFORM EXPRESSION

Burchett, Mary University of Kentucky College of Medicine Class of 2012, Lexington, KY, USA,

Ling, I-Fang Ph.D., Department of Physiology, University of Kentucky, Lexington, KY, USA,

Estus, Steven Ph.D., Department of Physiology, University of Kentucky, Lexington, KY, USA

Objectives

Mutations in FBN1 cause Marfan syndrome, a heritable disorder of connective tissue. FBN1 encodes the extracellular matrix protein, fibrillin. Our objective was to elucidate the extent that inefficient exon splicing contributes to fibrillin mRNA variants.

Methods

To identify splice variants of *FBN1* mRNA, we scanned each of its 66 exons using overlapping primer pairs in 20 pooled human brain cDNA samples. We then quantified expression of the identified isoforms using RT-PCR with cDNA from multiple tissues, including adult human skeletal muscle and brain, as well as fetal human skeletal muscle, brain, liver, aorta, lung, skin, and heart.

Results

FBN1 splicing is generally efficient as we identified only two splice variants, which include (i) a novel isoform containing a 105 basepair insertion between exons 54-55 and (ii) a previously identified isoform containing a cryptic exon between exons 57 and 58. Quantification revealed that this latter splice variant represents 8-44% of *FBN1* mRNA in a tissue- and developmental-specific fashion. Expression of the 57A-containing isoform in fetal tissue was high in brain (27%) and low elsewhere, e.g., skin, aorta and lung. In adult tissue, the 57A-containing isoform represented 39 ± 3 (% , mean \pm SD) of *FBN1* mRNA in brain, and 19 ± 2 (% , mean \pm SD) in skeletal muscle.

Conclusions

A significant portion of *FBN1* is expressed as the 57A-containing isoform. Since the 57A insertion creates a premature stop codon that could significantly impact fibrillin function, the biological function of this splice variant merits investigation.

INCREASED T-HELPER 1 CELL RESPONSE IN MARFAN SYNDROME IS MODIFIED BY LOSARTAN

Teodora Radonic, MD¹, Piet de Witte, MD², Rene Lutter, Ph.D³, H., Baars Marieke, MD, Ph.D⁴, Yvonne Hilhorst-Hofstee, MD⁵, Peter J. van Tintelen, MD, Ph.D.⁶, Ben C.J. Hamel MD, Ph.D.⁷, Barbara J.M. Mulder, MD, Ph.D.⁹, Maarten Groenink, MD, Ph.D.¹⁰, Aeilko H. Zwinderman, Ph.D.⁸

^{1,8} Dept. of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center Amsterdam, Netherlands

^{2,9,14} Dept. of Cardiology AMC Amsterdam and Interuniversity Cardiology Institute of the Netherlands

³ Dept. of Pulmonology and Experimental Immunology, Academic Medical Center Amsterdam, Netherlands

⁴ Dept. of Clinical Genetics, Academic Medical Center Amsterdam, Netherlands

⁵ Dept. of Human Genetics, Leiden University Medical Center, Leiden, Netherlands

⁶ Dept. of Clinical Genetics, Groningen University Medical Center, Groningen, Netherlands

⁷ Dept. of Clinical Genetics, St. Radboud University Medical Center, Nijmegen, Netherlands

Objectives

We investigated gene expression in Marfan patients in order to define genes and pathways that change after losartan treatment and modify the aortic dilatation.

Methods

Punch skin biopsies were obtained in participants of the COMPARE trial before therapy (baseline), after 4 weeks and one year of losartan therapy. In 88 samples RNA was isolated and Whole Transcriptome Gene Expression (WTGE) was measured using Human Exon 1.0 ST Arrays (Affymetrix). Results were validated using rtPCR. Baseline WTGE measurements were correlated with aortic dilatation rate and aortic distensibility change over 12 years measured with MRI. Results were validated in another 13 patients. We measured levels of 48 cytokines in 160 blood samples of these patients.

Results

Analysis of gene expression after 4 weeks of losartan therapy revealed 20 differently expressed genes ($\Delta=0.54$, $p<10^{-7}$), 2 of which in the TGF- β pathway: CIDEA and ENG. Losartan therapy changed splicing of ACSM3 and ADCY6 ($\Delta=0.3$, $p<10^{-7}$).

