

# TABLE OF CONTENTS

Welcome	1
Program	3
Thursday, May 3, 2018	3
Friday, May 4, 2018	6
Saturday, May 5, 2018	10
Poster Presentation List	13
Abstracts of Oral Presentations	19
Abstracts of Poster Presentations	87
General Information	138
Author Index	139

## ORGANIZING COMMITTEE

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Silvolde, The Netherlands

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**Marlies J.E. Kempers, MD, PhD**  
Radboud University Medical Center  
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## PROGRAM COMMITTEE

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Vanderbilt University Medical Center  
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**Bart Loeys, MD, PhD**  
University of Antwerp  
Antwerp, Belgium

**Daniel B. Rifkin, PhD**  
NYU Medical Center  
New York, USA

# Words of welcome...

On behalf of the local and program organizing committee, we would like to welcome you to Amsterdam, The Netherlands for the 10th International Research Symposium on Marfan Syndrome and Related Disorders. This three-day, state-of-the-art meeting presents and discusses new cutting-edge research on the multifaceted aspects of Marfan syndrome and related disorders. 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 2014 in Paris, France. This year we are celebrating the 10th International Research Symposium. Since the first meeting in Baltimore, USA in 1988, the symposium has offered common ground for basic scientists, applied scientists, and clinicians to better understand the:

- molecular etiology of these disorders
- the biochemical abnormalities produced by the underlying mutations in connective tissue genes
- the clinical consequences of these mutations
- and the medical and surgical management on the natural history in Marfan syndrome and related disorders

The symposium brings together a panel of the world's experts for constructive discussions and debate on research and clinical therapies for Marfan syndrome and related disorders covering all major disciplines including, cardiology, cardiovascular surgery, orthopedics, genetics and ophthalmology.

This symposium includes ten sessions with oral presentations and two poster sessions, all exclusively selected from submitted abstracts. Additionally, we are excited to see many young researchers presenting their work this year. The talks will be relatively short in order to promote discussion. The meeting objectives will be aimed at unveiling molecular mechanisms underlying Marfan syndrome and related disorders; deciphering genetic and environmental modifiers; recognizing the broad clinical range of aneurysm related syndromes; insights derived from new animal models and new technology; discovering advances in imaging and biomarkers; and updates on management for aortic, ocular and orthopedic issues.

The Marfan Foundation and the Contactgroep The Netherlands are hosting the 10th International Symposium for Marfan Syndrome and Related Disorders.

Welcome and enjoy science!

Josephine Grima, PhD, Chief Science Officer, The Marfan Foundation  
Michael Weamer, President and CEO, The Marfan Foundation  
Bart Loeys, MD, PhD, University of Antwerp, Chair



**Backpack Health**

## Empowering Patients

Backpack Health supports those living with complex health conditions like Marfan Syndrome and related disorders, providing tools for health management and contributions to research.

## Global Support

Backpack Health profiles are viewable in English, Spanish, French, Portuguese and German, with more languages in the works.



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# PROGRAM

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## THURSDAY **MAY 3** 2018

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- 8:30 AM WELCOME AND INTRODUCTION  
Josephine Grima, PhD, The Marfan Foundation  
Michael Weamer, President and CEO, The Marfan Foundation  
Bart Loeys, MD, PhD, University of Antwerp, Belgium
- 8:45-10:15 AM SESSION 1A  
**MOLECULAR PATHOGENESIS IN MARFAN SYNDROME AND RELATED DISORDERS**
- Chair:* Bart Loeys, MD, PhD, University of Antwerp, Belgium
- 8:45 AM S1 **Massive Aggrecan Accumulation in Thoracic Aortic Aneurysm is Associated with Dissection in a Mouse Model of Marfan Syndrome**  
Suneel Apte, MBBS, PhD, Cleveland Clinic, USA
- 9:00 AM S2 **The Importance Of Endothelial Function to Losartan's Anti-Remodeling Effects: of Marfan Mice and Men**  
Pascal Bernatchez, PhD, University of British Columbia, Canada
- 9:15 AM S3 **Blockade Of TGF- $\beta$ 1 Signaling by the TGF- $\beta$  Receptor Peptide P144 Attenuates the Progression of Aortic Aneurysm in Marfan Syndrome**  
Gustavo Egea, PhD, University of Barcelona School of Medicine, Spain
- 9:30 AM S4 **Lineage-Specific Sensitivity to TGF- $\beta$ -Signaling Perturbation Drives Aortic Root Aneurysm in Loeys-Dietz Syndrome**  
Elena Gallo, PhD, Johns Hopkins School of Medicine, USA
- 9:45 AM S5 **Application of a Conditional Allelic Series of SKI to Mechanistically Dissect the TGF Vasculopathies**  
Benjamin Kang, PhD, Johns Hopkins School of Medicine, USA
- 10:00 AM COFFEE BREAK

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THURSDAY **MAY 3** 2018

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10:30AM-12NOON SESSION 1B

**MOLECULAR PATHOGENESIS IN MARFAN SYNDROME AND RELATED DISORDERS***Chair:* Daniel Rifkin, PhD, New York University, USA10:30 AM S6 **Fibrillin-1 Directly Regulates Macrophage NO Production and Polarization**

Turney McKee, MSc, McGill University, Canada

10:45 AM S7 **New Mediators and Therapeutic Targets of Aortic Disease**

Juan Miguel Rodondo, PhD Spanish National Cardiovascular Centre, Spain

11:00 AM S8 **TGF- $\beta$  and Aortic Disease Severity in Marfan Syndrome**

Marjolijn Renard, PhD, Ghent University, Belgium

11:15 AM S9 **BMP Driven Mechanisms in Aortic Aneurysm Formation Caused By Fibrillin-1 Deficiency**

Gerhard Sengle, PhD, University of Cologne, Germany

11:30 AM S10 **Activation Of Xanthine Oxidoreductase In Vascular Endothelial Cells Mediates Aortic Aneurysm Formation In Marfan Syndrome**

Hiroki Yagi, MD, PhD, University of Tokyo, Japan

12:00 NOON

LUNCH: DIAMOND ROOM

1:00-2:45 PM

SESSION 2

**GENETIC AND ENVIRONMENTAL MODIFIERS OF MARFAN PHENOTYPIC VARIATION***Chair:* Hal Dietz, MD, Johns Hopkins School of Medicine, USA1:00 PM S11 **Association of Modifiers And Other Genetic Factors Explain Marfan Syndrome Clinical Variability**

Melodie Aubart, MD, PhD, INSERM, France

1:15 PM S12 **Oxytocin Antagonism Prevents Pregnancy-Associated Aortic Dissection in a Mouse Model of Marfan Syndrome**

Jennifer Habashi, MD, Johns Hopkins School of Medicine, USA

1:30 PM S13 **Epigenetic Modulation in the Pathogenesis and Treatment of Marfan Syndrome and Related Disorders**

Benjamin Kang, PhD, Johns Hopkins School of Medicine, USA

1:45 PM S14 **Functional Relevance Of Fibulin-4 Interactions With Ltbp-4 In The Context Of Fibrillin-1 And Fibronectin**

Dieter Reinhardt, PhD McGill University, Canada

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 THURSDAY **MAY 3** 2018
 

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- 2:00 PM    **S15 Sexual Dimorphism in SMAD3 Mutation Carriers**  
Julie Richer, MD, Children's Hospital of Eastern Ontario, Canada
- 2:15 PM    **S16 Functional Characterization Of Modifier Loci For Marfan Syndrome Reveals Novel Therapeutic Strategies**  
Robert Wardlow II, BS, Johns Hopkins School of Medicine, USA
- 2:30 PM    **S17 Mechanistic Interrogation Of A Gene-By-Environment Interaction Informs The Pathogenesis And Treatment Of Marfan Syndrome**  
Nicole Wilson, PhD, Johns Hopkins School of Medicine, USA
- 2:45–3:45 PM    **SESSION 3  
NEW ANIMAL MODEL, TECHNOLOGIES AND THERAPEUTICS**
- Chair:*      Suneel Apte, MBBS, DPhil, Cleveland Clinic, USA
- 2:45 PM    **S18 Mechanistic Interrogation Of A Novel Mouse Model Of Vascular Ehlers-Danlos Syndrome**  
Caitlin Bowen, BS, Johns Hopkins School of Medicine, USA
- 3:00 PM    **S19 An iPSC-Derived Drug Screening Platform To Identify Therapeutic Compounds For Marfan Syndrome**  
Madeline McNamara, PhD, University of Cambridge, UK
- 3:15 PM    **S20 Effect Of Losartan And Beta-Blockers On Aortic Root Dilatation In Patients With Marfan Syndrome — Results Of The Extended COMPARE Trial**  
Maarten Groenink, MD Academic Medical Center, The Netherlands
- 3:30 PM    **S21 Introduction to Backpack Health — Marfan Foundation Registry Initiative**  
Brett Collinson, Backpack Health, USA
- 3:45–5:45 PM    **BREAK/POSTER SESSION: DIAMOND/STATEN/NASSAU ROOMS**
- 6:30 PM      **PRINS VAN ORANJE DINNER CRUISE**  
Buses depart from hotel for the Prins van Oranje (Prince of the Orange) dinner cruise at 6:30 PM. Boat departs from Steiger 14, just behind Amsterdam Central Station.
- An exclusive and delicious fine dining experience while taking in the views of the IJ River in Amsterdam. The dinner cruise will last from 7:00–10:00 PM during which the chef will prepare a wonderful three-course buffet. Beer, wine, and soft drinks are included. Transportation back to the hotel will also be provided

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# FRIDAY **MAY 4** 2018

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8:30–10:15 AM

## SESSION 4A

**GENOTYPE/PHENOTYPE CORRELATION IN MARFAN SYNDROME AND RELATED DISORDERS***Chair:* Peter Byers, MD, University of Washington, USA

8:30 AM S22 **Next Generation Sequencing In The Diagnosis Of Marfan and Related Disorders: An Efficient Global Approach In The SNV/CNV Detection**

Pauline Arnaud, PharmD, AP-HP, France

8:45 AM S23 **Arterial Tortuosity Syndrome: 40 New Families and Literature Review**

Aude Beyens, MD, Ghent University Hospital

9:00 AM S24 **Simple Renal Cysts and Aortic Disease in Marfan Syndrome and Matched Controls**

Claire Bouleti, MD, PhD, Bichat Hospital, France

9:15 AM S25 **Diagnosis of Marfan Syndrome in Children Requires Modification of the Revised Ghent Criteria**

Lauren Grote, MS, Mercy Children's Hospital, USA

9:30 AM S26 **Pathogenic Variants In LTBP3 And ARIH1 Predispose To Thoracic Aortic Diseases**

Dongchuan Guo, PhD, The University of Texas Medical School at Houston, USA

9:45 AM S27 **Risk for Thoracic Aortic Disease, Associated Complications, and Expanded Spectrum of SMAD3 Pathogenic Variants from the Montalcino Aortic Consortium**

Ellen Hosteltler, BA, The University of Texas Medical School at Houston, USA

10:00 AM S28 **Novel SMAD2 Mutations In Five Families With Arterial Aneurysm And Dissection: Further Delineation Of The Phenotype**

Marlies Kempers, MD, PhD, Radboud University Medical Center, The Netherlands

10:15 AM

COFFEE BREAK



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FRIDAY **MAY 4** 2018

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10:30AM-12NOON SESSION 4B

**GENOTYPE/PHENOTYPE CORRELATION IN MARFAN SYNDROME AND RELATED DISORDERS***Chair:* Julie DeBacker, MD, PhD, University of Ghent, Belgium

10:30 AM S29 **Candidate Gene Resequencing in a Large Bicuspid Aortic Valve-Associated Thoracic Aortic Aneurysm Cohort: SMAD6 as An Important Contributor**

Ilse Luyckx, MSc, Antwerp University Hospital, Belgium

10:45 AM S30 **Congenital Contractural Arachnodactyly: Establishment of a Clinical Scoring System and Confirmation of Molecular Heterogeneity**

Ilse Meerschaut, MD, Ghent University Hospital, Belgium

11:00 AM S31 **The Role Of Genetic Variation In Phenotype Variability And Response To Treatment In Marfan Syndrome**

Josephina Meester, PhD, University of Antwerp, Belgium

11:15 AM S32 **TMEPAI Mutation in MFS/LDS-Like Patients In Two Japanese Families**

Takayuki Morisaki, MD, PhD, Tokyo University of Technology, Japan

11:30 AM S33 **Results Of Next Generation Sequencing Gene Panel Diagnostics Including Copy Number Variation Analysis in 810 Patients Suspected of Heritable Thoracic Aortic Disorders**

Eline Overwater, MD, VU Medical Center, The Netherlands

11:45 AM S34 **The Clinical Implications of Variation in FBN1 in a Health System**

Reed Pyeritz, MD, PhD, University of Pennsylvania, USA

12:00 NOON LUNCH: DIAMOND ROOM

1:00-2:00 PM SESSION 4C

**GENOTYPE/PHENOTYPE CORRELATION IN MARFAN SYNDROME AND RELATED DISORDERS***Chair:* Maarten Groenink, MD, PhD, Academic Medical Center, The Netherlands

1:00 PM S35 **Montalcino Aortic Consortium: Thoracic Aortic Disease Outcomes Among 987 Individuals With ACTA2, PRKG1, TGFB1, TGFB2 AND SMAD3 PATHOGENIC VARIANTS**

Ellen Regalado, MS, University of Texas Health Science Center at Houston, USA

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 FRIDAY **MAY 4** 2018
 

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- 1:15 PM     **S36 Expert Consensus Recommendations On The Cardiogenetic Care For Patients With Thoracic Aortic Disease And Their Relatives**  
Ingrid van de Laar, MD, PhD, Erasmus University Medical Center, The Netherlands
- 1:30 PM     **S37 MYLK Mutations: Aortic Disease Presentation, Pregnancy Risk, And Characterization Of Pathogenic Missense Variants**  
Stephanie Wallace, MS, University of Texas Health Science Center at Houston, USA
- 1:45 PM     **S38 Variants In LMOD1 Causing Thoracic Aortic Aneurysm And Dissection (TAAD) In a Collaborative International Cohort**  
Yui Wan, MRes, St. George's University of London, UK

2:00-4:00 PM

## SESSION 5

**ADVANCES IN IMAGING AND BIOMARKERS**

- Chair:*     Arturo Evangelista, MD, Hospital Universitario Vall d'Hebron, Spain
- 2:00 PM     **S39 Increased Visceral Arterial Tortuosity in Marfan Syndrome: A Possible New Approach To Risk Stratification**  
Bence Agg, MD, Semmelweis University, Hungary
- 2:15 PM     **S40 Factors Associated with Type A Aortic Dissection and with Dissection at Aortic Dimension <5cm in Marfan and Loeys-Dietz Syndrome**  
Shaine Morris, MD, MPH, Texas Children's Hospital, Baylor College of Medicine, USA
- 2:30 PM     **S41 Circulating Fibrillin-1 Fragments In Children And Young Adults With Marfan Syndrome: Biomarkers For Marfan Syndrome**  
Lynn Sakai, PhD, Shriners Hospital/Oregon Health & Science University, USA
- 2:45 PM     **S42 Aortic Wall Inflammatory Activity is Independent of Aortic Dilation in Marfan Syndrome: A Hybrid PET-MRA Imaging**  
Parmanand Singh, MD, New York Presbyterian Hospital/Weill Cornell Medical Center
- 3:00 PM     **S43 Aortic Geometry Is Related To Abnormal Flow Pattern And To Proximal Descending Aorta Dilation In Marfan: A 4D-Flow MRI Study**  
Gisela Teixido, MD, PhD, Hospital University Vall d'Hebron, Spain

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FRIDAY **MAY 4** 2018

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- 3:15 PM      **S44 Aortic Microcalcification Associates With Elastin Fragmentation In Marfan Syndrome**  
Shaynah Wanga, MD, PhD, Academic Medical Center, The Netherlands
- 3:30 PM      COFFEE BREAK
- 4:00-5:00 PM      **SESSION 6**  
**INSIGHTS FROM OCULAR FINDINGS IN ANIMAL MODELS AND HUMANS**
- Chair:*      Rachel Kuchtey, MD, PhD, Vanderbilt University, Medical Center, USA
- 4:00 PM      **S45 Role of Fibrillin 1 in the Structure And Stability of the Ciliary Zonule**  
Steven Bassnett, PhD, Washington University School of Medicine, USA
- 4:15 PM      **S46 Long-Term Stable Intraocular Pressure (IOP) Control Following Ocular Gene Therapy in A Canine Model Of ADAMTS10-WMS-OAG**  
Andras Komaromy, VMD, PhD, Michigan State University, USA
- 4:30 PM      **S47 Correlation of Visual Function and Optic Nerve Structure in Mice With FBN1 Mutation**  
Rachel Kuchtey, MD, PhD, Vanderbilt University Medical Center, USA
- 4:45 PM      **S48 Knockdown of ADAMTS10 in Zebrafish Results In Shortened Body and Abnormal Retinal Development**  
John Kuchtey, PhD, Vanderbilt University Medical Center, USA
- 5:00-7:00 PM      POSTER SESSION: DIAMOND/STATEN/NASSAU ROOMS

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# SATURDAY **MAY 5** 2018

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8:30–9:15 AM

## SESSION 7

**INSIGHTS REGARDING ORTHOPEDIC MANIFESTATIONS**

*Chair:* Marlies Kempers, MD, PhD, Radboud University Medical Center, The Netherlands

8:30 AM S49 Limb- And Tendon-Specific Deletion of ADAMTL2 Cause a Short-Limb Phenotype Recapitulating Geleophysic Dysplasia

Dirk Hubmacher, PhD, Icahn School of Medicine at Mt. Sinai, USA

8:45 AM S50 Involvement of TB5 Domain in Fibrillin-1 on Regulation of Chondrogenesis

Carine Le Goff, PhD, INSERM U1148, France

9:00 AM S51 Therapeutic Potential of Small N-Terminal Fibrillin-1 Fragments in Bone

Dieter Reinhardt, PhD, McGill University, Canada

9:15–10:00 AM

## SESSION 8

**ADVANCES IN SURGICAL TECHNIQUES**

*Chair:* Craig Miller, MD, Stanford University, USA

9:15 AM S52 Aortic And Mitral Valve Surgery In Pediatric And Young Adult Patients With Marfan Syndrome: Characteristics And Outcomes

Joseph Knadler, MD, Baylor College of Medicine, USA

9:30 AM S53 Inflammatory Response After Exovasc Personalized External Root Support Implantation. A Single Centre Experience

Radka Kockova, MD, PhD, Institute for Clinical and Experimental Medicine, Czech Republic

9:45 AM S54 Aortic Root Is Larger In Patients With Marfan Syndrome And Bicuspid Aortic Valve

Olivier Milleron, MD, APHP-Hopital Bichat, France

10:00 AM S55 Reinforcing The Dilated Aortic Root In Marfan Patients: Assessment Of The Use Of A Macroporous Exostent In Sheep

Emma Vanderveken, MS, KU Leuven, Belgium

10:15 AM

COFFEE BREAK

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SATURDAY **MAY 5** 2018

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10:45AM-12NOON SESSION 9

**NON-AORTIC CARDIOVASCULAR ISSUES***Chair:* Alan Braverman, MD, Washington University, USA10:45 AM S56 **Reduced Left Ventricular Function in Marfan Syndrome**

Richard Devereux, MD, Weill Cornell Medicine, USA

11:00 AM S57 **Incidence of Aortic Events in Marfan Syndrome. Multicenter Study in 406 Patients**

Carlos Martin Lopez, MD, PhD, Hospital Universitario Puerta de Hierro, Spain

11:15 AM S58 **Myocardial Disease and Arrhythmia in Marfan Syndrome**

Laura Muino Mosquera, MD, Ghent University Hospital, Belgium

11:30 AM S59 **Prevalence of Obstructive Sleep Apnea and Its Relation to Cardiovascular Disease In Marfan Syndrome**

Laura Muino Mosquera, MD, Ghent University Hospital, Belgium

11:45 AM S60 **Medium and Long-Term Results of the Mitral Valve Repair in Marfan Syndrome**

Susana Villar, MD, Hospital Universitario Puerta de Hierro, Spain

12:00 NOON LUNCH: DIAMOND ROOM

1:00-2:45 PM SESSION 10

**PAIN, EXERCISE AND QUALITY OF LIFE***Chair:* Janneke Timmermans, MD, Radboud University Medical Center, The Netherlands1:00 PM S61 **Children and Adolescents with Marfan Syndrome; Lessons Learned From Ehlers-Danlos Syndrome and Hypermobility Spectrum Disorder**

Raoul Engelbert, PhD, Academic Medical Center, The Netherlands

1:15 PM S62 **Effects Of Combination of Mild Aerobic Exercise and Angiotensin-II Receptor Type-I Blocker Losartan In A Mouse Model of Marfan Syndrome**

Miltra Esfandiarei, PhD, Midwestern University, USA

1:30 PM S63 **Male Fbn1C1039G/+ Mice Anxious-Like Profile as a Valuable Model to Study Anxiety in Marfan Syndrome And Related Disorders**

Lydia Gimenez-Lort, PhD, Universitat Autònoma de Barcelona, Spain

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**SATURDAY MAY 5 2018**

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- 1:45 PM    **S64 Children And Adolescents With Marfan Syndrome: 10,000 Healthy Steps and Beyond — Pilot Baseline Data**  
Seda Tierney, MD, Stanford University, USA
- 2:00 PM    **S65 A Comprehensive Study Of Adults with Marfan Syndrome; Psychosocial Aspects and Health Problems**  
Gry Velvin, PhD, Sunnaas Rehabilitation Hospital, Norway
- 2:15 PM    **S66 Parents Perspectives on the Impact Of Marfan Syndrome on Daily Life Functioning of Their Children**  
Jessica Warnink-Kavelaars, MD, Academic Medical Center, The Netherlands
- 2:30 PM    **S67 VASCERN, European Rare Disease Network (ERN) on Rare Vascular Diseases**  
Guillaume Jondeau, MD, PhD, Hôpital Bichat
- 2:45–3:15 PM    **CLOSING REMARKS**

# POSTER PRESENTATION LIST

**Poster Presentation Session I — Posters 1–25****Thursday, May 3, 2018, 3:45–5:45 PM****Poster Presentation Session 2 — Posters 26–50****Friday, May 4, 2018, 5:00–7:00 PM**

Presenting authors listed. For complete authorship, see full abstract.

## MOLECULAR PATHOGENESIS IN MARFAN SYNDROME AND RELATED DISORDERS

**P1 Mutations in the Signal Peptide of FBN1 Cause Accumulation of Fibrillin 1 in the Endoplasmic Reticulum and ER Stress**

Gerard Pals, PhD, et al, VU Medical Centre, Amsterdam, The Netherlands

**P2 Lentiviral-Mediated Silencing of the FBN1 Gene Leads to Aortic Dilation and Medial Degeneration**

Maria Jesus Ruiz-Rodriguez, et al, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

**P3 Microfibrillar Associated Protein Type 4 (MFAP4) Associates With Aortic Dissection In Marfan Syndrome and is Essential For Elastic Fiber Assembly**

S Wanga, MD, PhD Candidate, et al, Academic Medical Center Amsterdam, The Netherlands

**P4 Fibrillin-Mediated Regulation of Microrna Signaling and Cell Function**

Karina Zeyer, et al, McGill University, Montreal, Canada

## GENETIC AND ENVIRONMENTAL MODIFIERS OF MARFAN PHENOTYPIC VARIABILITY

**P5 New Insights into Fibrillinopathies in the Current Genomics Era**

Sylvan Caspar, MSc, Foundation for People with Rare Diseases, Schlieren-Zurich, Switzerland

**P6 Exploring the Influence of Maternal Versus Paternal Inheritance on Gene Modifiers and The Intrafamilial Penetrance of the Aortic Phenotype In Marfan Syndrome**

Michael Garcia, BSc. et al, St George's University of London, UK

**P7 Sex and Regional Differences in the Thoracic Aorta of a Murine Model of Marfan Syndrome**

Francesco Jiménez-Altayo, PhD, et al, Universitat Autònoma de Barcelona (UAB), Spain

**P8 Pregnancy Risk in Marfan Syndrome: The Cornell Experience**

Nupoor Narula MD, et al, Weill Cornell Medical Center, New York, USA

**P9 DNA Methylation as an Epigenetic Modifier of the FBN1 Transcription**

Jun Ohgane PhD, et al, Meiji University, Kanagawa, Japan

**P10 Obstetrical and Surgical Considerations in Loeys-Dietz Syndrome (Type 4):  
A Case Series**

Melissa Russo, MD, et al, Women and Infants Hospital of Rhode Island, USA

**NEW ANIMAL MODELS, TECHNOLOGIES  
AND THERAPEUTICS****P11 Interactions Between Stem Cell Derived Cardiomyocytes and Extracellular Matrix  
To Model Marfan Syndrome In Vitro**

Jeffrey Aalders, MSc et al., Ghent University, Ghent, Belgium

**P12 A Novel Therapeutic Strategy For Marfan Syndrome Utilizing Antisense  
Oligonucleotides**

Jessica Cale, BSc, et al, Murdoch University, Western Australia, Australia

**P13 Novel Read-Out System to Assess the Mechanical Integrity of the Thoracic Aorta  
in Murine Models**

Nicolo Dubacher MSc, et al, Foundation for People with Rare Diseases, Schlieren-Zurich, Switzerland

**P14 High-Throughput Methods to Interpret Genetic Variants of Uncertain Significance  
in Genes Causing Marfan Syndrome and Related Disorders**

Christina Gurnett, MD, PhD, et al, Washington University in St Louis, MO, USA

**P15 Chances and Challenges of High-Throughput Sequencing in Genetic Testing of  
Marfan Syndrome And Related Disorders**

Janine Meienberg. PhD, Foundation for People with Rare Diseases, Schlieren-Zurich, Switzerland

**P16 Phenotype of Homozygous Fibrillin-1 (FBN1) Mutant Pigs**

Kazuhiro Umeyama MS, et al, Meiji University International Institute for Bio-Resource Research (MUIBR), Kawasaki, Japan

**P17 A Novel Large Animal Disease Model Of Marfan Syndrome: Fbn1 Edited Pigs**

Andrew Roberts, et al, Meiji University Center for Collaborative Innovation and Incubation, Kawasaki, Japan

**P18 Using Zebrafish Models to Improve the Treatment of Marfan Syndrome**

Patrick Sips, PhD, et al, Ghent University Hospital, Ghent, Belgium

**GENOTYPE/PHENOTYPE CORRELATION IN  
MARFAN SYNDROME AND RELATED DISORDERS****P19 Evaluation of Genotype-Phenotype Correlations in Marfan-Syndrome For  
Predicting The Severity Of Cardiovascular Manifestation**

Kálmán Benke MD, PhD, et al, Semmelweis University, Budapest, Hungary



- P20 Ectopia Lentis Complicating Thoracic Aortic Aneurysm Disease: Not Always Marfan Syndrome**  
Alan Braverman, MD, et al, Washington University, St. Louis, Missouri, USA
- P21 3-Year Retrospective Analysis of NGS in Genetic Testing of Heritable Connective Tissue Disorders (HCTDS) in a Northern Ireland Cohort Between 2013-2016**  
Shirley V Heggarty et al, Belfast City Hospital, Belfast, Northern Ireland
- P22 Sequencing Of 30 Candidate Genes In A Czech Cohort Of Bicuspid Aortic Valve (BAV) Patients Proves Genetic Heterogeneity And Increased Detection Of Variants In Familial Cases**  
A Krebsová, MD, PhD, et al, IKEM, Prague, Czechia
- P23 Aortopathy in Sotos Syndrome**  
Julilen Marcadier, MD, et al, Alberta Children's Hospital, Canada
- P24 Altered Myogenic Differentiation in Cells from Patients with a TGFBR1 Mutation From a Family With Thoracic Aortic Aneurysms And Dissections**  
Dimitra Micha, PhD, et al, VU Medical Centre, Amsterdam, The Netherlands
- P25 The Cardiovascular Aspect Of Patients With Marfan Syndrome In Manitoba, Canada**  
Dionysios Pepelassis, et al, University of Manitoba, Winnipeg Manitoba, Canada
- P26 Case Report of Mistaken Identity: A Family with A COL1A1 Variant Masquerading**  
Andrea L. Rideout, MS, CGC, et al, IWK Health Centre, Halifax, Nova Scotia, Canada
- P27 Mutation Spectrum in the FBN1 Gene in the Russian Patients with Marfan Syndrome**  
YA Rogozhina, et al, Petrovsky National Research Centre of Surgery, Moscow, Russia
- P28 Case Report: A Complex Deletion Insertion in FLNA Causes a Loeys-Dietz-Like Phenotype**  
Lut Van Laer, PhD, et al, University of Antwerp and Antwerp University Hospital, Antwerp, Belgium
- P29 Aortic Aneurysm: An Underestimated Serious Finding in the EP300 Mutation Phenotypical Spectrum**  
Aline Verstraeten, PhD, et al, University of Antwerp/Antwerp University Hospital, Belgium
- P30 FBN1 Mutation Type Did Not Affect Aortic Events in Marfan Syndrome Patients**  
Hang Yang, MD, Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, China
- P31 The Project 101 Genomes Marfans (P101GM)**  
Romain Alderweireldt LLM, and Ludivine Verboogen, LLM, Foundation 101 Genomes, Brussels, Belgium

## ADVANCES IN IMAGING AND BIOMARKERS

**P32 Detection of Cardiomyopathy in Children with Marfan Syndrome With 2D Strain Echocardiography**

Yves Dulac, MD, et al, Hôpital des Enfants, CHU Toulouse, France

**P33 Investigation of Association Between Aortic Dimensions, Biophysical Properties, and Plasma Biomarkers In Children And Adults With Marfan Syndrome**

Mitra Esfandiarei, PhD et al, Midwestern University, Glendale, USA

**P34 8-Colour Multiplex Immunohistochemistry for Deep Phenotyping of the Immunological Response In Human Aortopathies**

Alexander Staal, MD, et al, Radboudumc, Nijmegen, The Netherlands

## INSIGHTS FROM OCULAR FINDINGS IN ANIMAL MODELS AND HUMANS

**P35 Vascular Defects of the Retinal Vasculature in a Mouse Model of Marfan Syndrome**

Florian Alonso, PhD, et al, Inserm U1045, University of Bordeaux, France

**P36 Novel Homozygous ADAMTSL4 Mutation in a Large Consanguineous British Ectopia Lentis (EL) Family**

Jose Antonio Aragon-Martin, BSc, PhD, et al., St George's University of London, UK

**P37 Disruption of the Elastic Fibers in the Ocular System of Mouse Model for Marfan Syndrome**

RB Souza, MSc, et al., Instituto de Biociências da Universidade de São Paulo, Brazil

## INSIGHTS REGARDING ORTHOPEDIC MANIFESTATIONS IN MARFAN SYNDROME AND RELATED DISORDERS

**P38 Mutation In Fibrillin-1 Results In Joint Degeneration In Tight Skin (TSK) Mice**

Blandine Poulet, et al., University of Liverpool, UK

## ADVANCES IN SURGICAL TECHNIQUES

**P39 Successful Aortic Root Reconstruction with Severe Lung Disease Due to Chest Deformity Case Presentation**

Miklos Polos, MD, Semmelweis University, Hungary

**P40 From Aortic Morphology to Diagnosis and Indication For Surgery**

Susanna Grego, MD, et al, Cardiocentro Ticino, Lugano, Switzerland

**P41 Aortic Contraction Versus The Windkessel Model**

Gerard Pals, PhD, et al, VU University Medical Centre, Amsterdam, The Netherlands

## NON-AORTIC CARDIOVASCULAR ISSUES

**P42 Prevalence of Dilated Cardiomyopathy in the Russian Patients with Marfan Syndrome**

VA Rumyantseva, et al, Petrovsky National Research Centre of Surgery, Moscow, Russia

**P43 Epidemiology of Marfan Syndrome in a Large, Integrated Health Care Delivery System, 2006-2017**

Matthew Solomon, MD, PhD, Kaiser Permanente Northern California, USA

**P44 A Heart for Fibrillin: Distribution- and Expression-Pattern in Adult Wild-Type Murine Myocardial Tissue**

Felke Steijns, MSc, et al, Ghent University Hospital, Ghent, Belgium

## PAIN, EXERCISE AND QUALITY OF LIFE

**P45 Holistic Care of Pain in Marfan Syndrome**

Anne Child, MD, FRCP et al, St George's University of London, UK

**P46 Adults with Vascular Ehler-Danlos, Loeys Dietz Syndrome and Other Genetic Aortic Disorders in Norway: Psychosocial Aspects and Challenges In Physical Activity**

Heidi Johnansen, MSc, Sunnaas Rehabilitation Hospital, Norway

**P47 Systematic Review of the Literature of Pain in Patients with Marfan Syndrome; Prevalence, Locations, Impact and Treatment**

Gry Velvin, PhD, Sunnaas Rehabilitation Hospital, Norway

**P48 Health Problems, Chronic Pain and Fatigue. Associations with Work Participation and Life Satisfaction in Adults with Marfan Syndrome**

Gry Velvin, PhD, Sunnaas Rehabilitation Hospital, Norway

**P49 How to Get Knowledge Into Practice? Challenges in the Knowledge Translation Process of Results from a Study of Psychosocial Aspects in Marfan Syndrome**

Gry Velvin, PhD, Sunnaas Rehabilitation Hospital, Norway

**P50 Psychosocial Aspects And Physical Function In Adults With Loeys Dietz Syndrome (LDS), Vascular Ehlers Danlos (vEDS) and Marfan Syndrome (MFS)**

Gry Velvin, PhD, Sunnaas Rehabilitation Hospital, Norway



# **ABSTRACTS**

## OF ORAL PRESENTATIONS

Listed in Order of Presentation

## S.1

# **MASSIVE AGGREGAN ACCUMULATION IN THORACIC AORTIC ANEURYSM IS ASSOCIATED WITH DISSECTION IN A MOUSE MODEL OF MARFAN SYNDROME**

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**Objectives:** Proteoglycan accumulation is a hallmark of medial degeneration in thoracic aortic aneurysm and dissection (TAAD), but is poorly understood. The objectives of this study were to define the ascending aorta “proteoglycanome” using mass spectrometry and investigate association of two aggregating proteoglycans, aggrecan and versican, with human TAAD and aortic disease progression in a mouse model of severe Marfan syndrome, *Fbn1*<sup>mgR/mgR</sup>.

**Methods:** We defined the human aortic proteoglycanome using mass spectrometry of affinity-isolated proteoglycans. The large aggregating proteoglycans aggrecan and versican were analyzed in human and mouse ascending aortas using immunofluorescence, RNA in situ hybridization, qRT-PCR and RNA microarray.

**Results:** The aortic proteoglycanome comprises 20 proteoglycans including the large aggregating proteoglycans aggrecan and versican. Antibodies to these proteoglycans intensely stained medial degeneration lesions in TAAD, contrasting with modest intra-lamellar staining in controls. Aggrecan, but not versican, was increased in longitudinal analysis of *Fbn1*<sup>mgR/mgR</sup> aortas. TAAD and *Fbn1*<sup>mgR/mgR</sup> aortas had increased aggrecan and versican mRNAs, and reduced expression of a key proteoglycanase, *ADAMTS5*, was seen in TAAD. *Fbn1*<sup>mgR/mgR</sup> ascending aortas with dissection and/or rupture had dramatically increased aggrecan staining compared to aneurysms without these complications.

**Conclusions:** Aggrecan and versican accumulation in ascending TAAD occurs via increased synthesis and/or reduced proteolytic turnover, and correlates with aortic dissection/rupture in *Fbn1*<sup>mgR/mgR</sup> mice. Tissue swelling imposed by aggrecan and versican is profoundly deleterious to aortic wall mechanics and smooth muscle cell homeostasis, predisposing to type-A dissections. These proteoglycans offer potential biomarkers for refined risk-stratification and timing of elective aortic aneurysm repair.

**S.2****THE IMPORTANCE OF ENDOTHELIAL FUNCTION TO LOSARTAN'S ANTI-REMODELLING EFFECTS: OF MARFAN MICE AND MEN.**

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**Objectives:** Despite promising Marfan mouse data and early clinical studies, prophylactic use of Losartan has shown underwhelming aortic root sparing effectiveness. Our objective is to understand the mechanisms of action and thereby optimize the therapeutic potential of Angiotensin II (AngII) Receptor Blockers (ARBs).

**Methods:** C1039G mice were bred to a strain that does not express Angiotensin II (AngII) receptor type 1a (ATR1a), the target of Losartan, based on the assumption that the resulting mice would be protected against MFS aortopathy. Arterial measurements were performed.

**Results:** C1039G mice lacking AngII/ATR1a are hypotensive and show no blood pressure (BP) response to AngII due to negligible ATR1b compensation. Nonetheless, they undergo unabated aortic root enlargement, suggesting that blood pressure and AngII signaling may play lesser roles than expected in MFS. More puzzling was the observation that these mice remained fully responsive to Losartan, indicating off-target effects. Instead, Nitric Oxide Synthase (NOS) inhibition rendered Losartan therapeutically inactive, indicating that Losartan can increase protective endothelial function in MFS mice, an effect not observed with atenolol. Preliminary analyses performed in a small number of MFS patients revealed that increased endothelial function/flow-mediated dilation during losartan therapy correlates with greater aortic root stability.

**Conclusions:** Our data suggest that increased endothelial function might be of greater therapeutic value than lower BP or ATR1 inhibition in MFS patients managed with Losartan. The prospect of titrating Losartan against endothelial function should be explored. If confirmed, identification of other ARBs with superior endothelial function-activating effects could result in greater aortic root stability.

**S.3****BLOCKADE OF TGFB-1 SIGNALING BY THE TGF-B RECEPTOR PEPTIDE P144 ATTENUATES THE PROGRESSION OF AORTIC ANEURYSM IN MARFAN SYNDROME MICE**

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Transforming growth factor- $\beta$  (TGF- $\beta$ ) has been suggested to be the molecular link between fibrillin-1 gene mutation and Marfan syndrome onset, and used as a predictive marker of aneurysm formation. However, this paradigm has recently been challenged and a dual role has been proposed. The role of TGF- $\beta$  in MFS has far mostly investigated in murine models. To date, the importance of TGF- $\beta$  is supported by evidence of increased signaling and neutralizing TGF- $\beta$  antibodies on the progress of aortic aneurysms. TGF- $\beta$  is bound to types I, II and III receptors, whose internalization triggers several signaling (activation/abrogation) and gene expression responses. We have investigated whether the chronic treatment with p144, a peptide synthesized from type III TGF- $\beta$  receptor, inhibits the aneurysm formation in a MFS murine model (*Fbn1*<sup>C1039C/+</sup>). To this aim, wild type and Marfan mice were injected with a single doses of a plasmid encoding peptide p144 linked to apolipoproteinA-I through a flexible linker (pApolinkerp144). The Apolinker sequence was incorporated into a hepatotropic adeno-associated vector (AAVApolinkerp144) to induce hepatocytes to produce HDLs containing the peptide to block TGF- $\beta$ , attenuating its signaling. Two-months old mice were treated for 4 months with AAVApolinkerp144 or its empty equivalent (AAVApolinker-LUC). Animals were subjected to echocardiography before their sacrifice (6 months old) to obtain ascending aorta for histology and protein extraction analysis. Results show that both the aortic root diameter and aortic wall disruption were significantly ameliorated in Marfan AAVApolinkerp144. We also evaluated TGF- $\beta$ 1, MMP2, MMP9 and PAI-1 levels in plasma. Marfan AAVApolinkerp144 showed unaltered levels of MMPs, higher levels of TGF $\beta$ -1 and reduced of PAI-1.

This work is supported by grants of The Marfan Foundation (USA) and MINECO (Spain).



## S.4

## LINEAGE-SPECIFIC SENSITIVITY TO TGF- $\beta$ SIGNALING PERTURBATION DRIVES AORTIC ROOT ANEURYSM IN LOEYS-DIETZ SYNDROME

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### Objectives

In this study we define lineage-specific events that reconcile both the anatomical location of aneurysm and the paradoxical enhancement of transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling that is observed in the aortic root of Loeys-Dietz syndrome (LDS) patients and mouse models that carry heterozygous loss-of-function mutations in positive effectors of this pathway.

### Methods

Using LDS mice that harbor a heterozygous kinase-inactivating mutation in the type I TGF- $\beta$  receptor,

*Tgfb1*<sup>M318R/+</sup>, we analyzed the signaling capacity of lineage-traced vascular smooth muscle cells (VSMCs) derived from the secondary heart field (SHF) and the cardiac neural crest (CNC), and assessed the effect of lineage-specific TGF- $\beta$  antagonism on disease progression.

### Results

*Tgfb1*<sup>M318R/+</sup> SHF-VSMCs but not *Tgfb1*<sup>M318R/+</sup> CNC-VSMCs show impaired induction of Smad-dependent pathways in response to TGF- $\beta$ . Loss of signaling in SHF-VSMCs associates with upregulation of angiotensin II type 1 receptor (*Agtr1a*) expression and increased angiotensin II-dependent expression of TGF- $\beta$ . Maintenance of signaling potential by CNC-derived VSMCs translates into enhancement of Smad2/3 signaling in vivo. Homozygous deletion of *Smad2* in CNC-VSMCs, but not in SHF-VSMCs, prevents aneurysm development, as also seen with ARBs that suppress TGF- $\beta$  ligand expression by SHF-VSMCs.

### Conclusions.

The clinical effects of mutations that impair but do not abrogate TGF- $\beta$  signaling stem from two interdependent processes related to *loss* of signaling in SHF-VSMC but to *gain* of signaling in CNCVSMCs - the ultimate effector of aneurysm progression. This pathogenic sequence has demonstrable relevance to other vasculopathies where disease focuses at the aortic root including Marfan syndrome.

**S.5****APPLICATION OF A CONDITIONAL ALLELIC SERIES OF THE SLOAN-KETTERING INSTITUTE PROTO-ONCOGENE (SKI) TO MECHANISTICALLY DISSECT THE TGF $\beta$  VASCULOPATHIES**

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**Objectives:** Marfan (MFS), Loeys-Dietz (LDS) and Shprintzen-Goldberg (SGS) syndromes show substantial phenotypic overlap including aortic root aneurysm. While all show an aortic wall signature for high TGF $\beta$  signaling, LDS is caused by heterozygous loss-of-function (LOF) alleles in genes encoding positive effectors of TGF $\beta$  signaling. This has engendered controversy regarding the precise role of TGF $\beta$  in aneurysm progression. We propose a reconciling model in which different VSMC lineages have variable vulnerability a relative perturbation of TGF $\beta$  signaling, resulting in compensatory upregulation of TGF $\beta$  ligand production and paracrine overdrive of signaling in its less vulnerable neighbor. In keeping with hypothesis, we showed that LDS second heart field (SHF)-derived VSMCs show signaling collapse and increased AT1R-dependent TGF $\beta$  expression, while neighboring cardiac neural crest (CNC)-derived cells remain signaling competent.

**Methods:** Crossing MFS mice with transgenic mice that overexpress wildtype SKI in a spatially- and temporally-conditional manner or a conditional *Ski* knock-out mouse allowed robust testing of the model.

**Results:** Postnatal overexpression of SKI specifically in CNC-derived VSMCs of MFS mice suppresses TGF $\beta$  target gene expression and completely prevents aneurysm. Deletion of *Ski* in the CNC of MFS mice exacerbates both TGF $\beta$  signaling and aneurysm growth. As predicted by the model, deletion of *Ski* in the SHF of MFS mice prevents aneurysm formation.

**Conclusions:** These data show that aneurysm severity in multiple conditions specifically titrates TGF $\beta$  signaling status in the CNC, support therapeutic strategies aimed at TGF $\beta$  antagonism, and highlight the importance of consideration of the microenvironments within which genetic alterations exert their phenotypic influence.

**S.6****FIBRILLIN-1 DIRECTLY REGULATES MACROPHAGE NO PRODUCTION AND POLARIZATION**

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**Objectives:** Marfan syndrome (MFS) is a heritable disease caused by mutations in fibrillin-1 that affects the cardiovascular, ocular, and skeletal systems. We have previously shown that cleavage of fibrillin-1 produces fragments that inhibit osteoclast differentiation through the calcium/calcineurin/NFAT pathway. Osteoclasts and macrophages share the monocytic origin, and calcium/calcineurin/NFAT pathway also regulates macrophage inflammatory function. Therefore, we hypothesized that fibrillin-1 or its degradation fragments regulate macrophage differentiation and/or inflammatory polarization.

**Methods:** Mouse bone marrow monocytes were cultured with macrophage colony stimulating factor (MCSF) and interferon- $\gamma$ , differentiation was assessed using immunofluorescence for a macrophage marker f480, inflammatory potential was examined by measuring NO production using Griess assay. Spleens were extracted from heterozygous C1039G mice, which develop classical manifestations of MFS, for RT-qPCR expression analysis of genes implicated in inflammation.

**Results:** Fibrillin-1 N-terminal or C-terminal halves did not affect the rate of macrophage formation. Fibrillin-1 N-terminal half, but not C-terminal, inhibited NO production in a dose-dependent manner. Using a panel of recombinant sub-fragments, we localized NO inhibitory activity to the 63 kDa subfragment comprising the N-terminal region of fibrillin-1. The spleens of C1039G mice demonstrated significant increases compared to wild type in the gene expression of macrophage markers, CD68 and f480, and pro-inflammatory Stat1 and CD163 genes. Typically anti-inflammatory genes, Arg-1 and interleukin (IL) 10, demonstrated opposing trends - expression of Arg-1 was significantly increased, IL10 was unchanged.

**Conclusions:** We identified a fibrillin-1 fragment as a novel anti-inflammatory compound that is relevant to both patients afflicted with MFS or chronic inflammation.

**S.7****NEW MEDIATORS AND THERAPEUTIC TARGETS OF AORTIC DISEASE**

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During the last few years, we have identified a number of genes that are involved in thoracic aortic aneurysm and dissection (TAAD) and in other diseases that occur with pathological wall remodeling. These genes include *Rcan1*, *Calcineurin*, *Adamts1*, *Nos2*, *C/EBP $\beta$*  and *Plk1*. We have found that *Adamts1* is a major regulator of vascular homeostasis whose genetic haploinsufficiency in mice causes a TAAD similar to Marfan Syndrome (MFS). Unexpectedly, aortic nitric oxide and *Nos2* levels are increased in *Adamts1*-deficient mice before TGF $\beta$  activation, and *Nos2* inactivation protects both *Adamts1*<sup>+/-</sup> mice and a mouse model of MFS from developing aortopathy. More importantly, pharmacological inhibition of NOS2 results in a rapid and sustained reversion of aortic dilation and medial degeneration in *Adamts1*-deficient mice and in MFS mice. MFS patients also show elevated NOS2 and downregulated ADAMTS1 in aorta, uncovering a possible causative role for this axis in human disease. We are currently investigating the relative roles and expression of different members and substrates of the ADAMTS family in aortic dilation and medial degeneration in MFS mouse models and MFS patients. We believe that these findings open new exciting avenues of research into TAADs pathogenesis and urge the evaluation of NOS2 inhibitors as a novel therapy.

## S.8

## TGFB AND AORTIC DISEASE SEVERITY IN MFS

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**Background:** Enhanced TGFβ signaling is observed in several heritable forms of thoracic aortic aneurysms and dissection, including Marfan syndrome (MFS). The precise role of the TGFβ signaling pathway in the disease process is currently unclear.

**Methods:** We used a genetic approach to investigate the role of the TGFβ pathway in aortic aneurysm formation and progression by crossing the *GT-8/+* MFS mouse model with *Tgfb1*<sup>+/-</sup>, *Tgfb2*<sup>+/-</sup> and *Fbn1*<sup>H1D/H1D</sup> mice. The latter model is characterized by an in-frame deletion of the hybrid 1 domain of fibrillin-1, essential for binding the latent TGFβ complex in the matrix. The aortas of male compound heterozygous mice and control littermates were then studied by means of ultrasound at 6 months of age.

**Results:** Ultrasound analyses show that compound heterozygous *GT-8/+;Fbn1*<sup>+/-H1D</sup> and *GT-8/+;Tgfb2*<sup>+/-</sup> mice present more severe aortic disease compared to *GT-8/+;Fbn1*<sup>H1D/H1D</sup>, and *Tgfb2*<sup>+/-</sup> mice at the age of 6 months. A fraction of the *GT-8/+;Fbn1*<sup>+/-H1D</sup> and *GT-8/+;Tgfb2*<sup>+/-</sup> mice dies prematurely (as early as 4-5 months of age) due to aortic rupture. In contrast, the aortic diameters of *GT-8/+;Tgfb1*<sup>+/-</sup> mice do not differ from *GT-8/+* mice at 6 months of age and the mice have a normal life span.

**Conclusion:** These data indicate that the effect of TGFβ on aortic aneurysm formation is isoform specific and that binding of the TGFβ complex to the extracellular matrix is required for correct homeostasis in the aorta in the context of MFS.

## S.9

### BMP DRIVEN MECHANISMS IN AORTIC ANEURYSM FORMATION CAUSED BY FIBRILLIN-1 DEFICIENCY

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**Objectives:** Fibrillin microfibrils (FMF) target and sequester growth factors of the TGF- $\beta$  superfamily within the extracellular matrix. Recently, we showed that fibrillin-1 binding to BMP prodomain-growth factor complexes induces a conformational change in the prodomain which renders the growth factor latent. So far, no information is available how BMPs can be activated from FMF-bound pools and how aberrant BMP activation induced by fibrillin-1 deficiency might contribute to pathomechanisms of Marfan syndrome.

**Methods:** Aortas from GT8 knock-in mice expressing a fibrillin-1 C-terminal truncation mutation were analyzed by echocardiography, immunofluorescence, immunohistochemistry, electron and second harmonic generation microscopy. Primary VSMCs from GT8 aortas were analyzed. Cleavage assays using recombinant proteins were performed. Genetic breeding experiments were conducted.