When correlated with the aortic dilatation rate, baseline expression of 2 genes was significant: HLA-DRB5 and HLA-DRB1 ($r=0.46$, $r=0.42$; $p<10^{-7}$), suggestive of immune response involvement. Prevailing cytokine profile was Th1 T-cell response. Remarkably, losartan significantly lowered levels of Th1 chemokines CTACK and SCGF- β ($p<0.05$) and up-regulated levels of IL-9 and MIG ($p<0.05$).

Conclusion

MFS is associated with increased Th-1 immune response. As 4 weeks of losartan therapy attenuates TH1 chemokine levels this may lead to reduced Th-1 responses with time. The involvement of TGF- β in these pathways is currently being analyzed.

REGULATORY ROLE OF FIBRILLIN IN BONE HOMEOSTASISKomarova Svetlana V.,¹ Tiedemann Kerstin,¹ Reinhardt Dieter P.^{1,2}McGill University, ¹Faculty of Dentistry and ²Faculty of Medicine, Montreal, Qc, Canada

Osteopenia and skeletal deformations in Marfan syndrome (MFS) and congenital contractural arachnodactyly are consistent with disrupted bone homeostasis. Changes in bone structure and composition are regulated by the activities of osteoblasts and osteoclasts. Fibrillin-1 and -2 are expressed throughout the skeleton, including long bones, ribs, vertebral bodies and cartilage. Here, we address the role of fibrillins in osteoblast and osteoclast differentiation and function.

Treatment of mouse bone marrow cells with ascorbic acid results in induction of osteoblast markers, alkaline phosphatase, osteopontin and Runx2. We have found that expression of fibrillin-1 is up-regulated throughout osteoblast differentiation, whereas fibrillin-2 is very strongly and transiently induced during the first week of culture. Fibrillin-1, but not fibrillin-2, was assembled into microfibrils in the extracellular matrix of the differentiated osteoblasts. Using established osteoclast differentiation and activity models with primary bone marrow-derived precursors and monocytic RAW 264.7 cells, we found that the soluble N-terminal (but not the C-terminal) half of fibrillin-1 inhibits osteoclastogenesis. In contrast, when recombinant fibrillin-1 fragments were coated on calcium phosphate plates, they did not affect osteoclast formation or resorptive activity. Using a panel of recombinant sub-fragments of the fibrillin-1 N-terminal half, we localized the inhibitory activity of fibrillin-1 on osteoclast differentiation to the N-terminal region of the protein. The same region acted as a specific inhibitor of cathepsin K and matrix metalloproteinase 9 expression by differentiated osteoclasts. In contrast, fibrillin-1 or its fragments did not affect differentiation of osteoblasts. We have found that osteoclast-specific protease cathepsin K is capable of cleaving fibrillin-1 *in vitro*. The observed cleavage sites are located at domain boundaries resulting in liberation of N-terminal fragments very similar to the N-terminal fibrillin-1 fragment exerting the inhibitory activity on osteoclast differentiation. Furthermore, fibrillin-1 fragments with neonatal MFS mutations were more susceptible for cleavage by cathepsin K than fragments with classical mutations. We obtained further data, indicating that the N-terminal region of fibrillin-1 exerts its anti-osteoclastogenic activity through sequestering "Receptor activator of nuclear factor kappaB ligand" (RANKL) and by inhibiting apoptosis of osteoclast precursors. These data demonstrate that fibrillin-1 can directly inhibit osteoclast formation and suggest that MFS mutations affect osteoclast-inhibiting properties of fibrillin-1 leading to osteoclast activation and osteopenia.

DIFFERENTIAL EFFECTS OF NEONATAL AND CLASSICAL MARFAN MUTATIONS IN FIBRILLIN-1

Hubmacher Dirk¹, Kirschner Ryan¹, Iyengar Garud¹, Bromme Dieter², Bartels Rainer³, Reinhardt Dieter P.^{1,4}

¹McGill University, ¹Faculty of Medicine and ⁴Faculty of Dentistry, Montreal, Qc, Canada

²University of British Columbia, Department of Biochemistry and Molecular Biology, Vancouver, Canada

³Forschungszentrum Borstel, Germany

Fibrillins belong to a family of extracellular matrix proteins which form supramolecular assemblies called microfibrils in various connective tissues including skin, blood vessels, and bone. Fibrillin-1 constitutes the backbone of these microfibrils, which are crucial for regulating elastic fiber biogenesis and TGF-beta bioavailability. Mutations in fibrillin-1 give rise to the Marfan syndrome (MFS) characterized by vascular, skeletal and ocular symptoms. Typical abnormalities include disorganized elastic fibers and an elevated level of active TGF-beta.

To investigate molecular consequences of mutations causing severe neonatal or the milder classical form of MFS, representative point mutations from each group were introduced in recombinant human fibrillin-1 fragments. Proteolytic susceptibility was probed with physiological proteases, including plasmin, thrombin, matrix metalloproteinases and cathepsins. The proteins harboring neonatal mutations were typically more susceptible to proteolytic cleavage than those with classical mutations. The cleavage sites were found both in close proximity and distant to the mutations, indicating structural changes which result in the exposure of cryptic cleavage sites. Neonatal mutations more severely affected the ability of fibrillin-1 to interact with heparin/heparan sulfate, which plays a critical role in microfibril assembly. Primary human dermal fibroblasts attached more efficiently to the fragments harboring the classical mutations as compared to the neonatal mutant and wild-type proteins, indicating a gain of function effect of these classical mutations. Such differential cell attachment properties were also observed with fibroblast cell lines derived from lung (WI38), skin (MSU1.1) and from different knock-out mice (perlecan, syndecan-4, NDST-1).

Our results suggest new molecular pathogenetic concepts for MFS including a differential susceptibility to proteolysis for relevant enzymes and changes in cell attachment. In most cases the neonatal mutations resulted in more severe effects and the biochemical variability correlates with the clinical variability observed in MFS.

CANDIDATE MODIFIERS OF *FBN1* ACTIVITY MAY BE ASSOCIATED WITH VARIABLE PHENOTYPE IN MARFAN SYNDROME.

Summers Kim M, BSc PhD PG Dip Human Biol, The Roslin Institute, University of Edinburgh, Edinburgh, UK.

Raza, Sobia, BSc, MSc, The Roslin Institute, University of Edinburgh, Edinburgh, UK.

Freeman, Thomas C, BSc, PhD The Roslin Institute, University of Edinburgh, Edinburgh, UK.

Hume, David A, BSc, PhD, The Roslin Institute, University of Edinburgh, Edinburgh, UK.

Objectives:

Although Marfan syndrome is inherited in an autosomal dominant fashion, the phenotype is very variable, even within families with the same *FBN1* mutation. We have used mouse cell line genome-wide expression data to identify candidate modifiers of *FBN1* activity, based on cluster analysis of gene expression patterns.

Methods:

Gene expression patterns in 44 mouse cell lines and two tissues were clustered at a Pearson correlation coefficient of $r \geq 0.9$, using Biolayout *Express*^{3D}, a program for visualisation and analysis of networks derived from biological systems.

Results:

Fbn1 expression clustered with genes showing high expression in cell types relevant to Marfan syndrome, including bone, adipose and muscle. Other genes in the cluster encoded known connective tissue proteins (for example several collagen genes, *Ltp2*, *Lox*, *Efemp2*, *Fn1*, *Has2*, *Lama4*, *Lamb2*), aortic proteins (*Aebp1*, *Acta2*) and growth factors (*Figf*, *Pdgfb*, *Pdgfl*, *Tgfb2*, *Tgfb3*). There were 17 genes for transcription factors, including *Prrx1*, *Prrx2*, *Twist 1* and *Wisp1*. A number of genes were unclassified, but their shared expression pattern with other genes in the cluster now implicates them in extracellular matrix function.

Conclusions:

Cluster analysis has identified 204 genes with an expression pattern similar to that of *Fbn1* in mouse, including novel transcripts with no current annotation. These genes are strong candidates as modifiers of *FBN1* activity in humans. If an epistatic role in determining final phenotype in the presence of *FBN1* mutations is confirmed, these genes could be integrated into genetic testing protocols, to provide information about aspects of the Marfan phenotype.