**Results:** At birth aortas from *Fbn1*<sup>+/<sup>GT8</sup></sup>, *Fbn1*<sup>GT8/GT8</sup> mice appear normal. After P7 we found progressive degradation of adventitial collagen and elastic lamellae. *Fbn1*<sup>+/<sup>GT8</sup></sup>, *Fbn1*<sup>GT8/GT8</sup> mice showed significant aortic root enlargement at P10 which correlated with the onset of increased BMP signaling and MMP-13 expression. BMPs upregulated MMP-13 expression in VSMCs. Utilizing a newly identified MMP-13 cleavage site within BMP prodomains resulted in release of bioactive BMPs from FMF-bound pools. Breeding the GT8 allele onto a *Mmp13* null background rescued aortic root enlargement.

**Conclusions:** Failed BMP sequestration to structurally impaired FMF leads to increased BMP signaling the moment structural compensation by fibrillin-2 ceases. Non-sequestered BMPs trigger a destructive feed-forward cycle by turning on MMP-13 activity which activates more fibrillin-bound BMPs but also other MMPs leading to gross destruction of the aortic architecture including collagen and elastin networks.

**S.10****ACTIVATION OF XANTHINE OXIDOREDUCTASE IN VASCULAR ENDOTHELIAL CELLS MEDIATES AORTIC ANEURYSM FORMATION IN MARFAN SYNDROME**

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**Objectives:** Marfan syndrome (MFS) is an inherited disorder caused by *FBN1* gene mutations. However, it still remains unclear how *FBN1* mutations lead to MFS-associated aortopathy. We investigated the roles of reactive oxygen species (ROS) generation in vascular endothelial cells (ECs) in the pathogenesis of aortic aneurysm in MFS.

**Methods and Results:** We observed a significant increase in ROS generation in ascending aorta of MFS patients and MFS mice harboring the *Fbn1* mutation (C1039G), which was associated with a significant increase in protein expression, enzymatic activity of xanthine oxidoreductase (XOR), a major source of ROS production. Next, we inhibited *in vivo* function of XOR in MFS mice either by *Tie2*-Cre-mediated disruption of *Xdh* gene in vascular ECs or by systemic administration of XOR inhibitor febuxostat in drinking water. In both EC-specific XOR knockout and febuxostat-treated MFS mice, the enlargement of aortic diameter was significantly suppressed, as compared with control MFS mice. Histologically, aortic wall thickening with degeneration of elastic fibers and proteoglycan deposition, and perivascular infiltration of macrophages were significantly attenuated in ascending aorta of both EC-specific XOR knockout and febuxostat-treated MFS mice. Additionally, the morphological and histological improvement of aortic aneurysm was associated with decrease in ROS generation and matrix metalloproteinase activation as well as a significant inhibition of activation of ERKs, p38MAPK, and Smads.

**Conclusion:** ROS generation by XOR in vascular ECs contributes to the pathogenesis of aortic aneurysm formation in MFS, and highlights an application of XOR inhibition as a potential therapy for aortic aneurysm in MFS patients.

## S.11

## ASSOCIATION OF MODIFIERS AND OTHER GENETIC FACTORS EXPLAIN MARFAN SYNDROME CLINICAL VARIABILITY

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**INTRODUCTION:** Marfan syndrome (MFS) is a rare autosomal dominant connective tissue disorder related to mutations in the *FBN1* gene. Prognosis is related to aortic risk of dissection following aneurysm. MFS clinical variability is notable, for age of onset as well as severity and number of clinical manifestations.

**OBJECTIVES AND METHODS:** To identify genetic modifiers, we combined genome-wide approaches in 1070 clinically well-characterized *FBN1* disease-causing variant carriers: 1) an *FBN1* eQTL analysis in 80 fibroblasts of *FBN1* stop variant carriers, 2) a linkage analysis, 3) a kinship matrix association study in 14 clinically concordant and discordant sib-pairs, 4) a genome-wide association study and 5) a whole exome sequencing in 98 extreme phenotype samples.

**RESULTS:** Three genetic mechanisms of variability were found. A new genotype/phenotype correlation with an excess of loss-of-cysteine variants ( $p=0.004$ ) in severely affected subjects. A second pathogenic event in another thoracic aortic aneurysm gene or the *COL4A1* gene (known to be involved in cerebral aneurysm) was found in 9 individuals. A polygenic model involving at least 9 modifier loci (named gMod-M1-9) was observed through cross-mapping of results. Notably, gMod-M2 which co-localizes with *PRKG1*, in which activating variants have already been described in thoracic aortic aneurysm, and gMod-M3 co-localized with a metalloprotease (proteins of extra-cellular matrix regulation) cluster.

**CONCLUSION:** Our results represent a major advance in understanding the complex genetic architecture of MFS and provide the first steps toward prediction of clinical evolution.



**S.12****OXYTOCIN ANTAGONISM PREVENTS PREGNANCY-ASSOCIATED AORTIC DISSECTION IN A MOUSE MODEL OF MARFAN SYNDROME**

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**Objectives:** Pregnancy increases the risk of aortic dissection in Marfan syndrome (MFS). Pathogenic models that singularly invoke hemodynamic stress are difficult to reconcile with the predominant postnatal occurrence of dissection. We hypothesized a role for oxytocin, a hormone known to initiate uterine contraction and milk letdown that is sustained postnatally during lactation. Oxytocin receptor is induced in the aorta during pregnancy and the hormone stimulates peripheral tissues through activation of ERK that has previously been implicated in the pathogenesis of aortic disease in MFS.

**Methods:** We explored the consequence of manipulation of oxytocin and downstream signaling events in a mouse model of MFS.

**Results:** The mgR mouse model of MFS shows ~90% death due to aortic dissection in the 4 weeks following delivery. Prevention of lactation through pup removal increased survival to 70% while treatment with a specific oxytocin receptor antagonist initiating at 14 dpc lead to greater than 90% survival, a performance that was mimicked upon treatment with hydralazine (that blocks ERK activation), but not propranolol (which does not). The risk of aortic dissection is directly proportional to the level of phosphorylated ERK1/2 in the aortic wall and near-complete survival of postpartum MFS mice was achieved upon treatment with trametinib, an FDA-approved inhibitor of ERK kinase (MEK) We have also shown that prevention of lactation eliminates postpartum dissection in a knock-in mouse model of vascular Ehlers-Danlos syndrome.

**Conclusion:** This therapeutic strategy has the strong potential to modify vascular risk in woman with MFS and other heritable connective tissue disorders.

## S.13

# EPIGENETIC MODULATION IN THE PATHOGENESIS AND TREATMENT OF MARFAN SYNDROME AND RELATED DISORDERS

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**Objectives:** Shprintzen-Goldberg syndrome (SGS) is caused by mutations in the *SKI* gene, which encodes a negative regulator of TGF $\beta$  signaling. SGS shares most features of Marfan and Loeys-Dietz syndromes (MFS and LDS) with the added finding of intellectual disability. SKI mutations occur in the Smad-binding domain, preventing co-recruitment and repressive activity of SKI at regulatory elements in TGF $\beta$  target genes, unopposed histone acetyltransferase (HAT) activity of EP300, and amplified TGF $\beta$  transcriptional responses.

**Methods:** We developed constitutive or conditional knock-in mouse models of SGS that express a heterozygous missense *Ski* allele (p.G34D) in all cells or specifically in VSMCs, respectively. These mice were assessed at baseline and in response to various treatment strategies.

**Results:** *Ski*<sup>G34D/+</sup> mice phenocopy SGS including skeletal, craniofacial, and neurodevelopmental manifestations. Both SGS mouse models show highly penetrant aortic root aneurysm and dissection in association with all of the hallmarks of MFS and LDS, including altered aortic wall architecture, an overt mRNA signature for a TGF $\beta$  synthetic repertoire (TSR), and complete protection with losartan. Systemic administration of C646 (a selective EP300 inhibitor) prevented aneurysm progression in both *Ski*<sup>VSMC:G34D/+</sup> and MFS (*Fbn1*<sup>C1039G/+</sup>) mice in association with preservation of aortic wall architecture, prevention of excessive H3K27 acetylation, and normalization of the TSR. C646 also normalized TGF $\beta$  target gene expression in SGS patient fibroblasts, with comparable efficiency as a TGF $\beta$  type I receptor (Alk5) kinase inhibitor.

**Conclusions:** These data unambiguously implicate the TGF $\beta$  transcriptional response in the pathogenesis of SGS and MFS and highlight the therapeutic potential of epigenetic modulation.

**S.14****FUNCTIONAL RELEVANCE OF FIBULIN-4 INTERACTIONS WITH LATENT TRANSFORMING GROWTH FACTOR BETA BINDING PROTEIN- 4 IN THE CONTEXT OF FIBRILLIN-1 AND FIBRONECTIN**

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Elastogenesis is an intricate and well-orchestrated cellular mechanism which involves several extracellular matrix (ECM) proteins such as Fibulin-4 (FBLN4), Latent TGF- $\beta$  binding protein-4 (LTBP4), Fibronectin (FN) and Fibrillin-1 (FBN1). These proteins play key roles in driving the assembly of tropoelastin into mature elastic fibers, which are essential for the elasticity of various soft tissues including skin and blood vessels. Surface plasmon resonance spectroscopy (SPR) showed that FBLN4 can interact with FN *in vitro*. Deletion of FN using fibroblasts from *Fn* knockout mice disrupts the assembly of FBLN4.

To analyze if the dependency of FBLN4 on FN is direct or is dependent on other ECM proteins which require FN for their assembly, fibroblasts from *Fbn1* knockout mice were utilized. Knocking out *Fbn1* (FN assembly is still intact) does not affect FBLN4 assembly. This data shows direct dependency of FBLN4 on FN for its assembly. FBLN4 assembly is also known to be dependent on LTBP4. Mouse studies also show the existence of a functional relationship between the two proteins. But how the two proteins physically interact and affect each other at molecular level is not clear. Atomic force microscopy (AFM) of samples of LTBP4 mixed with FBLN4 revealed that the interaction of FBLN4 with LTBP4 induces an unexpected conformational change in the structure of LTBP4, switching the conformation from a compact to an elongated structure. Furthermore, this conformational change triggered an increased binding of LTBP4 to FBN1, but a decreased binding to FN, as shown by SPR and solid phase binding assays. Immunofluorescence analyses revealed that the conformational change in LTBP4 induced by FBLN4 also affected LTBP4 assembly/deposition in cell culture.

Next we examined if FBLN4 acts as a chaperone to induce this conformational and functional change or it forms a complex with LTBP4. To test this, we passed LTBP4 over a covalently bound FBLN4 chromatography column. This facilitated a transient (30-45 min) interaction between the two proteins but prevented any permanent complex formation. It was observed that a transient exposure to FBLN4 is sufficient to induce a conformational change in LTBP4 as observed by AFM and dynamic light scattering experiments. The interaction also imposes a functional change in LTBP4 as observed SPR. These results confirmed that FBLN4 acts as a chaperone to induce conformation and functional change in LTBP4. These results highlight new interactions between the elastogenic proteins FBLN4 and LTBP4, their structural and functional consequences, and their dependency on FN and FBN1. These data provide a new paradigm of how elastic fibers assemble.

**S.15****SEXUAL DIMORPHISM IN *SMAD3* MUTATION CARRIERS**

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**Objective:** *SMAD3* mutation carriers are at-risk for aneurysms/dissections throughout the arterial tree. Based on prior reports of sex differences in thoracic aortic aneurysm/dissection, we investigated whether there is sexual dimorphism for vascular events in *SMAD3* mutation carriers.

**Methods:** We analysed two large pedigrees with  $\geq 82$  individuals segregating missense mutations in the MH2 domain of *SMAD3*. We excluded mutation-carrying individuals  $<40$  years without an aneurysm/vascular dissection/intervention as they were too young to be classified. We then subcategorized mutation-carrying individuals according to sex, the presence or absence of vascular lesions and the localisation of vascular involvement. For the latter, we distinguished two categories: either aneurysm/dissection without involvement of the aortic root/ascending aorta or aneurysm/dissection with disease affecting the aortic root/ascending aorta.

**Results:** In our two large pedigrees, 11/29 (38%) mutation-carrying females had no vascular involvement, whereas all 21 mutation-carrying males were affected ( $p=0.001$ ). This difference was not pregnancy-related as almost all women over 25 years of age with (16/17) and without (11/11) vascular involvement had children ( $p=0.41$ ). Of the 18 females with vascular involvement, 6 (33%) had vascular involvement without ascending or root aneurysm; only one of the 21 males (5%) did ( $p=0.02$ ).

**Conclusions:** (1) non-penetrance is more common in women and the proportion of women with completed pregnancies was not different between penetrant and non-penetrant women and (2) normal echocardiography in at-risk females is not as reassuring as in males in terms of risk of a) vasculopathy in other locations and b) being a transmitting female if mutation status unknown.

**S.16****FUNCTIONAL CHARACTERIZATION OF MODIFIER LOCI FOR MARFAN SYNDROME REVEALS NOVEL THERAPEUTIC STRATEGIES**

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**Objectives:** We sought genetic modifiers of Marfan syndrome (MFS) to inform disease pathogenesis and treatment.

**Methods:** A large cohort of intercrossed C57BL6J and 129 MFS mice was generated, stratified according to aortic root size, and genotyped to identify quantitative trait loci (QTLs). Functional characterization was performed.

**Results:** MFS mice displayed overt protection from aneurysm and death on the BL6 background compared to 129; this associated with marked attenuation or amplification of SMAD, ERK, p38 and EGFR activation in the aortic wall, respectively. Two QTLs achieved genome-wide significance in association with aortic root size, with marked epistasis between the loci. In comparison of strain-specific gene sequences, two functional candidates emerged - a stop-loss mutation in *Mmp17* and a non-conservative missense mutation (p.G76E) in *Map2k6*. Differential processing of MMP17 associated with excessive phosphorylation of EGFR and aggressive aneurysm progression on the 129 background. Mixed background mice selected only for null or 129 alleles at the two candidate loci perfectly recapitulated the biochemical and clinical performance of pure BL6 or 129 MFS mice, respectively. We also observed full prevention of aneurysm and ERK activation in 129/MFS animals treated with the FDA-approved EGFR antagonist Erlotinib. A parallel modifier study in patients with MFS identified protective variation in MAP3K4, a factor immediately upstream of the mouse modifier MAP2K6.

**Conclusions:** Unbiased methods in 2 species have revealed an unanticipated pathway of modification in MFS. Pharmacologic agents can be used to leverage nature's strategies to mitigate vascular disease in the care of MFS patients.

**S.17****MECHANISTIC INTERROGATION OF A GENE-BY-ENVIRONMENT INTERACTION INFORMS THE PATHOGENESIS AND TREATMENT OF MARFAN SYNDROME**

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**Objectives:** Marfan syndrome (MFS) and Loeys-Dietz syndrome (LDS) are characterized by aortic root aneurysm. Bicuspid aortic valve, the most common inherited aneurysm condition, associates with distal ascending aortic aneurysm (BAV/DAscAA), yet its etiology remains mysterious. We sought to understand whether these inherited aneurysm conditions show mechanistic overlap and common therapeutic vulnerabilities despite their anatomic differences.

**Methods:** We showed that mouse models of MFS or LDS develop ERK1/2-dependent hyperacute dilation of the DAscAo in response to calcium channel blockers (CCBs). We performed RNA sequencing on MFS mouse aortas, applying strict *a priori* filters to select for transcripts that display an acute change in response to CCBs that is abrogated upon ERK antagonism.

**Results:** The top pathways enriched in this dataset related to Notch and androgen signaling. The Notch inhibitor dibenzazepine (DBZ) accelerated DAscAA progression in CCB-treated MFS or LDS mice, an effect which was accentuated in males and remained ERK1/2- and Angiotensin II type 1 receptor (AT1R)-dependent. Androgen receptor antagonism was overtly protective in MFS/CCB/DBZ mice, informing the male gender bias inherent to many aneurysm conditions. We developed and rigorously tested a pathogenic model for DAscAA that integrates genetic predisposition, anatomical location of aneurysm, the protective influence of Notch, and the exacerbating effects of CCBs that collapses on the expression and activity of the regulator of G-protein signaling (RGS) proteins – potent modulators of AT1R signaling and ERK activation.

**Conclusions:** This work highlights the importance of Notch, androgen, and RGS protein activity in diverse hereditary aneurysm conditions and reveals novel therapeutic strategies.

## S.18

**MECHANISTIC INTERROGATION OF A NOVEL MOUSE MODEL OF VASCULAR EHLERS-DANLOS SYNDROME**

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**Objectives:** Patients with vascular Ehlers-Danlos syndrome (vEDS) demonstrate spontaneous dissection in medium-to-large vessels. Many features of vEDS are distinctly different from other heritable vasculopathies, such as Marfan Syndrome (MFS) and Loeys-Dietz Syndrome (LDS), which have been associated with excessive TGF- $\beta$  activity. These features include no particular predisposition for involvement of the aortic root and dissection without prior dilation. We created and characterized a knock-in mouse model of vEDS (*Col3a1*<sup>G209S/+</sup>), hypothesizing that comparison to MFS and LDS mice would inform pathogenic mechanisms and therapeutic strategies.

**Methods:** We evaluated the phenotype of *Col3a1*<sup>G209S/+</sup> mice at baseline and in response to pharmacologic and physiologic manipulations known to provoke aneurysm in other disease models.

**Results:** *Col3a1*<sup>G209S/+</sup> mice die suddenly due to arterial rupture without prior dilation or deterioration of aortic wall architecture. As in MFS, angiotensin-II (AngII) signaling and lactation-associated oxytocin exposure induce vascular rupture in *Col3a1*<sup>G209S/+</sup> mice and vulnerable vascular segments show elevated phosphorylation of ERK and increased expression of the AngII type 1 receptor (AT1); in contrast to MFS or LDS, expression profiling of vEDS aorta did not show a synthetic repertoire typical for high TGF $\beta$  signaling and negative regulators of the TGF $\beta$  transcriptional response (SKI, SKIL) were downregulated. Informatively, the low TGF $\beta$  signaling C57BL/6J background is protective in MFS but provokes early vascular death in vEDS; opposite results are observed for the high TGF $\beta$  signaling 129Sve background.

**Conclusion:** TGF $\beta$  may support compensatory vascular changes in vEDS, but AT1 signaling and ERK activation represent common pathogenic events and promising therapeutic targets.

**S.19****AN IPSC-DERIVED DRUG SCREENING PLATFORM TO IDENTIFY THERAPEUTIC COMPOUNDS FOR MARFAN SYNDROME**

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**Background:** Marfan syndrome (MFS) is a connective tissue disorder with pleiotropic manifestations including severe cardiovascular complications, such as aortic aneurysms and dissection. These are caused by mutations in *FBN1*, which codes for the extracellular matrix structural component, fibrillin-1. Currently, MFS treatments focus on minimising aortic wall stress by controlling blood pressure and haemodynamics. TGF- $\beta$  signalling blockade prevented aortic dilatation in a MFS mouse model (Habashi et al. 2006) but clinical trial attempts remain unsuccessful (Lacro et al. 2014).

**Objectives:** We recently identified p38 and KLF4 as novel disease drivers in our human induced pluripotent stem cell (iPSC) model (Granata et al. 2017). However, the signalling abnormalities in MFS remain complex and varied hence our decision to focus on downstream pathogenic phenotypes. One common phenotype is excessive matrix degradation coupled with increased expression of proteolytic enzymes. We hypothesised that MFS proteolytic dynamics may reliably identify novel therapeutic targets.

**Methods:** Patient iPSC-derived smooth muscle cells (SMCs) are treated with a phenotypic compound library (AstraZeneca) in 24-wells. Subsequently, proteolytic enzyme activity is measured using a fluorescent substrate based system.

**Results:** Our phenotypic assay reliably identified compounds inhibiting MMP activity. In addition, RNA sequencing on MFS and CRISPR-corrected isogenic SMCs further validated novel genes and pathways involved in MFS.

**Conclusions:** Our *in vitro* disease model offers a screening platform to identify putative hits. Further validation will be done by assaying the ability to also inhibit apoptotic and proliferation inhibition responses. These techniques will enable the identification of novel drugs to treat MFS.



**S.20****EFFECT OF LOSARTAN AND BETA-BLOCKERS ON AORTIC ROOT DILATATION IN PATIENTS WITH MARFAN SYNDROME – RESULTS OF THE EXTENDED COMPARE TRIAL**

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**Introduction:** The effect of losartan and  $\beta$ -blockers on aortic dilation rate in Marfan syndrome is still not entirely clear. The COMPARE trial showed a small but significant beneficial effect of losartan on top of regular medical treatment on aortic root dilatation. However, this effect was not reproduced by other studies. Aortic root dilatation rates were very low in all studies, suggesting selected study populations that were mildly affected. Therefore we extended the follow-up period of the COMPARE trial up to 10 years. We retrospectively assessed clinical events in the total study cohort and analyzed aortic root dilatation rates in relation to medication regimes in patients with a native aortic root at inclusion.

**Methods:** Patients previously enrolled in the COMPARE trial (mean age 38 years, 54% male, 73% native aortic root at inclusion) were retrospectively analyzed. Aortic root dilatation rate was calculated in patients with a native aortic root using transthoracic echocardiography (TTE). Patients who underwent aortic root replacement after randomization were included until the last preoperative TTE. The correlation between cumulative losartan or  $\beta$ -blocker use, as measured in days, and aortic root dilatation rate was assessed with scatter plots and Spearman's rho.

**Results:** During a median follow-up of 7.9 years, 14 dissections (12 type B, 1 type A, 1 unknown) and five deaths occurred in 221 patients (events in 8%). Causes of death were two complicated dissections (1 type B, 1 unknown), and ruptured thoracoabdominal aortic aneurysm, bowel ischemia, and cancer in the remaining three. No differences in events could be demonstrated between patients treated with and without losartan. A native aortic root was present in 125 patients at inclusion (13 on losartan, 29 on  $\beta$ -blockers, 73 on both and 10 without either). Of these patients, 48 (38%) underwent aortic root replacement during follow-up.

Median aortic root dilatation in patients with a native aortic root upon inclusion was 0.28 mm/y (interquartile range 0.08 – 0.60). Aortic root dilatation rate was negatively correlated with the number of losartan treatment days (rho -0.234, P = .009) and with number of  $\beta$ -blocker treatment days (rho -0.183, P = 0.043; Figure).

**Conclusions:** MFS patients from the COMPARE trial have mainly shown vascular events in the descending aorta, probably due to an aggressive prophylactic surgical regime. The association between both  $\beta$ -blockers and losartan with a lower aortic root dilatation rate may suggest beneficial effects of both agents in MFS.

**S.21****DESIGNING A PATIENT-DRIVEN PLATFORM AND REGISTRY TO INCREASE USER ENGAGEMENT IN RESEARCH FOR MARFAN SYNDROME AND RELATED DISORDERS****Authors:**

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**Objectives:**

Complex and rare conditions pose unique barriers for researchers interested in data collection, while also burdening patients and caregivers managing health information. Our goal was to design an innovative approach for increasing patient participation and engagement in studying Marfan syndrome and related disorders, and at the same time provide value for patients.

**Methods:**

Our team created a tool backed by over two dozen medical libraries in five languages to give users control over organizing, co-managing, and translating health information. Working with researchers, the Marfan Foundation, and early users, we built a secure and structured way for users to share health information with the Foundation.

**Results:**

The Marfan and Related Disorders International Patient Registry captures and aggregates health data. Balancing privacy and transparency, we designed Share Cards, a method of anonymously sharing information with the Foundation, while giving users control over what data is shared. The Foundation receives real time data through Share Cards. When users complete condition-specific surveys, answers load into their personal account, providing users immediate benefit to participation. Survey responses and information stored on a Backpack account can be reused for additional research, lowering the barrier of entry for subsequent studies.

**Conclusions:**

The Marfan Foundation will have a live, dynamic data registry to support research, and patients will have a tool they can use in their daily lives to keep track of their health information and that of their loved ones. In the future, we plan to evaluate how this platform influences patient and caregiver study participation.

## S.22

### NEXT GENERATION SEQUENCING IN THE DIAGNOSIS OF MARFAN SYNDROME AND RELATED DISORDERS: AN EFFICIENT GLOBAL APPROACH IN THE SNV/CNV DETECTION

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**Objectives:** The Genetic Laboratory in Bichat Hospital (Paris) receives each year samples for approximately 600 probands suspected for Marfan Syndrome (MFS) or Related Disorders (RD) nationwide. Genetic heterogeneity is high and new genes are regularly discovered. Initially, the strategy for molecular diagnosis was based on successive Sanger sequencing of the main genes, associated with MLPA analysis. The objective was to evaluate on these probands a NGS capture panel strategy, targeting 25 known disease causing genes.

**Methods:** Sequencing was carried out on Illumina MiSeq. Bio-informatical analysis was performed on CLC Genomics Workbench software; Single Nucleotide Variants (SNV) annotation was completed with a Python script. Copy Number Variation (CNV) were sought through comparison of coverage depths, standardized for each amplicon to those of a group of controls.