MARFAN SYNDROME AND BICUSPID AORTIC VALVE ANEURYSM ULTRASTRUCTURE ABNORMALITIES

¹Do Hong-Lien MD PhD, ¹West Malcolm MD PhD, ¹Nataatmadja Maria MDS PhD, ²Stenzel Deborah PhD, ²Theodoropoulos Christina PhD, ¹West Jennifer RN. - ¹The University of Queensland, Department of Medicine, Prince Charles Hospital, Brisbane, Australia; ²Queensland University of Technology, School of Life Sciences, Brisbane, Australia

Objectives: Tissue from Marfan syndrome (MFS) or bicuspid aortic valve (BAV) aneurysm is characterized by vascular smooth muscle cell (VSMC) loss, cystic medial necrosis and elastic tissue destruction. We examined morphological changes in aneurysm tissue using light microscopy (LM) and transmission electron microscopy (TEM).

Methods: Aortic tissue and cultured VSMCs from normal aorta (1M, 4F; age 39±14 yr), MFS (5M, 3F; age 35±11 yr fulfilling de Paepe criteria for MFS) and BAV (6M, 5F; age 57±16 yr identified with cardiac ultrasound) were processed and embedded in resin. Semi thin sections were cut and stained for LM examination (magnification of 1000x) and ultra thin sections for TEM analysis (magnification of 25000x). VSMCs were counted and characterised as normal, abnormal or apoptotic and elastic lamellae (EL) defined as normal or abnormal according to histological criteria.

Results: Light microscopy revealed generalized severe structural abnormalities of VSMCs unrelated to location of cystic medial necrosis. Many VSMCs located between intact EL were abnormal. VSMCs contained vacuoles and structural masses. Numbers of apoptotic VSMCs were significantly higher in patient groups compared to controls (MFS=8.1±2.8%; BAV=10.3±3.2%; control=1.4±0.9%; P<0.001 for aortic tissue). Examination by TEM showed loss of interdigitations between VSMCs and elastic lamellae due to abnormal EL and limited VSMC cytoplasmic projections. Cultured VSMCs showed bulging intracellular masses, tearing of the cytoplasmic membrane, cellular indentations and disintegration. Calcification of VSMCs and extracellular matrix were common in BAV.

Conclusions: The findings suggest that morphological abnormalities in VSMC precede recognizable alterations of EL, consistent with the hypothesis that the primary defect in the pathogenesis of MFS and BAV aneurysm arises within VSMCs.

COMPARISON OF PRAVASTATIN, LOSARTAN AND DOXYCYCLINE FOR ATTENUATION OF DILATION IN A MURINE MODEL OF MARFAN SYNDROME

McLoughlin Darren, MRCS, Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland
McGuinness Jonathan, PhD, MRCS, Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Byrne John Stephen, MD, MRCS, Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Terzo Elosia, MVB, Department of Veterinary Radiology, University College Dublin, Dublin, Ireland

Huuskonen Vehilmiine, MVB, Department of Veterinary Radiology, University College Dublin, Dublin, Ireland

McAllister Hester, MRCVS, Department of Veterinary Radiology, University College Dublin, Dublin, Ireland

Black Alex, MSc, Department of Anatomy, National University of Ireland Galway, Galway, Ireland

Kearney Sinead, BSc, Department of Anatomy, National University of Ireland Galway, Galway, Ireland

Hill Arnold D.K., MCh, FRCS, Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Redmond J.Mark, MD, FRCS, Department of Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland

Background: Statins inhibit biosynthesis of isoprenoids, essential for prenylation of proteins such as metalloproteinases. We hypothesised that pravastatin could reduce the transportation and secretion of metalloproteinases to prevent aneurysm formation. Using a murine model of Marfan Syndrome, we compared the effect of treatment and mechanism of action with Pravastatin, Losartan, and Doxycycline on aortic root dilation, combined with ultrastructural analysis of vascular smooth muscle (VSMC) cells using electron microscopy.

Methods: The effects of treatment in a mouse model of MFS with Pravastatin 0.5g/L, Losartan 0.6 g/L, and Doxycycline 0.24g/L on the end-points of aortic root diameter, and aortic pathology (using both light and electron microscopy) were assessed using untreated Marfan mice and normal mice as controls.

Results: The aortic root diameter of untreated Marfan mice are significantly increased in comparison to normal mice (1.61+/- 0.012 mm vs. 2.52+/-0.04 mm, P<0.05). Losartan produced a significant reduction in aortic root dilation (2.21+/-0.04 mm, P<0.05), as did Pravastatin (2.20+/-0.03 mm, P<0.05) compared to untreated marfan mice. Doxycycline treatment had no beneficial effect at 8 months (2.43+/-0.04 mm, P=0.1). Ultrastructural analysis of VSMC showed greater reduction of rough endoplasmic reticulum with Losartan compared to Pravastatin, while cisternal volume within these cells was equally reduced with both Pravastatin and Losartan. This suggests that Pravastatin acts by inhibiting excessive TGF-beta signalling mechanism at a post-transcriptional level.

Conclusions: We have now shown that Pravastatin is equally as effective as Losartan in reducing aortic root dilatation. Our findings suggest that marfan aortic pathology may be targeted through different pathways.

PRENATAL AND PREIMPLANTATION DIAGNOSES IN MARFAN SYNDROME: THE POINT OF VIEW OF FRENCH PATIENTS AND GENETICISTS

CORON Fanny (1), JONDEAU Guillaume (2), CUSIN Véronique (2), ODEnt Sylvie (3), DULAC Olivier (4), PLAUCHU Henri (5), COLLIGNON Patrick (6), DELRUE Marie-Ange (7), LEHEUP Bruno (8), CASSINI Cécile (1), THAUVIN-ROBINET Christel (1), BOILEAU Catherine (9), FAIVRE Laurence (1)

1 *Génétique, Hôpital d'Enfants, Dijon, France*

2 *Centre de Référence Maladie de Marfan, Hôpital Bichat, Paris, France*

3 *Génétique, CHU Rennes, France*

4 *Génétique, CHU Toulouse, France*

5 *Génétique, CHU Lyon, France*

6 *Génétique, CHU Marseille, France*

7 *Génétique, CHU Bordeaux, France*

8 *Génétique, CHU Nancy, France*

9 *Laboratoire de Biologie Moléculaire, Hôpital Ambroise Paré, Boulogne, France*

Objectives: Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder with manifestations mainly involving the skeletal, ocular, and cardiovascular systems. The phenotypic variability makes genetic counseling difficult. Prenatal (PND) and preimplantation diagnoses (PID) are technically feasible when a causal mutation is identified, but both raise many ethical questions. Little is known regarding opinions and practices in such reproductive issues in MFS. The goal of this study was to report on patients' points of view and geneticists' standard practices.

Methods: Two questionnaires were produced. Questionnaire 1 was sent to patients via the "Association Française pour le Syndrome de Marfan". Questionnaire 2 was dedicated to geneticists.

Results: 54 answers were collected for questionnaire 1, 65% from patients and 35% from unaffected relatives. Most of them (74%) thought that PND was acceptable, and that the choice should be given to the parents, but only 19% affirm that they would perform this technique in case of a pregnancy. 70% were aware of the possibility of performing PND in MFS, and 54% of PID. Fifty geneticists filled in questionnaire 2. 46% had already had to deal with patients requiring information regarding PND or PID. This information led to PND or PID in a minority of cases. 22% of geneticists thought that PND was acceptable, 72% debatable and 6% not acceptable. Significant differences were found depending on the experience of the practitioner.

Conclusions: This study showed that the majority patients were in favor of proposing PND although most of them would not use it, and that various opinions were found among practitioners.

FBN1, TGFBR1, TGFBR2, and SLC2A10 MUTATION ANALYSES IN PATIENTS WITH SUSPECTED MARFAN SYNDROME: A SWISS STUDY

Mátyás Gábor, PhD, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland

Arnold Eliane, MSc, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland

Alonso Sira, MSc, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland

Patrignani Andrea, PhD, ETH & University of Zurich, Functional Genomics Center Zurich (FGCZ), Zurich, Switzerland

Magyar István, MSc, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland

Henggeler Caroline, MSc, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland

Carrel Thierry, MD, Clinic for Cardiovascular Surgery, University Hospital, Berne, Switzerland

Berger Wolfgang, PhD, Division of Medical Molecular Genetics and Gene Diagnostics, Institute of Medical Genetics, University of Zurich, Schwerzenbach, Switzerland

Steinmann Beat, MD, Division of Metabolism and Molecular Pediatrics, University Children's Hospital, Zurich, Switzerland