**Results:** To date, more than 600 probands have been sequenced on this capture panel; a potentially pathogenic variant was found in approximately 200 patients. This led us to identify the disease causing variation in new genes in families in which the most frequent genes had already been excluded. This global approach enabled us to expand the phenotypic spectrum of pathogenic variants in some genes. Moreover, several pathogenic CNV have been pinpointed, either in some genes that were not screened for CNV before.

**Conclusion:** NGS technologies enable us to have a unique strategy in the molecular diagnosis of MFS and RD. The chosen technology is reliable for SNV and CNV detection and flexible since the targeted genes can easily be adapted as scientific knowledge develops.

## S.23

## ARTERIAL TORTUOSITY SYNDROME: 40 NEW FAMILIES AND LITERATURE REVIEW

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**Objectives:** We delineate the clinical spectrum and describe the histology in arterial tortuosity syndrome (ATS), a rare connective tissue disorder characterized by tortuosity of the large and middle-sized arteries, caused by mutations in *SLC2A10*.

**Methods:** We retrospectively characterize 40 novel ATS families (50 patients) and review the 52 previously reported patients. We perform histology and electron microscopy (EM) on skin and vascular biopsies and evaluate TGF $\beta$  signaling with immunohistochemistry for pSMAD2 and CTGF.

**Results:** Stenoses, tortuosity and aneurysm formation occur widespread. Severe but rare vascular complications include early and aggressive aortic root aneurysms, neonatal intracranial bleeding, ischemic stroke, and gastric perforation. Thus far, no reports unequivocally document vascular dissections or ruptures. Of note, diaphragmatic hernia and infant respiratory distress syndrome (IRDS) are frequently observed. Skin and vascular biopsies show fragmented elastic fibers (EF) and increased collagen deposition. EM of skin EF shows a fragmented elastin core and a peripheral mantle of microfibrils of random directionality. Skin and end-stage diseased vascular tissue do not indicate increased TGF $\beta$  signaling.

**Conclusions:** Our findings warrant attention for IRDS and diaphragmatic hernia, close monitoring of the aortic root early in life and extensive vascular imaging afterwards. EM on skin biopsies shows disease-specific abnormalities.

**S.24****SIMPLE RENAL CYSTS AND AORTIC DISEASE IN MARFAN SYNDROME AND MATCHED CONTROLS**

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**Objectives:** A link between simple renal cysts (SRC) and aortic aneurysm or dissection has been reported. The Marfan Syndrome (MFS) is associated with severe aortic diseases but very few data on SRC exist in this population.

**Methods:** All MFS patients with a mutation in the Fibrillin 1 gene who underwent a CT scan in the Reference Center for Marfan disease were included in the study. They were matched 1:1 for age and sex with controls without MFS.

**Results:** Between 2010 and 2016, 131 MFS patients and their matched controls were included. Mean age was 40±14 years with 42% of women.

The proportion of SRC in MFS patients was 41% versus 21% in controls ( $p<0.0001$ ). The prevalence of SRC was increased in MFS patients in all categories of age, the difference being more marked in young patients (FigureA). As severity of arterial disease increased, so did the prevalence of SRC: 59% of patients with dissection, 43% in aortic aneurysm surgery, 19% when no events and 21% in controls,  $p<0.009$  (FigureB).

In MFS patients, SRC were associated with higher rates of arterial dissection ( $p=0.02$ ). In multivariate analysis, 3 factors were independently associated with aortic surgery: male gender ( $p=0.02$ ), higher indexed aortic diameters ( $p<0.0001$ ) and the presence of SRC ( $p<0.05$ ).

**Conclusion:** This study reports for the first time an increased prevalence of SRC in MFS patients with *FBN1* mutation as compared with matched controls. SRC were associated with aortic events in this population and could be used as a marker of severity.

**S.25****DIAGNOSIS OF MARFAN SYNDROME IN CHILDREN REQUIRES MODIFICATION OF THE REVISED GHENT CRITERIA**

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**Objective:** Determine Revised Ghent Criteria (RGC) performance during childhood and propose modifications to enhance diagnostic accuracy.

**Methods:** A chart review of connective tissue disorder evaluations (n=481) performed in our Cardiovascular Genetics Clinic from May 2010-December 2015 was conducted. Patients were grouped into two cohorts (ages 0 to ≤10 and ages 11 to ≤20). Wilcoxon Rank Sum, Chi-square and Fisher's Exact tests were used to compare groups and logistic regression models were used to examine factors associated with diagnoses of Marfan syndrome (MFS).

**Results:** Of 421 patients, 42 received a diagnosis of MFS. Diagnosed patients had a higher systemic score (median 7 vs. 3). 48% of diagnosed patients had systemic scores ≤6 (median 4.5), predominantly in the younger cohort. The combination of RGC and molecular testing led to diagnosis in 100% of older patients and 54% of younger patients. When the aortic z-score requirement is modified to always be ≥2, the diagnostic rate increases to 68% in younger patients. If the systemic score criterion is changed from ≥7 to ≥5, the diagnostic rate increases to 77%. If RGC included patients with both a family history and a *FBN1* mutation, we would reach a diagnosis in all patients.

**Conclusion:** RGC was insufficient in diagnosing MFS during early childhood. We propose lowering the systemic score to ≥5, using consistent aortic z-score criterion, and allowing the combination of family history and a *FBN1* mutation to reach diagnostic significance. For improved diagnostic rate, modified RGC should be used when evaluating children ≤10 years for MFS.

## S.26

PATHOGENIC VARIANTS IN *LTBP3* AND *ARIH1* PREDISPOSE TO THORACIC AORTIC DISEASES

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Pathogenic variants in genes encoding proteins involved in vascular smooth muscle cell contraction, adhesion to the extracellular matrix, and the TGF- $\beta$  signaling pathway have been confirmed to lead to heritable thoracic aortic disease (HTAD), but are responsible for only ~30% of HTAD. To further identify genes for HTAD, we performed exome sequencing of DNA from individuals with HTAD and found rare variants in *LTBP3* and *ARIH1*. Rare recessive *LTBP3* variants were identified in two probands: compound heterozygous variants (p.Pro45Argfs\*25 and p.Glu750\*) and a homozygous variant (p.Asn678\_Gly681delinsThrCys) that introduces a cysteine in the epidermal growth factor-like domain of the corresponding protein, latent TGF- $\beta$  binding protein. Individuals with either the compound heterozygous or homozygous variants had early-onset thoracic aortic aneurysms and dissections, aneurysms of the abdominal aorta and other arteries, as well as dental abnormalities and short stature. Heterozygous carriers of the indel variant had late-onset thoracic aortic disease and variable dental and skeletal anomalies. *LTBP3* variants segregated with aortic disease in these families (combined LOD score 3.9). In mice with *Ltbp3* null variants, weight-adjusted aortic diameters were enlarged. Exome sequencing analysis involving a young patient with sporadic thoracic aortic aneurysm and dissection presenting at the age of 6 years identified a *de novo* nonsense *ARIH1* variant, p.Arg171\*. Additionally, rare, heterozygous *ARIH1* variants (p.Glu15Gln and Glu44Gly) were found in two probands with HTAD. *ARIH1* encodes a RING-between-RING E3 ligase that is involved in function of the linker of nucleoskeleton and cytoskeleton (LINC) complex that regulates nuclear positioning and shape in muscle cells. *Drosophila* with mutations of the *ARIH1* homolog, *Ari-1*, exhibited nuclear clustering and morphologic defects of the larval muscles. We postulate that loss of *ARIH1* affects mechanotransduction and weakens the aortic smooth muscle cells leading to thoracic aortic aneurysms and dissections. These studies show that recessive *LTBP3* variants and heterozygous loss of function *ARIH1* variants predispose to thoracic aortic aneurysms and dissections.

## S.27

# RISK FOR THORACIC AORTIC DISEASE, ASSOCIATED COMPLICATIONS, AND EXPANDED SPECTRUM OF SMAD3 PATHOGENIC VARIANTS FROM THE MONTALCINO AORTIC CONSORTIUM

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**Objectives:** We examined the differences in clinical presentation of aortic events with respect to age, variant type, and other risk factors in an international cohort of individuals with *SMAD3* variants.

**Methods:** Aortic disease, vital status, and clinical features were abstracted from medical records of 210 individuals from 60 families with 51 unique *SMAD3* pathogenic variants that result in haploinsufficiency (HI) and missense substitutions in the MH2 domain of the *SMAD3* protein, as well as novel in-frame deletions and missense variants in the MH1 domain.

**Results:** Aortic events were documented in 36% of cases, with dissections or ruptures accounting for 71% of events. The median age at first aortic event was significantly younger in individuals with *SMAD3* MH2 missense variants than those with HI variants (42 years versus 49 years;  $p = 0.003$ ) but there was no difference in frequency of aortic events by variant type. Overall risk of aortic event was 0.84 (95% CI 0.65-0.96) at 81 years of age. The 50% cumulative risk for an aortic event was at 54 years of age. No aortic events in childhood were observed.

**Conclusions:** *SMAD3* pathogenic variants cause thoracic aortic aneurysms and dissections in the majority of individuals, with variable age of onset and reduced penetrance. The type of underlying *SMAD3* variant was responsible for some of this variation. Novel variants in the MH1 domain were found to segregate with aortic disease. Later onset of aortic events and the unique phenotype associated with *SMAD3* variants support gene-specific management of this disorder.



## S.28

# NOVEL SMAD2 MUTATIONS IN FIVE FAMILIES WITH ARTERIAL ANEURYSM AND DISSECTION: FURTHER DELINEATION OF THE PHENOTYPE

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**Objective:** Determine the contribution of mutations in the gene coding for SMAD2, encoding a key transcriptional regulator of TGF- $\beta$  signaling, to the pathogenesis of aortic and arterial aneurysmal disease.

**Methods:** Next-generation targeted resequencing of *SMAD2* in patients with unknown genetic etiology.

**Results:** We identified the first *SMAD2* nonsense mutation and four additional *SMAD2* missense mutations, all affecting highly conserved amino acids in the functionally important MH2 domain. The premature stop codon, resulting from a single nucleotide duplication (c.612dup; p.(Asn205\*)), was identified in a marfanoid patient with aortic root dilatation and in his affected father. A p.(Asn318Lys) missense mutation was found in a Marfan syndrome-like case who presented with typical marfanoid habitus and aortic root aneurysm. Segregation analysis confirmed the presence of the mutation in an affected daughter with marfanoid features and mild aortic dilatation. In a LDS woman with a root dilatation and marked tortuosity of the neck vessels, another missense mutation, p.(Ser397Tyr), was identified. The mutation was present in her daughter with hypertelorism and arterial tortuosity and in the proband's mother with hypertelorism and marked skin anomalies but a normal echocardiogram. The third missense mutation, p.(Asn361Thr), was discovered in a male presenting with coronary artery dissection. Mutation analysis of three unaffected family members confirmed absence of the mutation. The last missense mutation, p.(Ser467Leu), was found in a male with significant dilatation of the aorta sinus and ascendens, aortic and mitral valve insufficiency, and multiple connective tissue findings.

**Conclusions:** Taken together, our data suggest that loss-of-function *SMAD2* mutations cause a wide spectrum of aortic and arterial aneurysmal disease, combined with connective tissue findings reminiscent of Marfan and Loeys-Dietz syndrome.

## S.29

# CANDIDATE GENE RESEQUENCING IN A LARGE BICUSPID AORTIC VALVE-ASSOCIATED THORACIC AORTIC ANEURYSM COHORT: *SMAD6* AS AN IMPORTANT CONTRIBUTOR

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**Objectives:** Bicuspid aortic valve (BAV) is the most common congenital heart defect. Although many BAV patients remain asymptomatic, at least 20% develop thoracic aortic aneurysm (TAA). Multiple lines of evidence suggest that genetic determinants contribute to the pathogenesis of both BAV and TAA in affected individuals. Despite high heritability, very few genes have been linked to human BAV or BAV/TAA (i.e. *NOTCH1*, *SMAD6*, and *MAT2A*), explaining a minority of patients. Yet, additional candidate genes have been suggested based on the presence of BAV in knockout mouse models (e.g., *GATA5*, *NOS3*) or in syndromic (e.g., *TGFBR1/2*, *TGFBR2/3*) or non-syndromic (e.g., *ACTA2*) TAA forms. We investigated if rare genetic variants in these genes are enriched in patients presenting with both BAV and TAA compared to controls.

**Methods:** Targeted resequencing of 22 candidate genes using Haloplex target enrichment was performed in a strictly defined BAV/TAA cohort (n=441; BAV in addition to an aortic root or ascendens diameter  $\geq 4.0$  cm in adults, or a Z-score  $\geq 3$  in children) and a cohort of healthy controls with normal echocardiographic evaluation (n=183). Subsequently, we selected rare protein-altering variants and did a burden analysis against the Exome Aggregation Consortium database.

**Results:** The strongest candidate susceptibility gene was *SMAD6* (p=0.002), with 2.5% (n=11) of BAV/TAA patients harbouring causal variants, including two nonsense, one in-frame deletion and two frameshift mutations. All six missense mutations were located in the functionally important MH1 and MH2 domains.

**Conclusion:** We report a significant contribution of *SMAD6* mutations to the genetic aetiology of BAV/TAA.

## S.30

### CONGENITAL CONTRACTURAL ARACHNODACTYLY: ESTABLISHMENT OF A CLINICAL SCORING SYSTEM AND CONFIRMATION OF MOLECULAR HETEROGENEITY

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**Objectives:** To develop a clinical scoring system for patients suspected with congenital contractural arachnodactyly (CCA) and to assess the molecular heterogeneity underlying the disorder.

**Methods:** We allocated 176 CCA probands referred for *FBN2* analysis in a *FBN2*-positive and *FBN2*-negative group. The sensitivity and specificity of 10 major clinical features formed the basis for a weighted 20 point clinical scoring system that predicts the likelihood of the clinical diagnosis. Because CCA shows substantial overlap with Bethlem myopathy (BM) and Ullrich congenital muscular dystrophy (UCMD), we analysed the genes encoding collagen 6 and collagen 12 in *FBN2*-negative ‘likely CCA’ patients.

**Results:** Forty-one different (likely) pathogenic *FBN2* variants were identified in a total of 51 patients (29% mutation uptake rate). Except for three missense variants and three multi-exon deletions, all variants were localized in the central region of the gene (exon 22-36). Clinical scores differed significantly between *FBN2*-positive and *FBN2*-negative patients ( $p < 0.001$ ). Only 2% of the variation in the score could be attributed to age. Cut point analysis using a ROC curve classified patients with scores equal to or above 7/20 as ‘likely CCA’. In 68 *FBN2*-negative ‘likely CCA’ patients we found five potentially deleterious variants in collagen 6 and three potentially pathogenic variants in collagen 12.

**Conclusion:** Our clinical scoring system may improve the diagnostic process in patients suspected with CCA. Our data confirm molecular heterogeneity and indicate clinical convergence between CCA, BM and UCMD. These results suggest that the pathophysiology underlying elastinopathies and collagenopathies at least partly overlaps.

**S.31****THE ROLE OF GENETIC VARIATION IN PHENOTYPE VARIABILITY AND RESPONSE TO TREATMENT IN MARFAN SYNDROME**

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**Objectives:** In a large cohort of 300 clinically diagnosed MFS patients, we addressed the following questions: Which proportion of classic MFS patients has an *FBN1* mutation? Are there genotype-phenotype correlations? Does the nature of the *FBN1* mutation predict cardiovascular treatment outcome?

**Methods:** Next-generation targeted resequencing of *FBN1* and related genes was followed by deletion/duplication testing (MLPA).

**Results:** A causal *FBN1* mutation was identified in 94% of patients (including 15 del/dups). Three percent of MFS patients had non-*FBN1* mutations. A similar small fraction had no causal variant but was clinically indistinguishable from the *FBN1* mutation positives. We confirm prior literature that mutations creating or deleting cysteines are significantly associated with lens dislocation ( $p=10^{-7}$ ). Premature termination codons are more often associated with skeletal findings, but not with cardiovascular severity. We did not observe a correlation between mutations in the middle region of *FBN1* and phenotypical severity. Mutations at the C- and N-terminal (exons 1-16 and exons 60-66) end tend to lead to a milder cardiovascular phenotype ( $p=0.047$ ). We observed no difference in aortic root size or progression nor in treatment outcome comparing dominant negative with haploinsufficient mutations. No effect of mutation location on treatment outcome could be detected. Patients that started treatment before age 10 had less aortic growth than older patients, irrespective of treatment type.

**Conclusion:** A comprehensive molecular analysis identifies an *FBN1* mutation in the overwhelming majority of MFS patients. With one exception, no major genotype-phenotype correlations can be identified. Importantly, early start of treatment has better outcome with regards to aortic root growth.

**S.32****TMEPAI MUTATION IN MFS/LDS-LIKE PATIENTS IN 2 JAPANESE FAMILIES**

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Genetic mutations in patients with Marfan / Loeys-Dietz syndrome (MFS/LDS) or similar disorder are known to cause high transforming growth factor (TGF)- $\beta$  signaling. To identify new genes responsible for these diseases, 304 patients with MFS/LDS-like features or young aortopathy negative in *FBN1*, *TGFBR1*, *TGFBR2*, *TGFB2*, *TGFB3* or *SMAD3* mutation were subjected to exome sequencing. Three patients were identified to have a novel mutation in *TMEPAI*. Proband was a 43y tall male with funnel chest, arachnodactyly and a history of osteosarcoma with leg amputation in childhood. His mother was also tall and had abdominal aortic aneurysm. His 13y daughter and 11y son were both tall and showed funnel chest, dural ectasia and arachnodactyly without lens dislocation. Daughter had scoliosis and mitral valve prolapse. Son showed mild aortic root dilatation. Exome sequencing revealed candidate variants in 12 genes and only 3 of them (*TMEPAI*: p.S209Qfs; *AP5M1*: p.I88L; *ESRRB*: p.H52Q) were segregated with similar stature and deformities. In addition, another 33y female patient in unrelated family, showing arachnodactyly with aortic and axillar arterial dissection, revealed to have the same mutation of *TMEPAI*. Then, we studied the expression of fibronectin after TGF- $\beta$ 2 stimulation and the patient's fibroblasts with the *TMEPAI* mutation showed upregulation of TGF- $\beta$  signals. Since *TMEPAI* gene is known to play an important role in regulation of TGF- $\beta$  signals, it is thought that *TMEPAI* mutation can cause MFS/LDS-like features. Therefore, we conclude that *TMEPAI* is essential not only for cancer progression but also for systemic connective tissue disorders similar to MFS/LDS.

**S.33****RESULTS OF NEXT GENERATION SEQUENCING GENE PANEL DIAGNOSTICS INCLUDING COPY NUMBER VARIATION ANALYSIS IN 810 PATIENTS SUSPECTED OF HERITABLE THORACIC AORTIC DISORDERS**

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Simultaneous analysis of multiple genes using next generation sequencing (NGS) technology has become widely available. Copy number variations (CNVs) in disease-associated genes have emerged as a cause for several hereditary disorders. CNVs are, however, not routinely detected using NGS analysis. The aim of this study was to assess the diagnostic yield and the prevalence of CNVs using our panel of Hereditary Thoracic Aortic Disease (H-TAD) associated genes. 810 patients suspected of H-TAD were analysed by targeted NGS analysis of 21 H-TAD associated genes. In addition, the eXome Hidden Markov Model (XHMM; an algorithm to identify CNVs in targeted NGS data) was used to detect CNVs in these genes. A pathogenic or likely pathogenic variant was found in 66 out of 810 patients (8.1%). Of these 66 pathogenic or likely pathogenic variants, six (9.1%) were CNVs not detectable by routine NGS analysis. These CNVs were four intragenic (multi-)exon deletions in *MYLK*, *TGFB2*, *SMAD3* and *PRKG1* respectively. In addition, a large duplication including *NOTCH1* and a large deletion encompassing *SCARF2* were detected. Given the clinical relevance of the identification of a genetic cause, CNV analysis using a method such as XHMM should be incorporated into the clinical diagnostic care for H-TAD patients.

**KEY WORDS**

Thoracic Aortic Aneurysm, Thoracic Aortic Dissection, Genetics, eXome Hidden Markov Model, Copy Number Variations

**S.34****THE CLINICAL IMPLICATIONS OF VARIATION IN *FBN1* IN A HEALTH SYSTEM**

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**Objective:** To study the phenotypes associated with *FBN1* variants reported to be causative of Marfan syndrome (MFS).

**Methods:** The PennMedicine Biobank contains electronic health record (EHR)-linked biospecimens of patients recruited from throughout the University of Pennsylvania Health System and is enriched for individuals with complex cardiovascular disease (CVD). Whole exome sequences of over 12,000 individuals were screened for variants in *FBN1* mutations that have been reported to be causal of MFS. The EHR was examined for diagnoses and family history (FHx).

**Results:** 13 nonsynonymous variants occurred in 68 subjects. Their ages varied considerably (mean age 64.5, range 28-89 yrs) and 54% were male. 7 variants occurred in only one individual (G1013R, C1672Y, T1908I, I2185T, P2278S, T2520M, I2585T); 2 had MFS and 4 others had severe CVD. R1170H, occurred in 40 subjects; 8 had aortic dilatation and 2 severe mitral valve regurgitation. For the other 30, no imaging was present in 10 and most lacked a pertinent FHx. Among the 66 not diagnosed with MFS, 15 had ascending aortic dilatation, and 11 of them were older than 60 years at the time of donating a specimen.

**Conclusions:** In a large adult patient population seen at an academic medical center, nonsynonymous point variants in *FBN1* thought to be causative of MFS are often associated with ascending aortopathy or mitral valve disease but not necessarily a diagnosis of MFS. Furthermore, variability in cardiovascular features was marked with respect to presentation, age and severity. Notably, *FBN1*-associated aortopathy was compatible with a long life-expectancy.

## S.35

### MONTALCINO AORTIC CONSORTIUM: THORACIC AORTIC DISEASE OUTCOMES AMONG 987 INDIVIDUALS WITH *ACTA2*, *PRKG1*, *TGFBR1*, *TGFBR2* AND *SMAD3* PATHOGENIC VARIANTS

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The Montalcino Aortic Consortium was established to characterize the phenotype associated with novel genes for heritable thoracic aortic disease (HTAD). We analyzed initial data from 987 individuals with pathogenic variants in *ACTA2* (n=319), *PRKG1* (n=37), *SMAD3* (n=190), *TGFBR1* (n=176), and *TGFBR2* (n=265) and examined age and type of first aortic event (aortic dissection or elective aortic aneurysm repair) and factors influencing disease variability. Forty-four percent had had an aortic event at median age of 34 years (IQR 24, 1-80). *SMAD3* patients were significantly older at first aortic event (median age 47 years, IQR 14) compared to individuals with *TGFBR1* (median 31, IQR 20; p<0.0001), *TGFBR2* (median 29, IQR 23; p<0.0001), *ACTA2* (median 35, IQR 21; p<0.0009), and *PRKG1* variants (median 32, IQR 23; p=0.009). Aortic events in childhood were observed only in individuals with *TGFBR2* (n=18), *TGFBR1* (n=5) and *ACTA2* (n=6) variants, mostly disrupting *ACTA2* arg179, *TGFBR2* arg528 and *TGFBR2* asp446. Elective aneurysm repairs were more frequent among *TGFBR1* and *TGFBR2* patients (50% and 53%, respectively) than *SMAD3* (32%), *ACTA2* (14%) and *PRKG1* (29%) patients. Only *PRKG1* patients presented with aortic dissections at significantly younger ages than elective aneurysm repairs. Event-free survival probability was highest among *SMAD3* patients at any age compared to patients with variants in other genes. When other risk factors were assessed, gender influenced event-free survival only among *TGFBR1* (p<0.0001) and *ACTA2* (p=0.02) patients, with women having higher event-free survival than men, and European patients had longer event-free survival than North American patients (p=0.002). These findings demonstrate that the underlying gene and, in some cases, the specific variant in the gene dictate the age and type of aortic disease onset, and gender and site of recruitment influence this risk. These data have significant implications for stratifying risk and managing HTAD patients. Importantly, the differences in disease presentation between geographic location needs to be further interrogated to determine if it is due to recruitment bias or more optimal management of HTAD patients in European countries.



## S.36

### EXPERT CONSENSUS RECOMMENDATIONS ON THE CARDIOGENETIC CARE FOR PATIENTS WITH THORACIC AORTIC DISEASE AND THEIR FIRST-DEGREE RELATIVES

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**Objectives:** Thoracic aortic aneurysm (TAA) is a potentially life-threatening disorder with a strong genetic component. The number of genes implicated in TAA has increased exponentially over the last decade. Approximately 20% of patients with TAA have a positive family history. As most TAA remain asymptomatic for a long time, screening of at risk relatives is warranted to prevent complications. Existing international guidelines lack detailed instructions regarding genetic evaluation and family screening of TAA patients. We aimed to develop a consensus document to provide medical guidance for all health care professionals involved in the recognition, diagnosis and treatment of patients with thoracic aortic disease and their relatives.

**Methods:** A multidisciplinary panel of experts including cardiologists, cardiothoracic surgeons, clinical geneticists and general practitioners, convened to review and discuss the current literature, guidelines and clinical practice on genetic testing and family screening in TAA.