Classical Marfan syndrome (cMFS) is caused by FBN1 mutations. Many of the features of MFS show overlap with related disorders such as MFS type 2 (MFS2), Loeys-Dietz aortic aneurysm syndrome (LDS), familial thoracic aortic aneurysms and dissections (TAAD), and arterial tortuosity syndrome (ATS). In cMFS patients, FBN1 genetic testing detects only ~80% of mutations. This may be due to technical limitations of currently used PCR-based screening methods and/or because the disease-causing mutation occurs in a different gene. Here, we investigated the impact of these possibilities. In a cohort of unrelated individuals with suspected MFS in whom previous analysis of FBN1 revealed no mutation, we sequenced TGFBR1, TGFBR2, and SLC2A10. We also screened for large deletions/duplications by multiplex ligation-dependent probe amplification (MLPA). The impact of novel sequence variants was assessed by in silico predictions and/or RT-PCR, and segregation analyses. The breakpoints of deletions identified by MLPA were narrowed down by using microarrays. In three MFS2, two LDS, and four TAAD patients, we identified heterozygous TGFBR1 or TGFBR2 nucleotide substitutions and in one ATS patient a homozygous SLC2A10 nonsense mutation. The deleterious alleles occurred de novo or segregated with the disease in the families, indicating a causative association between the sequence variants and clinical phenotypes. Neither a TGFBR1- nor a TGFBR2-specific phenotype could be detected. In two patients, MLPA revealed genomic rearrangements affecting FBN1. Our data demonstrate that TGFBR1 mutations are associated not only with LDS but also with MFS2 and TAAD, and that true FBN1 haploinsufficiency is sufficient to cause MFS.

CLINICAL SIGNIFICANCE OF UNUSUAL HORIZONTAL STRIAE OF THE BACK IN CHILDREN WITH POSSIBLE CONNECTIVE TISSUE DISORDER

Powell-Hamilton, Nina, MD, Medical Genetics, Alfred I. duPont Hospital for Children, Wilmington, Delaware, United States

Jenny, Kim, MS, Medical Genetics, Alfred I. duPont Hospital for Children, Wilmington, Delaware, United States

OBJECTIVES:

Clarify the relevance of unusual striae of the back in children with subdiagnostic marfanoid features.

METHODS:

Chart review was conducted for patients with horizontal striae involving the back, evaluated from 5 January 2009 to 1 June 2010.

RESULTS:

9 of 119 patients (7.6%) referred for suspected connective tissue disorder had stretch marks involving the back (striae atrophicus). The diagnosis of Marfan Syndrome was confirmed in 1 of those 9 (11.1%) and he underwent surgical repair at age 14 years following aortic dissection.

For the 8 individuals with subdiagnostic findings, the age range was 11 to 16 years. Height for age ranged from 25th to greater than 95th percentile; arm span to height 0.97 to 1.02; upper segment to lower segment 0.85 – 0.99; aortic root diameter measurements ranged from 2.2 to 3.5 cm; Z-score -1.2 to +3. 7 of the 9 patients (77.8%) were male.

CONCLUSION:

Striae atrophicus were noted in 7.6% of patients referred for a possible connective tissue disorder, all during the adolescent period. This finding was more common in males and the majority did not have significant anthropometric or cardiovascular findings at that time. Even though these individuals may fall within the marfanoid spectrum, review of a larger cohort may provide further information to help reduce the frequency of evaluations in a more clearly defined manner, help alleviate patient anxiety as well as decrease cost, while still ensuring appropriate care.

DELETION OF ACTA2 LEADS TO INCREASED SMOOTH MUSCLE CELL PROLIFERATION AND NEOINTIMAL FORMATION IN MICE.

Papke, Christina L., PhD, Department of Internal Medicine, The University of Texas Health Science Center, Houston, TX, USA

Cao, Jiumei, PhD, Department of Internal Medicine, The University of Texas Health Science Center, Houston, TX, USA

Lim, Soon-Mi, PhD, Department of Systems Biology and Translational Medicine and Medical Physiology, Texas A&M Health Science Center College of Medicine, College Station, TX, USA

Rees, Meredith, Department of Internal Medicine, The University of Texas Health Science Center, Houston, TX, USA

Trache, Andreea, PhD, Department of Systems Biology and Translational Medicine and Medical Physiology, Texas A&M Health Science Center College of Medicine, College Station, TX, USA

Wilson, Emily, PhD, Department of Systems Biology and Translational Medicine and Medical Physiology, Texas A&M Health Science Center College of Medicine, College Station, TX, USA