**Results:** There is a lack of high-quality evidence in the literature. This consensus statement, based on the available literature and expert opinions, summarizes our recommendations in order to standardize and optimize the cardiogenetic care for patients and families with thoracic aortic disease. In particular, we provide criteria to identify those patients most likely to have a genetic predisposition, and discuss the preferred modality and frequency of screening in their relatives.

**Conclusions:** Age, family history, aortic size and syndromic features determine who is advised to have genetic testing as well as screening of first-degree relatives. There is a need for more prospective multicenter studies to optimize current recommendations.

## S.37

**MYLK MUTATIONS: AORTIC DISEASE PRESENTATION, PREGNANCY RISK, AND CHARACTERIZATION OF PATHOGENIC MISSENSE VARIANTS**

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**Objective:** Heritable thoracic aortic disease can result from null variants in *MYLK*, which encodes myosin light chain kinase (MLCK). Data on whether *MYLK* missense variants cause the condition and information to guide aortic disease management are limited.

**Methods:** Phenotypic information was collected from 55 individuals in 7 families with variants in *MYLK*. Obligate carriers and individuals who tested positive for a variant were included in the analysis. MLCK functional assays were performed *in vitro* to characterize a missense variants. Wild type and mutant MLCK were expressed in HeLa cells, purified, and the kinase activity and activation with calmodulin binding determined

**Results:** Five *MYLK* null variants and two missense variants were classified as pathogenic. Twenty-three individuals (39%) experienced an aortic event (defined as aneurysm repair or dissection); the majority of these events (74%) were type A aortic dissections. Aortic diameters were minimally enlarged at the time of dissection in many cases. Time-to-aortic event curves showed missense carriers have an earlier-onset of aortic events than null carriers, suggesting a dominant-negative effect of missense variants. Note that in the null population, one individual had a type A dissection at the age of 23, while the remainder did not dissect until after the age of 43. An *MYLK* missense variant segregated with disease over five generations with a LOD score of 4 but did not alter MLCK kinase activity, suggesting additional pathogenic pathway is involved.

**Conclusions:** These data further define *MYLK* variants that cause thoracic aortic disease. Given minimal aortic enlargement prior to dissection, an alternative approach to guide patients and their physicians as to the timing of aortic repair is proposed based on mutation type and the probability of a dissection at a given age.

## S.38

### VARIANTS IN *LMOD1* CAUSING THORACIC AORTIC ANEURYSM AND DISSECTION (TAAD) IN A COLLABORATIVE INTERNATIONAL COHORT

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**Objectives:** The aim of our study was to identify the cause of disease in a cohort of 99 (36 familial TAAD and 63 sporadic) probands diagnosed with thoracic aortic aneurysm and dissection (TAAD) through clinical evaluation.

**Methods:** A whole exome sequencing (WES) approach was utilized to assess the profiles of the familial TAAD group for known TAAD genes and novel candidates using ACMG guidelines. For the largest FTAAD families, three distantly related affected individuals were processed for WES. Patient cells and zebrafish were utilized to assess the function of candidate genes.

**Results:** A missense variant, c.1784T>C, p.(V595A) in *LMOD1* was identified to segregate with affected individuals in a British family. *LMOD1* was further assessed in UK and international TAAD cohorts and 18 other variants were discovered of which eight were assessed as pathogenic using variant interpretation tools. Patient cells presented with mislocation of the *LMOD1* protein and reduced contraction of cells compared to that of controls. Knock-down of both *lmod1a* and *lmod1b* paralogs in zebrafish revealed a reproducible abnormal phenotype involving the pharyngeal arches. Injection of the human mRNA carrying the variant from our study family demonstrated a likely dominant negative effect and illustrated a loss of function cause.

**Conclusions:** We describe variants detected in the smooth muscle gene *LMOD1*, which predisposes individuals to TAAD. Mutations found in the WH2 actin-binding domain of *LMOD1* may delay migration of the protein and subsequent actin polymerization and therefore compromise actin length, dynamics and interaction with myosin in the smooth muscle contraction pathway.

**S.39****INCREASED VISCERAL ARTERIAL TORTUOSITY IN MARFAN SYNDROME: A POSSIBLE NEW APPROACH TO RISK STRATIFICATION**

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**Objectives:** Clinical evidence suggests that the currently recommended approach to estimate the risk of aortic dissection in Marfan syndrome (MFS) by mainly focusing on aortic diameters and aortic growth rate is not reliable enough. According to previous findings assessing arterial tortuosity could improve risk stratification. In this study we investigated the tortuosity of renal and splenic arteries that are not influenced by the skeletal features of MFS.

**Methods:** In our retrospective analysis the centerline of splenic and renal arteries was exported for 46 MFS patients and 92 age and gender matched control subjects using helical thoracic and abdominal CT angiography imaging. To measure tortuosity distance metric (DM) and the 3D version of sum of angles metric (SOAM) and inflection count metric (ICM) were calculated. Mann-Whitney U-test was used for statistical analysis.

**Results:** DM of the right and left renal, and splenic artery was significantly higher in MFS patients than in controls ( $1.24 \pm 0.21$  vs.  $1.13 \pm 0.11$   $p=0.002$ ;  $1.54 \pm 0.39$  vs.  $1.19 \pm 0.15$   $p<0.001$ ;  $2.39 \pm 0.83$  vs.  $2.00 \pm 0.80$   $p<0.001$ ). A similar tendency was observed for ICM values. SOAM of the right and left renal artery was significantly lower in the MFS group compared to controls ( $0.55 \pm 0.14$  vs.  $0.63 \pm 0.15$   $p=0.005$ ;  $0.53 \pm 0.44$  vs.  $0.61 \pm 0.32$   $p<0.001$ ).

**Conclusions:** To our knowledge this is the first demonstration of increased arterial tortuosity in MFS on visceral arteries. Visceral arterial tortuosity, dominated by curves of lower frequency but higher amplitude according to the observed opposite tendency between the DM and SOAM metrics, could be a possible new predictor of serious manifestations of MFS.

## S.40

### FACTORS ASSOCIATED WITH TYPE A AORTIC DISSECTION AND WITH DISSECTION AT AORTIC DIMENSION <5CM IN MARFAN AND LOEYS-DIETZ SYNDROMES: THE ROLE OF ARTERIAL TORTUOSITY

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**Background:** Prior research suggests that increased vertebral artery tortuosity is a biomarker of earlier cardiovascular events in young patients with Marfan syndrome (MFS) and Loeys-Dietz syndrome (LDS) due to *TGFBR1* or *TGFBR2* mutations. The objective of our study was to examine the change in tortuosity with aging, and to evaluate if increased tortuosity is associated with Stanford Type A dissection (TAD) overall and/or at a small aortic dimension in this population.

**Methods:** Patients were included from the National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions (GenTAC) or our institutional Cardiovascular Genetics Clinic, with MFS or LDS due to a *TGFBR1* or *TGFBR2* mutation who underwent magnetic resonance or computed tomography angiography < age 50 years that included the vertebral arteries. The vertebral artery tortuosity index (VTI) was calculated, and outcomes were compared using Cox regression with time-dependent covariates.

**Results:** A total of 204 patients were included, with median age at study entry of 18.0 years (IQR 8.1, 30.8). Studies of serial VTI in individuals demonstrated that VTI decreases with increasing height (mean change -0.5% per cm increase, 95%CI -0.9, -0.2,  $p=0.004$ ). Given this, height-adjusted VTI (VTI-h) was calculated for all patients. VTI-h was highest in those with infantile MFS and *TGFBR2* mutations. By univariate analysis, VTI-h  $\geq 68$  was associated with TAD (HR 3.10, 95%CI 1.44-6.71). In multivariate analysis, VTI-h  $\geq 68$  remained associated with TAD (HR 2.89, 95%CI 1.26-6.64). Among those with TAD and aortic dimensions available ( $n=13$ ), 8 had TAD at a dimension of <5.0 cm (MFS  $n=6$ , *TGFBR2*  $n=2$ ), with a minimum diameter of 4.0 cm. All of these had either VTI-h  $\geq 68$ , prior Type B dissection, or were peripartum; when classifying any of these variables as “high-risk”, 8/27 high-risk patients had a TAD between 4.0 and 5.0 cm, while 0/29 low-risk patients reached 5.0 cm without TAD ( $p=0.002$ ).

**Conclusions:** Given the decrease in VTI as height increases, VTI-h may be most optimal for use in analyses. VTI-h  $\geq 68$  is associated with Type A dissection, and the presence of either VTI-h  $\geq 68$ , prior aortic dissection, or pregnancy was associated with higher likelihood of dissection <5 cm.

**S.41****CIRCULATING FIBRILLIN-1 FRAGMENTS IN CHILDREN AND YOUNG ADULTS WITH MARFAN SYNDROME: BIOMARKERS FOR MARFAN SYNDROME**

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**Objectives:** High concentrations of circulating fibrillin-1 fragments have been associated with thoracic aortic aneurysm and dissection in the general adult population. This study aims to test whether a distinctive profile of fibrillin fragments is found in at-risk children and young adults with Marfan syndrome compared to related disorders and to unaffected controls. This study also aims to identify fibrillin fragments associated with aortic root size.

**Methods:** 114 children and young adults ages 4-26 diagnosed with Marfan syndrome or a related disorder were enrolled at our clinic sites. Clinical data, including echocardiography, and blood samples were collected at the time of the clinic visit. Plasma was frozen and shipped to Portland for testing. In addition, 214 plasma samples were obtained from the GenTAC registry, and 58 plasma samples, from unaffected controls. The majority of our enrolled participants (75%) and GenTAC registrants (67%) had Marfan syndrome.

**Results:** Significantly lower levels of fibrillin-1 fragment 15-201 distinguish children and young adults with Marfan syndrome from those with related disorders or unaffected controls. The percentage of Marfan children (ages 4-18) with aortic root z-scores  $\geq 2$  was two-fold greater among those with detectable levels of fibrillin-1 fragment 15-78 (73%) compared to those in whom this fragment was not detected (33%), and this difference was statistically significant ( $p < 0.001$ ). Levels of fibrillin-1 fragments 15-78 and 201-78 were significantly lower among participants who had previous aortic root surgery compared to those who had not had surgery.

**Conclusions:** Circulating fibrillin-1 fragments may be useful biomarkers for Marfan syndrome.

**S.42****AORTIC WALL INFLAMMATORY ACTIVITY IS INDEPENDENT OF AORTIC DILATION IN MARFAN SYNDROME: A HYBRID PET-MRA IMAGING**

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**Background:** Aortic size and inflammation are adverse prognostic markers in Marfan syndrome (MFS); links between the two are poorly understood. This study tested whether MFS patients manifest aortic inflammation independent of dilation.

**Methods:** Hybrid PET-MRA was prospectively performed in MFS and control patients (n=16): Aortic size on MRA was quantified in pre-specified (root, ascending, arch, descending) non-grafted aortic segments; dilation was categorized by established age/BSA adjusted cutoffs. Aortic inflammation on PET was measured in co-localized segments using <sup>18</sup>F-fluorodeoxyglucose (FDG); increased uptake was defined by standard criteria ( $\geq 2$  SUV [standardized uptake value]). MRA and PET were analyzed independently.

**Results:** 15 MFS patients (40 $\pm$ 19yo, 69%M) underwent PET-MRA, yielding 68 non-grafted analyzable aortic segments. All patients had increased FDG uptake, comprising 60% of segments (root 5/12 | ascending 8/13 | arch 15/15 | mid-descending 11/14 | distal-descending 3/14); 80% patients (12/15) had either dilated native segments or previously replaced dilated/dissected segments (root 9/15 | ascending 5/15 | arch 0/15 | mid-descending 1/15 | distal-descending 1/15). Aggregate aortic inflammation was 63% higher than the normative control segments (2.04 $\pm$ 0.17 vs. 1.28 $\pm$ 0.19, p=0.004). Regarding distribution, FDG uptake was highest in the arch (2.29 $\pm$ 0.13) and lowest in the descending aorta (1.85 $\pm$ 0.18). PET-evidenced aortic inflammation did not correlate with MRI-quantified aortic size (r= -0.68, p=0.58; Figure). Mean FDG uptake was similar between dilated and normal caliber segments (1.90 $\pm$ 0.20 vs. 2.00 $\pm$ 0.31, p=0.388).

**Conclusions:** Aortic inflammation occurs commonly independently of anatomic dilation in MFS, as evidenced by increased PET inflammatory activity in regions of normal aortic caliber by MRA.

**S.43****AORTIC GEOMETRY IS RELATED TO ABNORMAL FLOW PATTERN AND TO PROXIMAL DESCENDING AORTA DILATION IN MARFAN PATIENTS: A 4D-FLOW MRI STUDY**

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**BACKGROUND AND OBJECTIVES:** Disease of the descending aorta (DAo) has emerged as a clinical issue in Marfan syndrome (MFS). Although aortic diameter has been identified as a risk marker for these complications, many type B aortic dissections happen with non-advanced aortic diameters. Recently MRI studies have revealed the existence of marked flow vortex in the proximal DAo which were related to local dilation, but the causes are unknown. We aimed to investigate the relationship between aortic geometry and flow characteristics in the thoracic aorta of MFS by 4D flow MRI.

**METHODS:** Fifty-three MFS with no valve dysfunction and 40 age-matched healthy volunteers were recruited. All participants underwent non-contrast-enhanced 4D flow-MRI, obtaining flow field and angiography. Geometric (Aortic diameter, ellipticity and curvature) and flow parameters (in-plane rotational flow, IRF, and systolic flow reversal ratio, SFRR) were determined at 20 planes positioned from sinotubular junction to proximal DAo (figure 1).

**RESULTS:** Proximal ascending aorta (AAo) and proximal DAo diameters and ellipticity were significantly larger in MFS. Aortic curvature was lower in the aortic arch and larger in the proximal DAo (figure 2). IRF (helicity) was lower in MFS all along the aorta while SFRR (vorticity) was increased in the regions with larger diameters. Arch IRF was inversely related to arch ellipticity ( $p=0.016$ ) as and proximal DAo peak curvature ( $p=0.015$ ). Maximum proximal DAo diameter was negatively correlated with local IRF ( $p=0.038$ ) and positively correlated with local SFRR ( $p<0.001$ ).

**CONCLUSIONS:** Abnormal aortic ellipticity and curvature are present in MFS and are related to a reduction of flow helicity. Reduced helicity and increased vortex strength was related to local dilation.



**S.44****AORTIC MICROCALCIFICATION ASSOCIATES WITH ELASTIN FRAGMENTATION IN MARFAN SYNDROME**

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Marfan syndrome (MFS) is a genetic connective tissue disorder, in which aortic rupture is the major cause of death. MFS patients with an aortic diameter below the advised limit for prophylactic surgery (<5cm) may unexpectedly experience an aortic dissection or rupture, despite yearly monitoring. Hence, there is a clear need for improved prognostic markers to predict such aortic events. We hypothesize that elastin fragments play a causal role in aortic calcification in MFS and that microcalcification serves as a novel marker for aortic disease severity. To address this hypothesis, we analyzed MFS patient and mouse aortas. MFS patient aortic tissue showed enhanced microcalcification in areas with extensive elastic lamina fragmentation in the media. A causal relationship between medial injury and microcalcification was revealed by studies in vascular smooth muscle cells (SMCs); elastin peptides were shown to increase the activity of the calcification marker alkaline phosphatase (ALP) in human SMCs. In murine Fbn1<sup>C1039G/+</sup> MFS aortic SMCs, ALP mRNA and activity was upregulated when compared to wildtype SMCs. The elastin peptide-induced ALP activity was prevented by incubation with lactose or a neuraminidase inhibitor which inhibit the elastin receptor complex, and a MEK1/2 kinase inhibitor, indicating downstream involvement of ERK1/2 phosphorylation. Histological analyses in MFS mice revealed macrocalcification in the aortic root, while the ascending aorta contained microcalcification, as identified with the near-infrared fluorescent bisphosphonate probe OsteoSense-800. Significantly, microcalcification correlated strongly with aortic diameter, aortic distensibility, elastin breaks and phosphorylated ERK1/2. In conclusion, microcalcification colocalizes with aortic elastin degradation in MFS aorta of man and mice, where elastin-derived peptides induce a calcification process in SMCs via the elastin receptor complex and ERK1/2 activation. We propose microcalcification as a novel imaging marker to monitor local elastin degradation and thus predict aortic events in MFS patients.

**S.45****ROLE OF FIBRILLIN 1 IN THE STRUCTURE AND STABILITY OF THE CILIARY ZONULE**

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**Objectives:** To develop mice that model the Marfan Syndrome ectopia lentis phenotype.

**Methods:** We used multiplexed fluorescence in situ hybridization to visualize the expression of zonule genes. The *Fbn1* locus was disrupted in the non-pigmented ciliary epithelium (NPCE) by crossing *Fbn1*<sup>fl/fl</sup> mice with *pax6-αCre* mice. Zonular structure was visualized by confocal microscopy and high resolution SEM. Lens position was visualized in vivo by OCT and the strength of zonular fibers measured using a lens pull-up technique.

**Results:** *Fbn1* was expressed in the NPCE until at least 1 year of age. In *Fbn1*<sup>fl/fl</sup>; *Pax6αCre*<sup>tg/-</sup> animals, Fbn1 was largely absent from zonular fibers projecting from the nasal and temporal segments of the NPCE, although the density of fibers in Fbn1-deficient regions was not noticeably reduced. Mechanical measurements suggested that the tensile strength of the zonule was lessened in knockout animals. At about 6-weeks-of-age, the Fbn1-depleted fibers began to break, followed by the Fbn1-rich superior and inferior fibers. Thus, by eight weeks of age, lenses were completely dislocated. In the following months, the ectopic lenses became cataractous. In severe cases, particulate material released from the cataractous lenses was visible by OCT. This was sometimes associated with a sharp increase in IOP and development of phacolytic glaucoma. IOP remained low in most knockout animals. Nevertheless, we measured a significant increase in axial length compared to wild type.

**Conclusions:** We developed a mouse model of ectopia lentis that will be useful for studying structure/function relationships in the zonule and clinical sequelae of lens dislocation.

**S.46****LONG-TERM STABLE INTRAOCULAR PRESSURE (IOP) CONTROL FOLLOWING OCULAR GENE THERAPY IN A CANINE MODEL OF *ADAMTS10*-WEILL MARCHESANI SYNDROME-ASSOCIATED OPEN-ANGLE GLAUCOMA (WMS-OAG)**

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**Objective:** Elevated IOP is a key risk factor of OAG caused by increased aqueous humor (AH) outflow resistance via the trabecular meshwork (TM). The purpose of this study was to achieve long-term, stable IOP control by TM-targeted gene replacement therapy using adeno-associated virus (AAV) in a well-established canine model of WMS-OAG based on an *ADAMTS10* missense mutation.

**Methods:** Ten dogs were treated unilaterally by intracameral injection of the single stranded, capsid mutant vector AAV2(Y444F)-smCBA-*hADAMTS10*, at doses of either  $1 \times 10^{11}$  (n=7) or  $1.4 \times 10^{12}$  (n=3) vector genomes. Clinical outcome measures were monitored for up to 18 months and included diurnal IOP (rebound tonometry) and AH outflow facility (pneumotonography). *hADAMTS10* transgene expression within the TM was followed by qPCR in 6-12-month intervals for 36 months.

**Results:** While no therapeutic effect was observed over 6 months at the lower vector dose, a significant decrease in IOP ( $p < 0.0001$ ) was observed by 76 days post-treatment in 2/3 dogs treated with the higher dose: Mean diurnal IOPs were maintained between 12-15 mmHg over the 14-18-month study period compared to 26-41 mmHg in the untreated fellow eyes. Outflow facility was 70-114% higher in the treated vs. untreated fellow eyes in these 2 responding animals. The treatment was well tolerated, and *hADAMTS10* transgene expression was robust and stable for  $\geq 36$  months.

**Conclusions:** Long-term IOP control combined with robust transgene expression in a well-established large animal model of WMS-OAG supports the potential value of ocular AAV-gene therapy.

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**S.47****CORRELATION OF VISUAL FUNCTION AND OPTIC NERVE STRUCTURE IN MICE WITH *FBN1* MUTATION**

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**Objectives:** To investigate visual function and correlate with optic nerve structural changes in a mouse line carrying the tight skin *Fbn1* mutation (*Fbn1*<sup>Tsk/+</sup>).

**Methods:** Ocular phenotypes were characterized in young and old mice, age ranged from 3 months to 16 months old. Visual acuity was assessed by Optomotor Response. Intraocular pressure (IOP) was measured with Tonolab. Optical coherence tomography (OCT) was used to measure the central corneal thickness (CCT). Retinal function was evaluated by electroretinography (ERG) responses. Whole mount retinas were stained for Brn3a to assess number of retinal ganglion cell (RGC) and cross-sections of the optic nerves were stained with P-Phenylene Diamine for axon quantification and area measurement. Luna staining was used to evaluate optic nerve sheath.

**Results:** IOP was not affected by *Fbn1*<sup>Tsk/+</sup> mutation at all ages. *Fbn1*<sup>Tsk/+</sup> mice had significantly thinner CCT compared with controls, which was detectable at 3 months of age. At advanced age, *Fbn1*<sup>Tsk/+</sup> mice developed functional deficits, including reduced visual acuity and RGC responses in ERG. While RGC soma density was not reduced in *Fbn1*<sup>Tsk/+</sup> mice, the density of axons in the optic nerve was significantly reduced accompanied with expanded optic nerve areas compared to control mice. The axons in the optic nerves of *Fbn1*<sup>Tsk/+</sup> mice were significantly enlarged. Thinning of the elastic fiber network was observed in the pia mater of the optic nerve of *Fbn1*<sup>Tsk/+</sup> mice.

**Conclusions:** Our results suggest that *Fbn1* mutation leads to retinal functional deficits and optic nerve axonal loss and expansion, which accelerate with aging.

**S.48****KNOCKDOWN OF *ADAMTS10* RESULTS IN SHORTENED BODY AND ABNORMAL RETINAL DEVELOPMENT IN ZEBRAFISH**

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**Objectives:** Mutations in *ADAMTS10* cause Weill-Marchesani syndrome, characterized by short stature. Previously we showed that a Gly661Arg mutation in *ADAMTS10* causes glaucoma, and have proposed dysregulation of transforming growth factor  $\beta$  (TGF $\beta$ ) signaling as a possible disease mechanism. The purpose of this study is to investigate the role of *adamts10* in retinal development and TGF $\beta$  regulation.

**Methods:** Morpholino antisense oligos targeting *adamts10* mRNA, or control morpholinos, were injected into fertilized embryos from zebrafish lines with fluorescent protein expression driven by a Brn3a promoter specific for retinal ganglion cells or a Smad3 responsive element (12xSBE) as a read-out for TGF $\beta$  signaling. Embryos were examined at 48 h post-fertilization. Retinal morphology was determined in toluidine blue stained sections of epon-embedded embryos. Specificity of *adamts10* morpholino was tested by co-injecting normal *ADAMTS10* mRNA or *ADAMTS10* mRNA carrying the Gly661Arg mutation.

**Results:** Embryos injected with the *adamts10* morpholino developed significantly shorter bodies (49% of controls,  $p < 10^{-6}$ ) and displayed incomplete retinal lamination. *Adamts10* morpholino reduced fluorescence in the inner retina and optic nerve driven by the Brn3a promoter and Smad3-driven fluorescence in the eyes. These phenotypes were rescued by co-injection of normal *ADAMTS10* mRNA, but not *ADAMTS10* mRNA encoding the Gly661Arg mutation.

**Conclusions:** Similar to Weill-Marchesani syndrome, knockdown of *adamts10* in zebrafish causes pronounced reduction of body length. TGF $\beta$  signaling in the eye is reduced by *adamts10* knockdown, in contrast to fibrillin-1 deficiencies. *Adamts10* is required for normal development of the retina. The Gly661Arg mutation has a deleterious effect on the developmental functions of *adamts10*.

**S.49****LIMB- AND TENDON-SPECIFIC DELETION OF *ADAMTSL2* CAUSE A SHORT-LIMB PHENOTYPE RECAPITULATING GELEOPHYSIC DYSPLASIA**

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**Objectives:** Geleophysic dysplasia (GD) is caused by mutations in *ADAMTSL2*, *FBN1*, or *LTBP3*. Musculoskeletal manifestations of GD include short stature, brachydactyly, stiff joints, and a "pseudomuscular" build. To study the role of *ADAMTSL2* in postnatal limb development we analyzed limb-specific *ADAMTSL2* knock-out mice.

**Methods:** *Adamtsl2* expression was determined using an intragenic lacZ reporter. We deleted *Adamtsl2* with *Prx1*-Cre or *Scx*-Cre in the limb bud mesenchyme or tendons, respectively. Limb development was analyzed by morphometry and by histology. *ADAMTSL2* localization in the extracellular matrix of cultured fibroblasts was determined with immunocytochemistry.