Zimmer, Warren E., PhD, Department of Systems Biology and Translational Medicine and Medical Physiology, Texas A&M Health Science Center College of Medicine, College Station, TX, USA

Milewicz, Dianna M., MD, PhD, Department of Internal Medicine, The University of Texas Health Science Center, Houston, TX, USA

Mutations in *ACTA2* (smooth muscle cell (SMC)-specific α -actin) cause diffuse vasculopathy characterized by thoracic aortic aneurysms/dissections and early-onset coronary artery disease and stroke. Heterozygous *ACTA2* mutations result in aortic medial degeneration and vasa vasorum stenosis due to increased SMCs. Explanted *ACTA2* mutant SMCs show loss of α -actin filaments and proliferate more rapidly than controls, suggesting that *ACTA2* mutations cause aneurysms through loss of SMC contractility but occlusive lesions through gain of SMC proliferative function. Focal adhesions link α -actin filaments with the extracellular matrix, generating contractile force and activating multiple signaling pathways. We therefore hypothesized that loss of α -actin filaments drives SMC proliferation through altered localization and signaling of adhesion components. *Acta2*^{-/-} mice were used as a model for the study. *Acta2*^{-/-} mice develop aneurysms but do not show typical elastic fiber loss and proteoglycan accumulation. Surprisingly, direct counting of nuclei showed increased SMCs in *Acta2*^{-/-} aortas ($p < 0.05$). *Acta2*^{-/-} SMCs proliferated and migrated more than wildtype both *in vitro* and post carotid injury *in vivo*. Total Internal Reflection Fluorescence microscopy revealed increases in vinculin, activated focal adhesion kinase (FAK), and overall adhesion size in *Acta2*^{-/-} SMCs ($p < 0.05$). Immunoblotting showed increased FAK, ERK, and Akt activation, and specifically inhibiting these pathways prevented *Acta2*^{-/-} SMC proliferation ($p < 0.05$). Our data suggest that *Acta2*^{-/-} SMC proliferation is dependent at least in part on FAK signaling *in vitro*. Further study will determine putative mechanisms by which α -actin filament loss activates FAK, identify additional proliferative pathways, and elucidate the role of SMC proliferation in aneurysm formation.

8TH INTERNATIONAL RESEARCH SYMPOSIUM
ON THE MARFAN SYNDROME AND RELATED DISORDERS

GENERAL INFORMATION

THE SYMPOSIUM WILL BE HELD AT THE AIRLIE CENTER LOCATED
IN WARRENTON, VA AT THE FOLLOWING ADDRESS:

Airlie Conference Center
6809 Airlie Road
Warrenton, VA 20187
Telephone: 540-347-1300
Fax: 540-341-3207
www.airlie.com

The Center's natural setting serves as a scenic backdrop for jogging, biking, fishing, volleyball, horseshoes, swimming, tennis or skeet shooting. Airlie's campus provides opportunities for visitors to enjoy its diverse flora and fauna in our gardens and on acres of beautifully maintained grounds.

The center is located 50 miles southwest of Washington, DC, approximately 1 hour from Dulles International Airport and 1.5 hours from Regan National Airport. Rental cars or cab services are available at area airports. Rental cars may be more a more economical means of transportation from Regan National Airport. Please check car rental rates. Ample complimentary parking is available. If you are planning to arrive late, it is advised to ask the cab to wait until you check so that they can drive you to your hotel accommodation within the Airlie complex.

TRANSPORTATION

The following are taxi services which can provide transportation from the airports to the Airlie Conference Center. Return cab services to the airport can be coordinated in group form at the meeting.

Washington Flyer Cab Service: Serves Dulles International Airport Only. Dispatch counter located one level below baggage claim. No reservation necessary. These are metered cabs so fares will vary but without traffic, fare should be approximately \$70.00–\$80.00 for one person. Additional passengers incur a nominal additional fee (\$1.00–\$3.00) and a nominal baggage handling fee. Accepts credit cards, cash or US check. *Telephone: 703-661-6655.*

Timely Express Service: Dulles International Airport and Regan National Airport. Reservations Only. This is a van service that holds up to six people for single fare. Dulles to Airlie Fare: \$95.00 plus gratuity. Regan National to Airlie Fare: \$120.00 plus gratuity. Accepts cash and credit cards. *Telephone: 1-540-347-6488.*