**Results:** *Adamtsl2* was expressed in the superficial layer of articular cartilage, tendons, and skeletal muscle, but not in the growth plate or bone. *Adamtsl2*-Prx long bones were disproportionally shortened with a greater distal impact and anomalies of bone sculpting were evident in *Adamtsl2*-Prx mice. *ADAMTSL2*depleted Achilles tendons were shorter and thicker, with tenocyte disorganization and pericellular *FBN1* microfibril accumulation. Upon *ADAMTSL2* deletion in tendons, shortening in several limb bones was also observed, but without a disproportional shortening of distal limb elements. In cell culture, *ADAMTSL2* localized predominantly to extracellular *FBN1* microfibrils.

**Conclusions:** These results suggest that *ADAMTSL2* depletion in mice causes primary alterations in tendon properties which appear to restrict bone elongation and result in a short limb phenotype as a secondary effect. We hypothesize that the restriction in bone elongation is probably due to tendon-derived compressive forces limiting growth plate extension. Mechanistically, *ADAMTSL2* bound to *FBN1* microfibrils and may regulate microfibril formation in the extracellular matrix.

**S.50****INVOLVEMENT OF TB5 DOMAIN IN FIBRILLIN-1 ON REGULATION OF CHONDROGENESIS**

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The geleophysic dysplasia (GD) is a rare disorder characterized by short stature, short extremities, joint stiffness and cardiac defects which lead in early death in the first years of life. The GD shows multiple skeletal abnormalities suggesting defects in skeletal development. We identified heterozygous mutations in the gene coding for Fibrillin-1 (*FBN1*) responsible for the autosomal dominant form of the GD, all located in exons 41-42, encoding TGF $\beta$  binding protein-like domain 5 (TB5) of FBN1. Strikingly, FBN1 is also a major causative gene for the Marfan syndrome, which presents opposite features to the GD. We previously identified mutations in *ADAMTSL2* in the autosomal recessive form of the GD, which is a partner of FBN1, Latent TGF $\beta$  Binding Protein 1 (LTBP1), and fibrillin-2 (FBN2). and our recent work demonstrated the pivotal role of Adamtsl2 in the scaffold of the growth plate ECM and the column structure. Then, we generated a knock-in mouse model, FBN1<sup>TB5</sup> in order to decipher the role of the TB5 domain in skeletal development. We observed that Fbn1<sup>TB5</sup> mice displayed skeletal abnormalities, reminiscent of the human phenotype. Mutant mice exhibited shorter stature with Fbn1 mutation leading to a profound alteration of the growth plate formation and an abnormal organization and structure of chondrocytes. In addition, mutated Fbn1 chondrocytes failed to establish a microfibrillar network. Our findings reveal the link between Fbn1 microfibrillar network and the growth plate organization suggesting a role of Fbn1 in chondrogenesis regulation.

**S.51****THERAPEUTIC POTENTIAL OF SMALL N-TERMINAL FIBRILLIN-1 FRAGMENTS IN BONE**

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Marfan syndrome is the most common type-I fibrillinopathy characterized by severe skeletal complications, including low bone density (osteopenia), long bone overgrowth, scoliosis, and kyphosis. While it is clear that mutations in the fibrillin-1 gene cause MFS, it is poorly understood how these mutations lead to the clinical skeletal manifestations. Bone undergoes continuous remodeling by coupled activities of osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells). The N-terminal fragment of fibrillin-1, rF23 (63kDa) and its sub-fragment rF31 (32 kDa) were previously identified as strong inhibitors of osteoclastogenesis *in vitro* and *in vivo* in healthy animals. To examine if N-terminal fibrillin-1 fragments exhibit sufficient anti-resorptive activity in disease associated with osteoclast activation, we used ovariectomized mice as an osteoporotic model. Mice were randomized into i) a control group (ovariectomized, untreated), ii) a positive control group treated with zoledronic acid (ZA), and iii) an experimental group intraperitoneally injected with the rF31 fragment 3 times within the second week after surgery. Mice were sacrificed at 2.5 and 6 weeks after surgery, and bone density and quality were analyzed by microCT and 3-point bending analysis. Bone mineral density and bone volume/tissue volume analyzed after 2.5 weeks showed as expected that ZA significantly rescued bone loss, and rF31 showed a strong tendency for improvement. The trabecular thickness significantly improved for both, ZA and rF31, at 2.5 weeks. Trabecular bone quality parameters after 6 weeks did only improve for ZA but not for rF31. However, 3-point bending analysis at this time point showed a significant improvement in bone quality for rF31 similar or even better compared to ZA, indicating a beneficial effect of rF31 on cortical bone. This pilot analysis demonstrates that the ovariectomy-induced low bone mass phenotype was partially reversed upon treatment with the rF31 fibrillin-1 fragment, and cortical bone quality was improved at the later time point. These data suggest that N-terminal osteoclast-inhibiting fibrillin-1 fragments have the potential for use as novel anti-resorptive agents.



**S.52****AORTIC AND MITRAL VALVE SURGERY IN PEDIATRIC AND YOUNG ADULT PATIENTS WITH MARFAN SYNDROME: CHARACTERISTICS AND OUTCOMES**

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**Objectives:** We utilized a national database to evaluate outcomes in pediatric and young adult patients with Marfan syndrome undergoing mitral valve (MV), aortic valve (AV), or thoracic aortic procedures, and describe factors associated with mortality.

**Methods:** Patients under 25 years old with a diagnosis of Marfan syndrome (ICD-9 759.82) who had undergone MV, AV, or thoracic aortic interventions from 2004 to 2016 were queried in the Pediatric Hospital Information System database, a multi-institutional administrative database of 45 pediatric hospitals. Perioperative complications, comorbidities, and outcomes were assessed. Univariate analysis was performed to evaluate variables associated with mortality.

**Results:** Sample included 321 encounters in 294 patients. Ninety patients underwent 97 MV procedures and 253 patients underwent 267 AV/aortic procedures. Patients underwent MV and AV/aortic procedures in the same hospitalization in 43 cases. Median age was 11.1 y (range 0.4-23.4) for MV procedures and 15.7 y (range 0.1-24.3) for AV/aortic procedures. Aortic dissection/rupture was reported in 3.4%. Perioperative complications were reported in 49.2%. Aortic aneurysm, arrhythmia, heart failure, and post-operative hemorrhage were the most frequently reported complications/comorbidities. In-hospital mortality was 3.1% for MV procedures and 1.5% for AV/aortic procedures. Death after MV surgery was associated with heart failure, post-operative hemorrhage, and respiratory complications. Death after AV/aortic surgery was associated with post-operative ECMO, neurologic complications, gastrointestinal complications, post-operative hemorrhage, concurrent coronary artery procedure, and younger age.

**Conclusions:** Pediatric and young adult patients with Marfan syndrome commonly have perioperative complications and comorbidities after undergoing MV and AV/aortic surgeries, but have relatively low in-hospital mortality.

**S.53****INFLAMMATORY RESPONSE AFTER EXOVASC®PERSONALISED EXTERNAL ROOT SUPPORT IMPLANTATION. A SINGLE CENTRE EXPERIENCE**

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**Objectives:** The ExoVasc® Personalised External Root Support (PEARS) implantation is a novel method of prophylactic aortic root surgery in patients with aortic root dilatation due to Marfan syndrome or other related genetic disorders. We aim to study an inflammatory response after this innovative operation.

**Methods:** This is a single centre, retrospective analysis based on patient's hospital record analysis of all patients who underwent PEARS implantation between 2015-2017. The C-reactive protein (CRP), white blood count (WBC) and echocardiography were performed prior and during each hospital admission. Fever was defined as  $\geq 38^{\circ}\text{C}$ . Pericarditis criteria included a minimum of 3: chest pain, pericardial effusion, ST elevation, elevated CRP and/or temperature.

**Results:** The PEARS was successfully implanted in all 13 patients. FBN1 mutation, bicuspid aortic valve or a strong family history of aortic dissection were present in 8, 3 and 2 patients. The average age was 37 (23-50) years, 11 males, the average aortic diameter 49 (40-55) mm. The average CRP and WBC prior and within first 6 days after the PEARS implantation were 2.2 versus 264.5 mg/L and 6.9 versus  $15.2 \times 10^9/\text{L}$ . The average body temperature after surgery was  $37.8^{\circ}\text{C}$  reaching fever in 10 patients. Late fever requiring hospital admission was present in 2 patients up to 251 days after the surgery. Early pericarditis was present in 3 patients and late recurrent pericarditis requiring rehospitalisation occurred in 4 patients up to 299 days after surgery.

**Conclusion:** PEARS is extremely promising surgical technique, but post-operative inflammatory response is a relatively frequent complication and warrants further investigation.

## S.54

**AORTIC ROOT IS LARGER IN PATIENTS WITH MARFAN SYNDROME AND BICUSPID AORTIC VALVE**

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**Background:** Although bicuspid aortic valve (BAV) is common and frequently associated with a dilatation of the ascending aorta (aortic root and/or tubular ascending aorta), the inherent risk of aortic dissection remains unclear. Marfan syndrome (MFS) is associated with a risk of both aortic dilatation and dissection. Comparing aortic dissection rates in MFS patients with and without BAV may help understand if the presence of BAV is a risk factor. We sought to evaluate aortic dissection and aortic surgery occurrence in MFS patients with and without BAV.

**Method:** All patients with identified *FBN1* mutation evaluated in our clinic since 1996 were included. BAV were classified using the Sievers classification. During prospective follow up, aortic surgery and aortic dissection were gathered.

**Results:** Out of the 1437 MFS patients with *FBN1* mutation, 26 patients (1.8%) had a BAV. Aortic surgery was performed in 10 patients (38%), because of symptomatic aortic valve disease in 2 and aortic dilatation requiring prophylactic aortic root replacement in 8. There was a trend towards a younger age for prophylactic aortic surgery in MFS patient with BAV as compared with others MFS patients. Aortic root diameter and normalized z-score were larger at all ages in patients with BAV (figure) when compared with patients with tricuspid aortic valve whereas both groups did not have a significantly different variation of z-score with age suggesting an anatomic modification of aortic root related to the bicuspid valve. No dissection of the aorta was observed in MFS patients with BAV.

**Conclusion:** In MFS patients with *FBN1* mutation, BAV is associated with larger aortic root diameters with a trend towards earlier prophylactic aortic surgery but not with an increased risk of aortic dissection.

**S.55****REINFORCING THE DILATED AORTIC ROOT IN MARFAN PATIENTS:  
ASSESSMENT OF THE USE OF A MACROPOROUS EXOSTENT IN SHEEP**

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**Objectives:** Since the development of personalized external aortic root support (PEARS), more than 100 patients with Marfan syndrome successfully underwent this surgery. However, data concerning the incorporation of the exostent mesh and its influence on the histological properties of the aorta is very limited. To this end, sheep were treated with the macroporous exostent.

**Methods:** In ten sheep, an aortic aneurysm was induced by translocating the pulmonary artery in the descending thoracic aorta. The autograft was reinforced with a polyethylene terephthalate mesh (n=7) or left unreinforced (n=3). After six months, a CT-scan was taken and the aorta was excised and examined both histologically and mechanically.

**Results:** In the unreinforced group, the autograft/aortic diameter ratio was 1.59, while only 1.11 in the reinforced group (p<0.05). Histological examination of the reinforced autograft and the adjacent aorta revealed thinning of the vessel wall due to atrophy of the smooth muscle cells (SMC). A fibrotic sheet, variable in thickness and containing fibroblasts, neovessels and foreign body giant cells, covered the mesh entirely. Mechanical analysis revealed little influence of the mesh at physiological stresses and only a restriction of motion at higher pressures.

**Conclusions:** Reinforcing the pulmonary autograft after the Ross procedure with a macroporous mesh showed promising results in limiting autograft dilatation in this sheep model. Atrophy of SMC, and consequently thinning of the vessel wall was seen in all the wrapped samples. However, no loss of strength was measured in relation to this thinning.

**S.56****REDUCED LEFT VENTRICULAR (LV) FUNCTION IN MARFAN SYNDROME**

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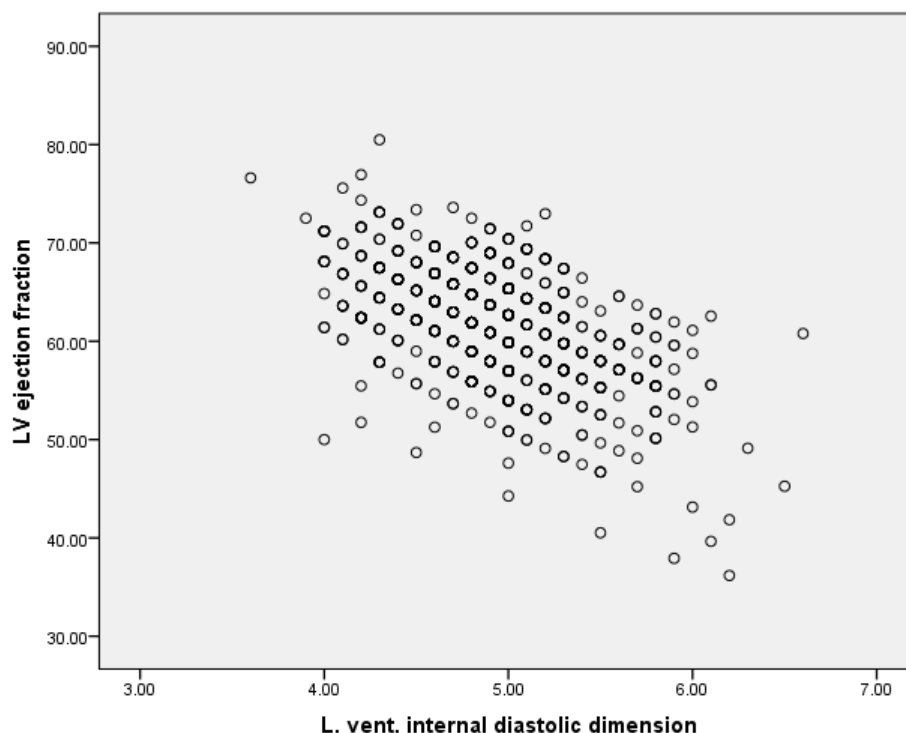
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We compared echo LV function in MFS participants in the GenTAC registry at Cornell to those in a previously published population of apparently normal adolescents and adults. The 115 MFS patients without prior graft or valve surgery or >mild native valve disease were similar to the 1207 apparently normal adults in gender (55 vs. 51%,  $p=0.38$ ), but were younger ( $34\pm12$  vs.  $41\pm14$  years) and taller ( $182\pm11$  vs.  $167\pm10$  cm, both  $p<0.001$ ). LV ejection fraction (EF) was lower in women than men in the reference population ( $62\pm6$  vs.  $64\pm6\%$ ,  $p<0.001$ ) without significant difference among MFS patients ( $59\pm8$  vs.  $60\pm8\%$ ,  $p=0.43$ ). LV end-diastolic dimension (LVIDd) was lower in women in the reference population ( $4.7\pm0.4$  vs.  $5.1\pm0.4$  cm,  $p<0.001$ ) with a parallel trend in MFS patients ( $4.6\pm0.8$  vs.  $4.9\pm0.7$  cm,  $p=0.06$ ).

LV EF was negatively related to LVIDd and height in the reference population ( $r=-0.43$  and  $-0.15$ , both  $p<0.001$ ), with virtually identical regression lines between EF and LVIDd in women and men (Figure). Among MFS patients, Z score for EF was  $<-2$  in 37 (32%). Compared to other MFS patients ( $n=78$ ), those with subnormal LZ EF by Z score were similar in gender (54 vs. 55%), age ( $34\pm12$  vs.  $32\pm12$ ,  $p=0.27$ ), height ( $182\pm11$  vs.  $182\pm10$  cm,  $p=0.71$ ) and LVIDd itself ( $4.6\pm1.0$  vs.  $4.9\pm0.6$ ,  $p=0.10$ ).

Thus, LV EF is reduced in MFS independent of coexisting valve disease, prior graft/valve surgery, age, gender, height or LV size.



**S.57****INCIDENCE OF AORTIC EVENTS IN MARFAN SYNDROME. MULTICENTER STUDY IN 406 PATIENTS**

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**Objective:** In the Marfan syndrome (MS), the indication for surgery on the aortic root remains controversial because of the lack of data on the risk of aortic events (death, dissection or aortic rupture) associated with this disease. We analyzed the annual incidence of aortic events as a function of aortic diameters, in order to propose the optimal time for prophylactic surgery.

**Methods:** From January-04 to June-15, 406 patients from Marfan Units were studied by echocardiographic and angio-CT/MRI of the initial aorta and periodic annual monitoring. The mean age at the first visit was  $28.4 \pm 14.5$  years, with a mean diameter of the sinuses of Valsalva of  $37.1 \pm 6.6$  mm. The mean follow-up was  $5.6 \pm 2.7$  years.

**Results:** During follow-up, there were 11 aortic events (seven exitus and four acute aortic dissections). The mean annual risk of aortic event was 0.5% (risk of death of 0.32% and risk of aortic dissection of 0.18%). The increase of the aortic diameters was associated with increased risk (0.2%/year (CI:0.03-0.5) with diameters <40 mm, 0.3%/year (CI 0.1-1, 4) between 40-44 mm, 1.3%/year (CI:0.3-4.6) between 45-49 mm and 5.2%/year (CI:0.4-12.4) with diameters  $\geq 50$  mm). Fifty-six aortic patients were underwent to elective surgery without in-hospital mortality. Overall survival at 3, 5 and 10 years was  $99 \pm 5\%$ ,  $98.6 \pm 6\%$  and  $95.5 \pm 2.5\%$ , respectively.

**Conclusions:** The centralization of patients with MS in specific units reduces substantially the incidence of aortic events. In experienced centers, prophylactic surgery with aortic root diameters  $\geq 45$  mm could be indicated in the MS.

**S.58****MYOCARDIAL DISEASE AND ARRYTHMIA IN MARFAN SYNDROME**

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**Background:** The risk of sudden death, independent of aortic disease, in patients with Marfan syndrome (MFS) seems increased. Ventricular dysfunction and arrhythmia might be important underlying causes. This prospective case-control study aimed to investigate these aspects in MFS patients.

**Methods:** We included 86 patients (48 women, 36.3±14.3yrs) and 40 age and gender-matched controls. Blood analysis of NT-proBNP and echocardiography was performed in all subjects. MFS patients also underwent resting electrocardiogram and 24h-Holter monitoring.

**Results:** Compared to controls, MFS patients showed mild LV impairment evidenced by higher indexed LVEDD (25.7±3.7 versus 23.9±4.6mm/m<sup>2</sup>, p<0.001) and LVESD (17.3±3.1 versus 15.9±3.7mm/m<sup>2</sup>, p=0.034), increased septal E/E' ratio (8.9±3.4 versus 6.5±2.3, p= 0.001) and higher NT-proBNP levels (68.5 IQR 35.3-149.3 versus 30pg/ml IQR 19-44.5, p=0.003). Indexed LVEDD and LVESD were not significantly higher after adjusting for valvular disease.

24h-Holter in the MFS group evidenced >10 ventricular extrasystoles (VES)/h in 33 and >100VES/h in 6 patients. Ventricular couplets, triplets and non-sustained ventricular tachycardia were present in 18, 10 and 1 patient, respectively. Patients having >100VES/h were significantly older (50.2 IQR 41.8-60.2 versus 32.8 IQR 21.5-46.2yrs, p=0.016), had a higher incidence of mitral valve prolapse (66.7 versus 25.5%, p=0.010) and increased indexed LVEDD (29.1±4.5 versus 25.4±3.6mm/m<sup>2</sup>, p=0.022) and LVESD (20.5±4.8 versus 17.1±2.9mm/m<sup>2</sup>, p=0.010). In multivariate analysis, age was the only independent predictor of >100VES/h (p=0.001).

**Conclusions:** Our study confirms mild myocardial impairment in MFS and suggests a higher incidence of ventricular events in older patients. 24h-Holter monitoring in the control population would be necessary to validate these results.

**S.59****PREVALENCE OF OBSTRUCTIVE SLEEP APNEA AND IT'S RELATION TO CARDIOVASCULAR DISEASE IN MARFAN SYNDROME**

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**Background:** Increased prevalence of obstructive sleep apnea (OSA) in Marfan syndrome (MFS) and its relation to cardiovascular risk has earlier been suggested, but it's still controversial. This prospective study aimed to further investigate these aspects.

**Methods:** Patients were recruited from an ongoing longitudinal study in 86 MFS patients. A priori risk for OSA was evaluated with the Epworth Sleepiness Scale (ESS) and the STOPbang questionnaires. An attended polysomnography (PSG) was performed in patients giving consent. OSA was defined as an apnoe-hypopnoe index >5events/h. Blood analysis of NT-ProBNP, echocardiography, resting electrocardiogram and 24h-Holter monitor were performed in all subjects.

**Results:** 40 patients consented for PSG (24 female, 37±12.8yrs). The ESS score was slightly higher but normal in patients undergoing PSG compared to the others (7/24 IQR 4-11 versus 4/24 IQR 2-8, p=0.029). All other baseline characteristics were similar.

The prevalence of OSA was 42.5% (47% female). Age and BMI were significantly higher in the OSA group (44.7±14.3 versus 32.1±11.4yrs, p= 0.004 and 25.6±4.8 versus 19.5±3.6kg/m<sup>2</sup>, p=0.001, respectively). After adjusting for age and gender, BMI was the only independent predictor of OSA (p<0.001).

Univariate analysis showed higher systolic blood pressure (136.2±16.3 versus 122.9±15mmHg, p=0.014), higher distal aortic diameters (descending 21 IQR 18-23.3 versus 17 IQR 16-18.5mm, p=0.002 and abdominal 19.7±3.6 versus 16.1±2.8mm, p=0.002) and higher prevalence of ventricular ectopy (53.3% versus 13%, p=0.012) in the OSA group. However, when adjusting for other factors, OSA did not show an independent relation with these variables.

**Conclusions:** Our study confirms a high prevalence of OSA in MFS patients. Higher BMI is associated with OSA. OSA might increase the cardiovascular risk but we have not been able to demonstrate an independent relation.



**S.60****MEDIUM AND LONG-TERM RESULTS OF THE MITRAL VALVE REPAIR IN MARFAN SYNDROME**

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**OBJECTIVES:** Mitral regurgitation in Marfan syndrome has similarities with the myxomatous disease, but with higher fibroelastic affectation. The results of the reparative surgery are very limited and it continues being debated. We reviewed our experience of mitral valve repair in Marfan syndrome.

**METHODS:** From Feb-05 to Dec-17, 21 Marfan syndrome's patients and mitral regurgitation were undergone to mitral valve repair. Mean age was  $30.67 \pm 12.75$  years and 12 patients (57.1%) were men. In 13 procedures (61.9%), it was associated to aortic valve-sparing (David operation). The 57.1% of the procedures were complex mitral repair techniques by ring or band annuloplasty with the implantation of artificial chords or valve resections. The mean follow-up was  $59.29 \pm 38.97$  months. We analyzed our results in terms of survival and freedom of reoperation.

**RESULTS:** The hospital mortality was 0%. The survival at 1, 5 and 10 years was 100%, 94.12% and 82.35%, respectively. Only one patient required reoperation because of severe mitral regurgitation after 23 months of follow-up. At last echocardiography monitoring, 80.9% of patients had mitral regurgitation <III and 57.1% had no regurgitation or it was trivial. There was no endocarditis or cerebrovascular events.

**CONCLUSIONS:** Mitral valve repair surgery in Marfan syndrome presents good clinical and functional results in medium and long-term and it should be considered as first choice procedure in the mitral regurgitation in these patients. Due to the high technical complexity, these patients should be centralized in experienced centers.

**S.61****CHILDREN AND ADOLESCENTS WITH MARFAN SYNDROME; LESSONS LEARNED FROM EHLERS-DANLOS SYNDROME AND HYPERMOBILITY SPECTRUM DISORDERS REGARDING CHRONIC PAIN AND FATIGUE.**

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**Objective:** Major issues in children with Marfan syndrome (MFS) are pain, fatigue and joint hypermobility which might influence functional ability. We recently studied these aspects in children with Ehlers-Danlos syndrome (EDS) and hypermobility spectrum disorders (HSD). What can be learned from this research regarding diagnostics and treatment for children with MFS?

**Methods:** We followed 101 children and adolescents with EDS and HSD for three years and studied the presence of pain, fatigue and functional ability as well as the presence of multi-systemic complaints (eg. dysautonomia). In 255 adolescents and adults with EDS and HSD, we studied the presence of pain, fatigue and functional ability.

**Results:** Children with a high incidence of multi-systemic complaints, and significant pain and fatigue at baseline were most likely to have a deteriorating trajectory of functional impairment. Besides joint hypermobility and chronic pain, generalized hyperalgesia was also found, possibly indicating central nervous system involvement.

We stated that in children and adolescents with EDS and HSD, identifying risk profiles are important to clarify the pathological mechanisms involved, and to develop interdisciplinary treatment strategies.

**Conclusion:** Based on lessons learned in EDS and HSD, we designed a longitudinal follow-up study in 150 children and adolescents with MFS in the Netherlands and Belgium. Disease characteristics, functional ability, exercise capacity, symptoms of pain and fatigue and psycho-social wellbeing will be studied. We will identify risk profiles for functional decline and develop therapeutic rehabilitation strategies.