007 Executive Sedan: Serves Dulles International, Regan National, Union Station, BWI. Dulles Fare: \$110.00 per car (up to 4 people) plus gratuity. Regan National to Airlie Fare: \$125.00 per car (up to 4 people). Accepts credit cards and cash. *Reservations: 1-888-320-6007. bond@007limo.com.*

Bailey's Taxi and Airport Express: There is no inside pick-up, so once you get to the airport, you must call the company and they will let you know where to meet the cab. Please note the price is the same if one or four people are in the car, so if you can arrange to travel with someone the cost per person will be reduced. Dulles International Airport to Airlie: Set Fare at \$80.00 plus gratuity (Cash or US check only); DCA to Airlie: Set Fare at \$110.00 plus gratuity (Cash or US check only). *Reservations 1-540-229-9052.*

Prime Executive Service: Services Dulles, Regan National, BWI and Union Station. Dulles International to Airlie Fare: \$126.00 per car (up to 4 people) including gratuity. Regan National to Airlie Fare: \$162.00 per car (up to 4 people) including gratuity. Only accepts credit cards. *Reservations: 1-800-241-9747.*

RESERVATIONS

The cost of accommodation is \$257.00 per night plus 7% tax and includes three meals per day. If overnight lodging is not required. Airlie offers a day rate of \$125.00/day plus 7% tax which includes admission to the meeting session and lunch. Fees are non-refundable after June 27, 2010.

REGISTRATION FEE AND DEADLINES

To register and reserve accommodations visit www.airlie.com or contact jgrima@marfan.org Reservations must be made by June 27, 2010.

Early Registration: Regular	\$350.00	June 27, 2010
Early Registration: Student/Fellow/VHO:	\$300.00	June 27, 2010
Late Registration:	\$450.00	June 28 – Sept.11, 2010

CALL FOR ABSTRACTS

Abstract Submission Deadline:	June 11, 2010
Abstract Notifications Sent:	June 21, 2010

ABSTRACT GUIDELINES:

Abstract must be written in English. Titles of abstracts should be in capital letters. The names of the authors should be listed in the following order: Last name, first name, degree, department, institution, city, state and country. The presenting author's names should be underlined. The abstract should be a maximum of 250 words and should include a section of objectives, methods, results and conclusions. Please use 10 pt Arial, single spaced with one inch margins. There is no limit to the number of abstracts a presenter can submit, but the scientific committee expects that all abstracts selected for presentation will be presented by the first author either as a platform presentation or poster. All authors will receive notification by June 21, 2010 and must complete registration and hotel lodging by June 2, 2010.

Abstracts should be submitted electronically by midnight June 11, 2010 to abstract@marfan.org.

POSTER NUMBER AND SESSION ASSIGNMENTS

Posters have been assigned a number and poster presentation session. Several abstracts were chosen for oral presentation but authors have also been given a poster board presentation slot to allow for further discussion of their work. For poster number and session assignments, see the of posters presentation list and abstracts.

POSTER SESSION I

Jefferson Room: Sunday, September 12, 2010, 11:30 AM – 1:00 PM

Poster Session I Setup: Poster boards are 4' x 8' feet with a cork backing. Please mount your poster between the hours of 7:00 – 11:00 AM on the board that corresponds to your poster number. Please remove the poster immediately after the session.

POSTER SESSION II

Jefferson Room: Sunday, September 12, 2010, 5:00 – 6:30 PM

Poster Session II Setup: Please mount your poster between the hours of 1:30 – 5:00 PM on the board that corresponds to your poster number. Poster boards are 4' high x 8' wide with a cork backing. Please remove the poster immediately after the session.

SPEAKER PRESENTATIONS

Because of the tight scheduling between talks, all speakers are requested to tailor their talks to the allotted time allowed. Additionally all speakers will be required to load their power point presentations onto a single computer at the Airlie Center. Please bring your presentation on a USB. Alternatively, presentations can be e-mailed to jgrima@marfan.org prior to the meeting.

Presentation loading will only be allowed between 7:30 and 9:00 AM on Sunday, Monday and Tuesday mornings. Only limited opportunities will be available during lunch and breaks.

8TH INTERNATIONAL RESEARCH SYMPOSIUM
ON THE MARFAN SYNDROME AND RELATED DISORDERS

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