**S.62****EFFECTS OF COMBINATION OF MILD AEROBIC EXERCISE AND ANGIOTENSIN-II RECEPTOR TYPE-I BLOCKER LOSARTAN IN A MOUSE MODEL OF MARFAN SYNDROME.**

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**Objective:** We have shown that mild aerobic exercise improves aortic elasticity and blocks aneurysm progression in Marfan (MFS) mice. Here, we investigated the effects of combination of mild exercise and blood pressure lowering drug losartan on aortic dimensions and stiffness in MFS mice using high resolution ultrasound imaging.

**Methods:** Four-week old control and MFS mice were subjected to sedentary life style or mild exercise (8m/min, 30min/day, 5days/week) with or without losartan treatment (full dose of 0.6/L or half dose of 0.3g/L in drinking water). Longitudinal imaging of the aorta was performed when mice reached 3 and 6 months of age to determine aortic dimensions and stiffness (pulse wave velocity, PWV).

**Results:** Aortic dimensions at the aortic annulus, sinuses of Valsalva, and sinotubular junction were significantly larger in 3 and 6-month old sedentary MFS mice as compared to controls ( $P<0.005$ ). Combination of mild exercise with full dose losartan (0.6g/L) decreased aortic annulus dimension at 3 and 6 months old MFS mice ( $P<0.005$ ), but decreased sinus of Valsalva dimensions only in 6-month old MFS mice. Measurements for PWV in MFS mice were significantly higher in both 3 and 6 months old sedentary MFS mice as compared to controls ( $P<0.0005$ ). Combination of exercise with full dose (0.6g/L) and half dose (0.3g/L) of losartan markedly reduced PWV in MFS mice aorta ( $P<0.0005$ ).

**Conclusion:** Our results are the first to demonstrate the potential therapeutic value of combination of exercise and losartan to prevent progression of aortic root dilation and to improve aortic wall elasticity in MFS mice.

**S.63****MALE FBN1C1039G/+ MICE ANXIOUS-LIKE PROFILE AS A VALUABLE MODEL TO STUDY ANXIETY IN MARFAN SYNDROME AND RELATED DISORDERS**

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**Objectives:** The validation of animal models is one of the most powerful tools that have contributed to the advancement of research and improvement of knowledge of human diseases. The hallmarks of Marfan syndrome and related disorders (MFS) feature cardiovascular system as the main clinical and research target due to its life-threatening implications. However, psychosocial and mental health aspects, such as anxiety, have been largely neglected in spite of being observed in all cross-sectional studies. The fibrillin 1 *Fbn1*<sup>C1039G/+</sup> murine model, heterozygous for the most common class of mutation in MFS, recapitulates most cardiovascular aspects described in humans with MFS, but nothing is known about their behavioral phenotype. Here we present the first results of the characterization of their behavioral phenotype.

**Methods:** Male mice were studied at 3 and 9 month of age, and as compared to age-matched wild-type controls. Twenty-one mice were behaviorally assessed in two distinct anxiogenic environments: the mild neophobia to a new home-cage in the corner test and the confrontation of an open and illuminated field as opposed to their innate habitat preferences. **Results:** The pattern of their behaviors, in regards to the temporal sequence of events, thigmotaxis, locomotion, exploratory activity and emotionality differed from that exhibited by their age-matched wild-type counterparts, providing evidence of an anxious-like profile. Most importantly, major anxious-like patterns were already apparent since young adulthood.

**Conclusions:** The consistent profile of *Fbn1*C1039G/+ male mice can be a useful tool to study this neuropsychiatric feature associated to MFS and for preclinical assessment of preventive/therapeutical interventions.

**S.64****CHILDREN AND ADOLESCENTS WITH MARFAN SYNDROME: 10,000 HEALTHY STEPS AND BEYOND - PILOT BASELINE DATA**

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**Objectives:** Aortic dissection is the most life-threatening complication of Marfan syndrome (MFS), characterized by weakening of the aortic wall due to fragmentation of elastin fibers. To date, clinical emphasis has been on what exercises to avoid, due to high risk of aortic dissection, rather than promoting regular exercise. MFS patients also often have sedentary lives due to self-imposed limitations. However, regular physical activity could potentially improve aortic wall structure and function by significantly reducing elastin fiber fragmentation and aortic wall stiffness, as already shown in mouse models. Here we present the pilot baseline data for an intervention that is underway to determine if regular physical activity improves aortic stiffness in MFS patients.

**Methods:** MFS patients 8-19 years old followed in our center are eligible to participate. During the 6-month intervention MFS patients (n=25) are asked to take at least 10,000 steps a day, tracked by an activity tracker. Patients have weekly check-ins with the study team via phone call or text-messaging. At baseline and at the end of intervention, arterial stiffness and physical activity indices are collected. Arterial stiffness is assessed by arterial tonometry (pulse wave velocity) and echocardiography. Physical activity is measured by accelerometers to determine time spent in sedentary or vigorous activity during waking hours. Fasting lipid panel is obtained.

**Results:** Up to present time, 6 patients (15.3±2.7 years old, 4 males) have been enrolled in the intervention. All 6 MFS patients are on Atenolol and/or Losartan.

**Conclusion:** Our pilot baseline data show that pediatric MFS patients have stiff arteries and spend a significant percent of their awake time sedentary. Thus, this lifestyle intervention has great potential to improve level of physical activity and arterial health in this vulnerable patient population.

**S.65****1. A COMPREHENSIVE STUDY OF ADULTS WITH MARFAN SYNDROME; PSYCHOSOCIAL ASPECTS AND HEALTH PROBLEMS, CHRONIC PAIN AND FATIGUE**

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2. Norway Faculty of Social Sciences, Department of Social Work, Child Welfare and Social Policy, Oslo Metropolitan University, Norway.

**Objectives:** Present the comprehensive research project “Living with Marfan syndrome in Norway”

**Methods:** A combined methods study. Part 1: Systematic review on psychosocial issues and on chronic pain in Marfan syndrome (MFS). Part 2: Cross-sectional questionnaire study investigating self-reported health problems, psychosocial factors, chronic pain and fatigue in Norwegian adults with verified MFS (Ghent 1 criteria).

**Results:** Part 1. Systematic review on psychosocial issues ([Velvin et al 2014](#)) found 15 articles, reporting reduced health-related quality of life, and lower work participation in adults with MFS. Systematic review on pain ([Velvin et al 2016](#)), found 18 articles reporting high pain prevalence in adults with MFS.

Part 2. of 117 adults with verified MFS 73 adults (62%) answered questionnaires. Several reported chronic pain (64%) and severe fatigue (42 %), significantly higher than in the general Norwegian population (GNP) ([Bathen et al 2014](#)). Despite high education levels, MFS patients had lower work participation than the GNP. Fatigue was the factor having highest association to work participation. ([Velvin et al 2015](#)). Marfan-related health problems were not significantly associated with work participation, chronic pain or fatigue ([Velvin 2016](#)). Satisfaction with life was significantly lower than in the GNP and significantly associated with fatigue and aortic dissection ([Velvin et al 2015](#)).

**Conclusions:** Marfan syndrome is a disease with significant psychosocial challenges. Both pain and fatigue are frequent. This warrants more research on prevalence, associations and treatment. There is a need for focus on work participation and adaptations for adults with MFS.

**S.66****PARENTS' PERSPECTIVES ON THE IMPACT OF MARFAN SYNDROME ON DAILY LIFE  
FUNCTIONING OF THEIR CHILDREN**

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**Objectives:** The aim of this qualitative study was to gain an in-depth understanding of how parents of children with Marfan syndrome aged 4-12 years perceive the impact of Marfan syndrome on daily life functioning of their children.

**Methods:** 3 focus groups including 16 parents and semi-structured interviews in 10 parents were conducted. Audio recordings were fully transcribed, independently coded by two investigators using qualitative analysis software (MAXQda) and linked to the categories of the International Classification of Functioning Disability and Health for Children and Youth. Disagreement between the two researchers was resolved by discussion. All participants received a summary of the interviews.

**Results:** Key themes that emerged from the parents' qualitative responses included the impact on the child's daily life functioning of physical impairments (fatigue, pain, skeleton, eyes, aorta), physical appearance and activity limitations (walking, running, cycling, carrying, writing, dressing, sport activities). Parents reported their child incapable of fully participating in school, a social environment with peers, playing outside, leisure and sports. To keep the child's daily life manageable and balanced, parents have adjusted daily routines, school schedules, sports, leisure and holidays. Parents stated this has an impact on the child's daily life.

**Conclusions:** Parents perceive a large impact of Marfan syndrome on daily life functioning of their children. The underlying causes of limitations in activities and participation should be unraveled in order to develop treatment interventions. Professionals should be aware of the impact and discuss these issues with children and their parents.

## S.67

**VASCERN, European Rare disease Network (ERN) on rare vascular diseases**

**M Hurard(1), K Benke (2), E Arbustini (3), E Bjorck (4), M Groenink (5), M Kempers (6), B Loeys (7), B Mulder(5), L Murphy (8), G Pepe (9), A Pini (10), L Robert (11), J Roos-Hesselink (12), Z Szabolcs (2), I Van de Laar (12), Y Von Kodolitsch (13), J DeBacker (14), G Jondeau (1) for the HTAD RDWG of VASCERN**

(1)VASCERN Paris France, (2) Budapest, Hungary, (3) Pavia, Italy; (4) Stockholm, Sweden. (5) Amsterdam, Netherlands. (6) Nijmegen, Netherlands. (7) Antwerp, Belgium, (8) Swedish Marfan organisation; (9) Florence, Italy; (10) Milan, Italy; (11) London, UK (12) Rotterdam, The Netherlands; (13) Hamburg, Germany, (14) Ghent, Belgium, HTAD RDWG Chair;

VASCERN is one of the 24 European Rare disease Network (ERN)s funded by the EU, and launched in Mars 2017. VASCERN, the European Reference Network on Rare Multisystemic Vascular Diseases, is a network gathering the **best expertise** in Europe to provide accessible cross-border healthcare to patients with **rare vascular diseases**. More than 1.3 Millions of European patients are concerned, and the scope will enlarge.

Our ERN consists of 31 highly specialized multidisciplinary Healthcare Providers (HCPs) from 11 EU Member States in this area of expertise, and of various European Patient Organisations involved. It includes 5 Rare Diseases Working Groups (RDWGs):

- Heritable Thoracic Aortic Diseases (HTAD-WG),
- Hereditary Haemorrhagic Telangiectasia (HHT-WG) (Chair Claire Shovlin, London, UK),
- Medium Sized Arteries (vascular Ehlers Danlos Syndrome) (MSA-WG) (Chair Leema Roberts, London UK)
- Pediatric and Primary Lymphedema (PPL-WG), Chair Robert Damstra, Drachten, Netherlands
- Vascular Anomalies (VASCA-WG). Chair Miikka Vikkula, Brussels, Belgium

In addition, several Thematic Working Groups are established to better tackle transversal work packages on: eHealth (Chair A Pini) / training & education (Chair J Rosslen), ethics (Chair R Alderweireldt), patient registry (Chair L Federici), and communication (Chair M Hurard). Patients have a specific WG (ePAG – European Patient Advocacy Group) and are also part of all the other WGs. VASCERN enables patient representatives to work on all common issues and to be involved in all activities.



# **ABSTRACTS** OF POSTER PRESENTATIONS

**P.1****MUTATIONS IN THE SIGNAL PEPTIDE OF FBN1 CAUSE ACCUMULATION OF FIBRILIN 1 IN THE ENDOPLASMIC RETICULUM AND ER STRESS**

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**Objective:** Mutations in the FBN1 gene cause Marfan syndrome. For missense mutations the effect of the mutation on the protein and the phenotype are often difficult to predict. Mutations in the signal peptide, the first 27 amino acids of fibrillin1, are usually classified as variants of uncertain clinical significance. Our aim was to study the effect of these mutations *in vitro*, to assess a possible pathogenic effect. These mutations may lead to errors in trafficking of the protein, such as retention in the endoplasmic reticulum (ER).

**Methods:** Fibroblasts from skin biopsies of control individuals and Marfan syndrome patients with missense mutations in the signal peptide of *FBN1* were cultured from skin biopsies. The cells were studied using immunofluorescence microscopy, using antibodies against fibrillin 1 or GRP78, a marker of ER stress.

**Results:** Fibroblasts with the mutations c.59A>G (p.Tyr20Cys), c.31C>G (p.Leu11Val), or c.32T>G (p.Leu11Arg) showed accumulation of fibrillin 1 and GRP78 in the ER.

**Conclusion:** Mutations in the signal peptide of fibrillin1 exert a pathogenic effect by causing accumulation of fibrillin 1 in the ER, resulting in ER stress.

**P.2****LENTIVIRAL-MEDIATED SILENCING OF THE *FBN1* GENE LEADS TO AORTIC DILATION AND MEDIAL DEGENERATION**

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Thoracic aortic aneurysm and dissection (TAAD) is a life-threatening effect associated with Marfan Syndrome (MFS), a disease caused by mutations in the Fibrillin-1 (*FBN1*) gene. We recently identified the metalloproteinase Adamts1 as a major mediator of vascular homeostasis, whose genetic haploinsufficiency in mice induces a phenotype similar to MFS. Of note, aortic nitric oxide levels are higher in *Adamts1*-deficient mice and in the *Fbn1*<sup>C1039G/+</sup> mouse model of MFS. To identify molecular mechanisms and mediators involved in MFS-associated aortopathy, we have set up a model based on acute lentiviral-mediated silencing of the *Fbn1* gene. *Wild-type* mice infected with lentiviruses encoding various short hairpin RNA (shRNA) sequences specific for *Fbn1* show aortic dilation in the ascending aorta, as well as features of medial degeneration, including elastic laminae fragmentation and disarray. These results suggest that *Fbn1* silencing leads to an aortic phenotype that resembles that found in MFS genetic mouse models. We are using these *Fbn1* knock-down mice to uncover early pathogenic mediators of aortic dilation and to determine whether the mechanisms and mediators that underlie the aortic disease in this model are common to those mediating MFS aortopathy in the *Fbn1*<sup>C1039G/+</sup> mouse model.

## P.3

### MICROFIBRILLAR ASSOCIATED PROTEIN TYPE 4 (MFAP4) ASSOCIATES WITH AORTIC DISSECTION IN MARFAN SYNDROME AND IS ESSENTIAL FOR ELASTIC FIBER ASSEMBLY

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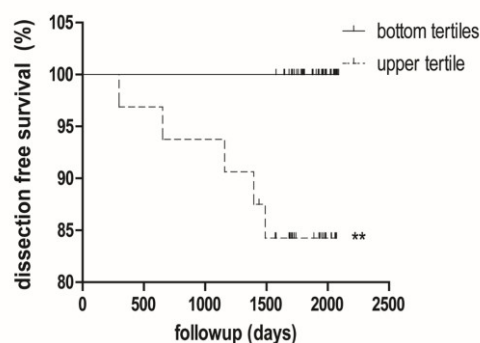
**Aim:** Marfan syndrome is a disorder with mutations in the fibrillin-1 gene, leading to elastic fiber degradation and increased TGF-beta signaling. The life-threatening feature of Marfan is aneurysm formation with a risk of fatal aortic dissections. In a proteomics screen, we identified MFAP4, an elastic fiber-associated protein, to be increased in the Marfan aorta. We aim to study the role of MFAP4 in Marfan aortic disease.

**Methods and results:** MFAP4 co-localizes in the aorta with elastin and collagen fibers. In vitro experiments show that MFAP4 expression is upregulated by TGF-beta, which could explain the increased MFAP4 protein levels in the Marfan aorta.

Plasma MFAP4 levels correlate with aortic root diameter in Marfan patients ( $r$  0.30,  $p$  0.01). High plasma MFAP4 associates with poor dissection-free survival, with all (type B) dissections occurring in the upper tertile (Figure 1). The aortic distensibility, as measure for elastic lamina integrity, was calculated throughout the aorta. The aortic distensibility in the descending thoracic aorta, where type B dissections occur, is lower in Marfan patients with high plasma MFAP4, suggesting increased elastic lamina damage. To further investigate the specific function of MFAP4, siRNA-mediated knockdown of MFAP4 was performed in fibroblasts. It reveals that MFAP4 is essential in elastic fiber assembly, which could not be attributed to alteration of elastin mRNA or protein expression.

#### Conclusion:

1. High plasma MFAP4 predicts type B aortic dissections.
2. MFAP4 is essential for elastic fiber formation in vitro.



**Figure 1**

**P.4****FIBRILLIN-MEDIATED REGULATION OF MICRORNA SIGNALING AND CELL FUNCTION**

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Fibrillin-1 and fibronectin are two principal extracellular matrix (ECM) components in elastic and non-elastic tissues. Both proteins contain one evolutionarily conserved ArgGly-Asp (RGD) sequence which mediates cell-matrix interactions through integrins. In turn, integrin ligation triggers intracellular signal pathways, and consequently guides cellular functions. Small non-coding microRNAs (miRNAs) post-transcriptionally control about 30% of mammalian gene expression, including several genes relevant in the pathogenesis of Marfan syndrome. However, whether fibrillin-1 and fibronectin regulate miRNAs and downstream cellular functions through integrin ligation is largely unknown. This study addresses how cell interaction of fibrillin-1 regulates miRNA-mediated cell function relevant to Marfan syndrome including growth factor signalling, focal adhesion formation, cell proliferation and differentiation, and compares the specificities of the RGD regulation between fibrillin-1 and fibronectin.

A miRNA global microarray of fibroblasts seeded on fibrillin-1 demonstrated more than 100 miRNAs that are differentially regulated between the fibrillin-1-bound and unbound states. Comparative messenger RNA (mRNA) analysis by a global microarray of the same fibroblasts identified differentially regulated mRNAs involved in growth factor activities, actin cytoskeleton and integrin dynamics upon fibrillin-1 ligation. qPCR results showed that the miRNA expression profile is both similar and distinct when cells interact with fibrillin-1 versus fibronectin, demonstrating a level of specificity between the respective RGD sites. To study the cellular consequences, miRNAs controlled by fibrillin-1 ligation were further analyzed by mRNA target analysis and functional assays. Bioinformatical prediction and experimental validation of target mRNAs highlighted their roles in the transforming growth factor signal pathway, Erk signal pathway and focal adhesion formation as the most important functional groups regulated upon fibrillin-1 ligation. All of these are relevant to cellular mechanisms in Marfan syndrome. Among the validated miRNAs, miR-503 was shown to participate in p-SMAD2 regulation upon RGD ligation. Immunofluorescence analysis indicated that miR-1208 is involved in the regulation of total Erk1/2 and cell proliferation. miR-29b-1\*, miR-612, miR-1231 and miR-1208 play differential roles in the focal adhesion formation process regulated by fibrillin-1. Furthermore, miR-1208 was shown to be involved in fibroblast to myofibroblast differentiation.

In summary, we show that fibrillin-1 and fibronectin interaction with fibroblasts regulate a set of miRNAs through integrin-mediated mechanisms. Some of these miRNAs in turn are relevant regulators for Marfan-related molecular mechanisms, most prominently the TGF-beta and Erk1/2 signaling pathways.

**P.5****NEW INSIGHTS INTO FIBRILLINOPATHIES IN THE CURRENT GENOMICS ERA**

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\*These authors contributed equally to this work

**Objectives:** Fibrillinopathies such as FBN1-related Marfan syndrome (MFS) and FBN2-related congenital contractural arachnodactyly (CCA) are characterized by a wide and overlapping range of clinical signs with neonatal to adult onset. Although the underlying molecular etiology of MFS and CCA has been known since 1991 and 1995, respectively, their prevalence, co-occurrence, and genetic modifiers have only been estimated or are unknown. Here we address these issues by assessing the frequency of pathogenic FBN1 and FBN2 sequence variants in ExAC/gnomAD and the largest Swiss database of Marfan genomes

**Methods:** By focusing on a priori pathogenic sequence variants in ExAC/gnomAD, we calculated conservative prevalences for MFS and CCA. Moreover, we screened whole genomes (60× WGS) of 420 Swiss patients with MFS or rare (aortic) disorders for sequence variants in FBN1, FBN2, and other related genes.

**Results:** We show the presence of clearly pathogenic FBN1/FBN2 variants in the apparently healthy reference cohort ExAC/gnomAD, providing prevalence estimates for MFS and CCA. In our Swiss cohort, we identified two families with dual (FBN1/FBN2) mutations, explaining the variable phenotype within these families including clinical features of MFS and CCA.

**Conclusions:** Our results not only demonstrate that apparently healthy reference data sets may include individuals with late-onset or unrecognized disease (pitfall in variant filtering and interpretation!) but also show that MFS may occur more frequently than expected and provide an estimate for the yet unknown prevalence of CCA. Furthermore, we emphasize the importance and increasing possibility of detecting modifying genes and sequence variants in the current genomics era.

**P.6****EXPLORING THE INFLUENCE OF MATERNAL VERSUS PATERNAL INHERITANCE ON GENE MODIFIERS AND THE INTRAFAMILIAL PENETRANCE OF THE AORTIC PHENOTYPE IN MARFAN SYNDROME**

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**Objectives:** To provide evidence that maternal versus paternal inheritance influences the observed intrafamilial variation in Marfan syndrome (MFS) penetrance. Sex-linked genetic modifiers have reportedly been found to contribute protective or additive effects for the penetrance of MFS in mice.

**Methodology:** Using pedigrees produced in the Madeline2 program, 4 families were selected on the following criteria; 2-3 generations in size, and complete medical records, for the screening of an *FBN1* positive (exons 20-44) mutation and/or clinical demonstration of Ghent positive MFS. Targeted branches with inheritance from either gender probands to offspring were analysed, and deep phenotyped for; **cardiovascular (CVS)**, **ocular**, and **skeletal** features. **Mutation type** and **exon location** were also provided.

Affected (A), unaffected (U), possibly affected (?) and unknown status (.) for each phenotype, was recorded and compared between either proband gender and associated severity of MFS. Aortic parameters (**CVS**) were a key focus. SPSS was used to determine statistical significance.

**Results:** Initially only 10 maternal and 16 paternal offspring were extracted from 4 families and analysed. 44% (n=7) of paternal offspring developed abnormalities in the aorta, in contrast to 40% (n=4) of maternal offspring. Similarly 30% (n=3) of maternally inherited subjects were unaffected in the Aorta, compared to only 12% (n=2) of paternally inherited subjects ( $P = 0.536$ ).

**Conclusion:** Albeit small in study size, the findings here warrant further investigation. Whole exome sequencing in large families holds promise for discovery of gene modifiers. Greater insight into gene modifiers could provide therapeutic avenues to decrease disease severity.

**P.7****SEX AND REGIONAL DIFFERENCES IN THE THORACIC AORTA OF A MURINE MODEL OF MARFAN SYNDROME**

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**OBJECTIVES:** Marfan syndrome (MFS) is equally prevalent in men and women, but epidemiologic evidence suggests that men are at a higher risk of aortic complications than non-pregnant women. Aneurysmal expansion initiates at the aortic root and can progress into the ascending portion. However, neither the presence of sexual dimorphism nor regional differences in thoracic aorta function have been investigated experimentally.

**METHODS:** Ascending and descending thoracic aorta reactivity was evaluated by wire myography. Ascending aorta mRNA and protein levels, and elastic fiber integrity were assessed by qRT-PCR, Western blotting, and Verhoeff-Van Gieson histological staining, respectively, in 3- and 6-month-old MFS (*Fbn1*<sup>C1039G/+</sup>) mice.

**RESULTS:** MFS either increased or decreased phenylephrine contractions in the ascending and descending thoracic aorta, respectively. These alterations were only seen in males, where ascending aorta contractions increased progressively from 3 to 6 months of age. In contrast, MFS did not alter ascending aorta endothelium-dependent relaxation in either sex, as a result of augmented endothelium-dependent hyperpolarization-type dilations. Non-selective cyclooxygenase inhibition prevented the MFS-induced enhancement of male phenylephrine contractions, an effect that was linked to increased cyclooxygenase-2 expression. Negative feedback of nitric oxide on phenylephrine contractions was observed in MFS mice of both sexes, which was associated with endothelial nitric oxide synthase upregulation in females. Finally, MFS ascending aortas from males showed a greater number of elastic fiber breaks than females.

**CONCLUSIONS:** The presence of more pronounced thoracic aorta alterations in male mice provides experimental evidence to support that male MFS patients are at increased risk of suffering aortic complications.

This work is supported by grants of The Marfan Foundation (USA) and MINECO (Spain).



**P.8****PREGNANCY RISK IN MARFAN SYNDROME: THE CORNELL EXPERIENCE**

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**Objectives:** The magnitude of risk of aortic and coronary dissection in Marfan women has been confounded by lack of knowledge of diagnosis before pregnancy. In addition, serial peri-partum imaging data on large numbers of women are lacking. We aim to describe our experience with pregnancy in Marfan women to assess risk and correlates of vascular complications and to provide prospective peri-partum imaging data.

**Methods:** Among 196 women fulfilling Ghent diagnostic criteria for Marfan syndrome, 128 have consented to participate in this study and have evaluable data (26 have yet to consent, and 42 are either deceased or lost to follow-up with incomplete data). 75 of 128 (59%) have never been pregnant and 53 (41%) have been pregnant (total live births=82 [range 0-5]). 26 dissections have occurred, including 5 peripartum dissections.

**Results:** Following completion of enrollment, we will compare prevalence and timing of vascular complications (aortic or coronary dissection, need for prophylactic surgery) in patients according to pregnancy status (never vs. ever) and knowledge of diagnosis. Among ever-pregnant women, we will compare those with and without pregnancy-related vascular complications to quantify risk, risk factors, timing and type of dissection. We will report aortic growth rates in women followed prospectively with peri-partum serial imaging data. We will assess the accuracy of different aortic diameters that have been proposed to heighten pregnancy risk.

**Conclusions:** Forthcoming.

**P.9****DNA METHYLATION AS AN EPIGENETIC MODIFIER OF THE *FBN1* TRANSCRIPTION**

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**Objectives:** Haploinsufficiency is a cause of autosomal dominant genetic diseases, which cause pathological varieties, termed “reduced penetrance” or “variable expressivity” among affected individuals. However, the cause of such pathological varieties cannot be explained by simple differences of nucleotide sequences via classical genetics. On the other hand, epigenetic gene regulation such as DNA methylation, resulting in transcriptional repression, can further modify genetic information.

**Methods:** *FBN1*, mutations of which are responsible for Marfan Syndrome, has a CpG island within the promoter region, and the CpG island ‘shore’ exhibits variable DNA methylation status. Here, we examined the relationship between gene expression and DNA methylation patterns of the *FBN1* CpG island shore focusing on transcriptionally active hypomethylated alleles (Hypo-alleles) in porcine tissues.

**Results:** The porcine *FBN1* was highly expressed in fetal skin fibroblasts but not in the liver. Consistent with this finding, the hypo-allele ratio of the *FBN1* CpG island shore was higher in fetal fibroblast cells than in the liver, and induced demethylation resulted in *FBN1* upregulation. Thus, the hypo-allele ratio of the *FBN1* CpG island shore correlated with expression levels in porcine tissues.

**Conclusions:** Changes in DNA methylation levels of the *FBN1* CpG island shore affected *FBN1* expression levels. We propose a new concept in that epigenetic fluctuation behaves as a modifier, which can act as a molecular mechanism to cause pathological varieties of haploinsufficiency diseases.

**P.10****OBSTETRICAL AND SURGICAL CONSIDERATIONS IN LOEYS-DIETZ SYNDROME (TYPE 4): A CASE SERIES**

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**Objectives:** Individuals with Loeys-Dietz syndrome (LDS) caused by *TGFBR1* and *TGFBR2* mutations are at increased risk for aortic dissection, especially during pregnancy. Pregnancy outcomes in individuals with LDS type 4 caused by *TGFβ2* have not been well reported.

**Methods:** Two sisters with a diagnosis of LDS type 4 were followed prospectively through their pregnancies. They have signed consent for publication of this report.

**Results:** The first case is a 16 year-old primigravida who presented with a family history of aortic dissection. LDS was suspected and genetic testing confirmed a pathogenic *TGFβ2* (c.988C>T) mutation. Her aortic root measured 3.6 cm at the beginning of pregnancy and remained unchanged. A cesarean delivery was performed at 36 weeks gestation with no cardiovascular complications. Significant tortuosity was noted of the uterine vessels at delivery. The second case her 19 year-old primigravida sister with a clinical diagnosis of LDS and pending confirmatory genetic testing. Her aortic root was 3.6 cm prior to pregnancy and 3.7 cm at late gestation. A cesarean delivery was performed at 37 weeks gestation with no cardiovascular complications. There was a dilated, abnormal vessel vertically in the uterine midline however the uterine vasculature appeared normal.

**Conclusions:** Women with LDS warrant special consideration in obstetrical management secondary to the risk for aortic dissection however can have successful pregnancies. A multi-disciplinary team is recommended for care in pregnancy. Vascular tortuosity and anomalous vessels may not be limited to thoracic and cerebral arteries but may extend to uterine vasculature and may complicate deliveries.

**P.11****INTERACTIONS BETWEEN STEM CELL DERIVED CARDIOMYOCYTES AND EXTRACELLULAR MATRIX TO MODEL MARFAN SYNDROME IN VITRO**

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Marfan syndrome (MFS) is a systemic disorder of connective tissue caused by pathogenic variants in the *FBN1* gene. Myocardial dysfunction has been demonstrated in MFS patients and mouse models but little is known about the interaction between the cardiomyocytes and extracellular matrix (ECM).

The aim of this study is to investigate the interactions between cardiomyocytes and the ECM, either derived from a healthy person or a MFS patient through an *in vitro* cell-matrix model. Concurrently the interactions between the healthy cardiomyocytes and the affected ECM and *vice versa* can be studied to verify if the healthy component can compensate the effect of the *FBN1* mutation.

For the cell component healthy or *FBN1* mutation affected cardiomyocytes will be obtained by differentiating the respective human induced pluripotent stem cells (hiPSCs). The differentiation will be performed with CHIR99021 which inhibits GSK3 and results in mesodermal commitment and IWP2, a Wnt inhibitor which induces cardiac mesoderm. The obtained cardiomyocytes will be purified based on their ability to survive when lactate is provided as single carbon source. The healthy or affected ECM component will be deposited by either fibroblasts from healthy volunteers or MFS patients respectively. Subsequently, the fibroblasts are removed so that only the ECM remains.

Extracellular fibrillin-1, collagen and elastin deposition will be analyzed by immunofluorescent stainings. Protein concentrations of TGF- $\beta$  will be studied using ELISA and qPCR will be used to assess the gene expression of MMPs. The cardiomyocytes will be assessed using a multielectrode array (MEA) for contraction and electric potential.

**P.12****A NOVEL THERAPEUTIC STRATEGY FOR MARFAN SYNDROME UTILISING ANTISENSE OLIGONUCLEOTIDES.**

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**Background:** Marfan syndrome (MFS) is a connective-tissue disorder, caused by *fibrillin-1* (*FBN1*) mutations. Fibrillin-1, is a large glycoprotein that aggregates into multimer units to form the backbone of microfibrils. The pathogenesis of MFS is not fully understood, however, *FBN1* mutations are hypothesised to result in monomers that are unable to form multimers, leading to a reduction in fibrillin-1 deposition and subsequent dysregulation of TGF- $\beta$ .

**Objective:** This study demonstrates a potential therapeutic strategy for a MFS patient carrying a *FBN1* silent mutation resulting in splicing of exon 52 from mature mRNA. Splice switching antisense oligonucleotides were designed to excise exon 52 from normal *FBN1* mRNA, re-establishing periodicity of fibrillin-1 monomers. We hypothesise that the resulting monomers will form multimers, increasing fibrillin-1 deposition and reducing disease severity.

**Methods:** Antisense oligonucleotides, designed to target regulatory splicing motifs within *FBN1* exon 52, were screened in normal and patient fibroblasts, to assess splicing of unaffected transcripts. Treated cells were also immunostained to reveal the abundance and morphology of fibrillin-1.

**Results:** The observed exon 52 skipping was dose dependant, with up to 99% of transcripts in patient fibroblasts missing exon 52. A corresponding increase in fibrillin-1 staining was observed in treated, compared to untreated patient cells.

**Conclusions:** The use of antisense oligonucleotides to induce targeted alternative splicing has garnered attention in recent years, particularly for treatment of Duchenne muscular dystrophy. We believe this technique is applicable to MFS, with preliminary *in vitro* data supporting the hypothesis that inducing homogeneity between fibrillin-1 monomers has therapeutic potential.

**P.13****NOVEL READ-OUT SYSTEM TO ASSESS THE MECHANICAL INTEGRITY OF THE THORACIC AORTA IN MURINE MODELS**

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**Objectives:** Patients with connective tissue disorders such as Marfan syndrome (MFS) or Ehlers-Danlos syndrome vascular type (EDS IV) are at increased risk for aortic ruptures. Using an EDS-IV-mimicking mouse model with *Col3a1* true haploinsufficiency, we established a read-out system for the assessment of the clinically highly relevant mechanical integrity of the thoracic aorta between heterozygous and wild-type mice. Moreover, we measured the effect of doxycycline on the mechanical integrity of the aorta as proof-of-principle test.

**Methods:** 1.5-mm-long sections of the ascending and descending murine thoracic aorta were mounted on a tissue puller (Danish Myo Technology) and uniaxially stretched until rupture while recording the tensile force (in mN). Furthermore, electron and multiphoton microscopy was used to assess collagen fiber microstructure and collagen distribution, respectively.

**Results:** The rupture force was significantly lower in heterozygous compared to wild-type mice and decreased with increasing distance from the heart. In samples from heterozygous mice, electron microscopy revealed higher variability in the diameters of collagen fibrils and multiphoton microscopy indicated reduced collagen volume and density, explaining the decrease in rupture force. Moreover, we showed that doxycycline increased the rupture force of the thoracic aorta in heterozygous mice to the level of wild-type, confirming the postulated beneficial effect of this drug.

**Conclusions:** Our novel read-out system is suitable for detecting significant differences in the rupture force of the murine thoracic aorta allowing the assessment of the effect of sequence variants in mouse models and evaluating the effect of candidate drugs on the mechanical integrity of the aorta.

**P.14****HIGH-THROUGHPUT METHODS TO INTERPRET GENETIC VARIANTS OF UNCERTAIN SIGNIFICANCE IN GENES CAUSING MARFAN SYNDROME AND RELATED DISORDERS**

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**Objectives:** Variants of uncertain significance are commonly identified during clinical testing for disorders associated with aortic aneurysms. Functional data are needed to provide evidence of pathogenicity, but individually testing the effects of every possible gene variant is cost-prohibitive. New methods to systematically evaluate the functional effect of every possible gene variant are needed to improve diagnostic accuracy.

**Methods:** We developed a low-cost method to create libraries of molecules, each containing only one single nucleotide variant. A library of every possible variant in exon 47 of COL3A1 (Ehlers-Danlos syndrome type IV) was created and introduced into the endogenous COL3A1 gene in U2OS cells using CRISPR-Cas9. Single-cell fluorescence assays were used to detect misfolded and intracellularly retained COL3A1. Engineered cells containing variants were sorted using flow cytometry, and captured for sequencing to identify variants in both high- and low-fluorescent cell populations.

**Results:** Quantitative effects of more than 450 variants in COL3A1 exon 47 were determined in a single experiment. As expected, single nucleotide variants causing glycine substitutions were enriched in cells with high compared to low fluorescence. In addition, COL3A1 variants predicted to be benign and present in the ExAC browser had no effect. However, some non-glycine substitutions were enriched in cells with high fluorescence, suggesting effects on COL3A1 trafficking.

**Conclusions:** High-throughput methods can be used to quantitatively and comprehensively determine functional effects of disease gene variants, and are being developed to improve diagnosis of Marfan syndrome and related disorders.

**P.15****CHANCES AND CHALLENGES OF HIGH-THROUGHPUT SEQUENCING IN GENETIC TESTING OF MARFAN SYNDROME AND RELATED DISORDERS**

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**Objectives:** High-throughput sequencing (HTS) is widely used for clinical applications such as the molecular diagnosis of Mendelian disorders like Marfan syndrome (MFS) and related disorders. As the applied technology/workflow substantially affects the diagnostic yield, knowledge about the pitfalls and advantages of HTS technologies and analysis pipelines is crucial for the successful application of hitherto unprecedented large-scale genetic testing.

**Methods:** Based on our experience from 420 Swiss whole genomes (WGS, 60× PE150), we address the chances and challenges of HTS in molecular diagnosis of MFS and related disorders. Furthermore, we assess sensitivity/recall, precision, computation time, and disk footprint of four corresponding HTS analysis pipelines.

**Results:** We exemplify the limitations of targeted (gene panel) and whole-exome sequencing (WES) and the potential of WGS in the detection of single nucleotide variants (SNVs) and copy number variations (CNVs). In addition, we elucidate limitations of short-read HTS on exemplary cases including the influence of homologous/repetitive regions (mappability <1) on variant calling and the impact of sequence composition on read depth, as well as show differences in the performance of whole-genome analysis pipelines.

**Conclusions:** We recommend to select the HTS method with care and to combine more than one independent bioinformatic pipeline for the most comprehensive analysis. The use of PCR-free WGS (>60×) instead of WES or panels and the inclusion of CNV analysis can contribute to increased diagnostic yield in molecular diagnosis with lifetime value. As long-read HTS may overcome limitations of short-read HTS, it is envisioned as the future of sequencing.



**P.16****PHENOTYPE OF HOMOZYGOUS FIBRILLIN-1 (*FBN1*) MUTANT PIGS**

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Fibrillin-1 (*FBN1*) is the causative gene for Marfan syndrome (MFS). In this study, the phenotype of pigs homozygous for a mutation in *FBN1* was investigated. The founder cloned pigs carrying a heterozygous *FBN1* mutation (+/Glu433AsnfsX98) were originally generated using zinc finger nucleases (Umeyama *et al.*, 2016). Crossing heterozygous *FBN1* mutant (male) founders and wild-type (WT) gilts resulted in heterozygous *FBN1* mutant offspring. Male and female heterozygotes were then crossed to produce homozygous *FBN1* mutant pigs.

Out of the total 25 piglets obtained in four litters by crossing heterozygous females with heterozygous males, 4 were WT, 16 were heterozygous and 5 were homozygous for the mutation. Transmission of the *FBN1* mutant trait followed the classical Mendelian pattern of inheritance. Homozygous mutant pigs exhibited symptoms typical of MFS, such as fragmentation of elastic fibers of ascending aorta, aortic dissection, ectopia lentis, and lipodystrophy. Homozygosity for the mutation proved to be a neonatal lethal condition, with the longest survival duration of 28 days, similar to MFS mouse models. In addition, *FBN1* mRNA was degraded in the fibroblast cultures established from the pigs, possibly due to nonsense-mediated mRNA decay.

In conclusion, the homozygous *FBN1* mutant pigs were found to manifest phenotypes similar to MFS patients, such as lesions, owing to the degradation of *FBN1* mRNA.

**P.17****A NOVEL LARGE ANIMAL DISEASE MODEL OF MARFAN SYNDROME: *FBN1* EDITED PIGS**

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Suitable animal models are essential for translational research and efficient study of MFS treatments. Studies of *FBN1* mutant mice have provided valuable information regarding causes but have proven inadequate for surgical interventions to address cardiovascular or skeletal system manifestations. A cattle model arising from spontaneous mutation has been described resulting in a condition that shares many of the clinical and pathological manifestations of human MFS.

Precise and efficient genome editing techniques enable the generation of tailored, bespoke models for MFS. Such techniques were applied in pigs to take advantage of short generation times and the physiological and anatomical similarities between the pigs and humans.

In this study, we generated heterozygous *FBN1* edited cloned pigs and their progeny and showed that they developed phenotypes resembling those of patients with MFS, such as scoliosis, *pectus excavatum*, delayed mineralization of the epiphysis and disrupted structure of elastic fibres of the aortic medial tissue.

These findings indicate the value and utility of *FBN1* edited pigs as a routinely generated 'on demand' model for deployment in studies for further understanding the pathogenesis of MFS and in developing treatments. The pathology of the edited pigs is described in this paper.

**P.18****USING ZEBRAFISH MODELS TO IMPROVE THE TREATMENT OF MARFAN SYNDROME**

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**Objectives:** Major challenges still exist for the management of patients with Marfan syndrome (MFS). On one hand there is a need for reliable risk estimation based on genomic patient data, and on the other hand there is a lack of disease-specific treatments besides symptomatic care. We set out to develop a zebrafish model for MFS to address these problems using an innovative approach.

**Methods:** We used both morpholino-based knockdown as well as targeted CRISPR/Cas9-induced indel mutation to disrupt gene function in zebrafish. For cardiovascular phenotyping of zebrafish embryos and larvae, we performed experiments in the *Tg(kdrl:GFP)* transgenic line, which expresses GFP specifically in endothelial cells, to enable efficient visualization of blood vessels *in vivo*.

**Results:** We first characterized the zebrafish fibrillin-1 ortholog, *fbn1*, on a genomic and transcriptional level. Based on newly obtained genetic sequence data, we generated novel zebrafish lines with independent mutations in four different *fbn1* loci. We also tested the effects of two distinct *fbn1*-specific morpholino oligonucleotides. Preliminary phenotyping showed that *fbn1* gene disruption in zebrafish embryos and larvae led to vascular defects, although variability was observed between individuals and between different genetic approaches.

**Conclusions:** We have generated new *fbn1* mutant zebrafish lines, which we are using to study the molecular pathogenesis of MFS. We plan to exploit the observed phenotypic variability to identify potential modifiers of the cardiovascular phenotype. Finally, we intend to use these zebrafish in a future *in vivo* chemical compound screen, using appropriate physiological or biochemical readouts to identify potential new treatment options.

**P.19****EVALUATION OF GENOTYPE-PHENOTYPE CORRELATIONS IN MARFAN-SYNDROME FOR PREDICTING THE SEVERITY OF CARDIOVASCULAR MANIFESTATION**

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**Objectives:** In Marfan syndrome (MFS), the most important, life-threatening phenomenon is aortic dissection, therefore identification and follow up of aortic dilatation, or a potential prophylactic surgery is essential. In most cases a mutation of the *FBN1* gene on chromosome 15 is responsible for the disease, leading to the reduction (haploinsufficiency=HI) or to abnormal structure (dominant negative type mutation=DN) of the fibrillin-1 protein. Our aim was to examine the correlations between the severity of the cardiovascular (CV) involvement and the genetic variations.

**Methods:** We analysed the relation between CV symptoms and genetic variations in the *FBN1* gene in case of 35 clinically proved MFS patients. Phenotypic evaluation was carried out according to the revised Ghent nosology, while molecular genetic analysis was performed using Next Generation Sequencing and Sanger sequencing techniques.

**Results:** Pathogenic mutations were identified in 20 out of 35 cases (57%) among them 14 mutations were previously not described in international mutation databases. Nine of the identified mutations are DN-type (missense) and 11 are HI-type (5 nonsense, 2 frameshift, 3 splice and 1 large deletion, affecting exons 2-4). There was no significant difference between major CV symptoms (aortic dilatation and/or dissection) in patient cohort with or without mutations ( $p=0,13$ ). There was no difference between the effect of DN and HI mutations on the major CV traits ( $p=0,07$ ), but among patients with non-cysteine missense mutations CV symptoms occurred with significantly lower probability ( $p=0,0035$ ).

**Conclusion:** Identification of disease-causing mutations in *FBN1* gene in MFS patients can improve the accuracy of the risk estimation of aortic disorders and planning prophylactic aortic root reconstruction surgeries.

**P.20****ECTOPIA LENTIS COMPLICATING THORACIC AORTIC ANEURYSM DISEASE: NOT ALWAYS MARFAN SYNDROME**

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**OBJECTIVES:** In patients with hereditary thoracic aortic aneurysm disease (H-TAD), ectopia lentis (EL) has been unique to Marfan syndrome (MFS). We report 2 cases of H-TAD and EL, one with a mutation in *TGFB2* and one with a homozygous *ADAMTSL4* mutation.

**METHODS:** Clinical case review.

**RESULTS:** A 43 year old woman was diagnosed with LDS4 due to a pathogenic variant in *TGFB2* (c.904C>T; p.Arg302Cys). Her mother had aortic dissection and other relatives with *TGFB2* mutation have TAA and cerebral aneurysm. She has a pectus carinatum, kyphosis, greyish sclera, translucent skin, and no hypertelorism or arachnodactyly. Imaging demonstrated basilar artery tortuosity and an aortic root of 3.6 cm. Ocular exam revealed bilateral posterior subcapsular polar cataracts and superonasal EL. No relatives have EL. A homocysteine level was mildly elevated (30 µmol/l). Whole exome sequencing (WES) is pending to determine if another etiology of EL is present.

A 48 year old man was previously diagnosed with MFS based on EL, mild skeletal features and TAA. His maternal GM's family has TAA disease. He has a 4.3 cm TAA, downsloping palpebral fissures, myopia, flat feet, and striae, but no arachnodactyly. *FBN1* testing was negative. WES revealed a homozygous pathogenic mutation in *ADAMTSL4* (c767\_786del20; p.Q256PfsX38).

**CONCLUSIONS:** Genetic testing is to be considered in H-TAD with EL to confirm *FBN1* mutation or alternative explanation, especially when the systemic score is low or LDS phenotype is present. Whether mild homocystinemia influenced EL or this represents a new ocular finding in *TGFB2* disease is being explored.

**P.21****3-YEAR RETROSPECTIVE ANALYSIS OF NGS IN GENETIC TESTING OF HERITABLE CONNECTIVE TISSUE DISORDERS (HCTDS) IN A NORTHERN IRELAND COHORT BETWEEN 2013-2016**

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HCTDs are a heterogeneous range of conditions with clinical overlap that include Marfan, Loeys-Dietz and Ehlers-Danlos syndromes, and Familial Thoracic Aortic Aneurysm and Dissection (FTAAD). The allelic and locus heterogeneity of HCTDs has limited the utility of genetic testing for these disorders due to the low clinical sensitivity, and high costs of single gene testing. Use of NGS targeted disease gene panels have enabled a genetic diagnosis to support and confirm a clinical diagnosis of these conditions.

A targeted NGS gene panel was developed and validated for routine diagnostic screening of the major genes implicated in causing these HCTDs (*ACTA2*, *FBN1*, *TGFBR1*, *TGFBR2*, *MYH11*, *SMAD3* and *COL3A1*).

Of 208 patients referred to Northern Ireland Regional Genetics Service (NIRGS) for screens to date, reportable variants were detected in 61 patients (29.3%, 45.9% were females). 175 variants were identified, 17.9% being assessed as either pathogenic or likely pathogenic. *MYH11* is highly polymorphic, 71 variants, but none were assessed as pathogenic. *FBN1* gene variants accounted for 93.3% of pathogenic and 62.5% of likely pathogenic variants and likely pathogenic variants were found in each of the other genes. Of the 64 variants reported 74.2% were missense, 19.4% frameshifts and 6.4% splice variants.

Cascade testing of 88 family members identified an additional 24 individuals at risk of developing a CTD. *De novo* variants were confirmed in 10 cases with one confirmed case of somatic and germline mosaicism.

NGS has enabled the introduction of a routine molecular diagnostic test for screening the main genes implicated in HCTDs and development of testing criteria preforms/pathway for the NIRGS.

## P.22

### SEQUENCING OF 30 CANDIDATE GENES IN A CZECH COHORT OF BICUSPID AORTIC VALVE (BAV) PATIENTS PROVES GENETIC HETEROGENEITY AND INCREASED DETECTION OF VARIANTS IN FAMILIAL CASES

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**Objectives:** Bicuspid aortic valve (BAV) is the most common congenital heart defect. Its presentation ranges from Mendelian forms to sporadic occurrence. In this study, we aimed to phenotypically and molecularly characterize a representative Czech BAV cohort.

**Methods:** From 386 BAV index patients seeking genetic counseling, we selected 53 probands with either BAV associated with thoracic aortic aneurysm (TAA > 50 mm) or a family history (1<sup>st</sup> or 2<sup>nd</sup> degree) for BAV and performed next-generation sequencing by a panel comprising 30 BAV/TAA candidate genes. All variants were confirmed by Sanger sequencing and segregation analysis was performed where possible.

**Results:** Overall, a positive family history of valve disease or documented aortic aneurysm/dissection was found in 162/386 cases (42%), of which 39 (10%) had confirmed BAV in ≥ 2 family members. A potentially causative DNA variant (class III or IV) was found in 9/53 (17%) patients of the sequenced cohort, while in familial cases the detection rate increased up to 26% (6/23). The most commonly mutated genes were *NOTCH1* (n=3), *FBN2* (n=2), *FBN1* (n=2) and a *COL5A2* and an *EMILIN1* variant were identified in 1 individual each. In the two *FBN2* families, the respective variants segregated with the disease.

**Conclusions:** A positive family history was detected in 42% of studied patients, while familial BAV was found in ~10% of cases through family cascade screening. There is a higher yield of genetic variants in familial cases of BAV. We are currently establishing population frequencies of variants in candidate genes in the Czechs and plan to carry out whole exome sequencing in familial cases who remain negative after candidate gene testing.

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**P.23****AORTOPATHY IN SOTOS SYNDROME**

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**Objectives:** Sotos syndrome is an autosomal dominant disorder caused by mutations in the *NSD1* gene. Overgrowth, macrocephaly, variable learning disability and characteristic facial features are near universal findings, while evidence of mild connective tissue dysfunction can be seen in a minority. Recent literature has described four cases with novel truncating *NSD1* mutations and severe connective tissue laxity, in which three demonstrated aortic dilatation - a novel association for Sotos syndrome.

**Methods:** We describe the clinical and cardiovascular features of four additional Sotos patients with pathogenic *NSD1* mutations, including a father-daughter pair. Ages range from 6 to 56 years.

**Results:** Thoracic aortic dilatation was detected in all patients. Presentations ranged from mild, stable enlargement to severe aneurysmal root dilatation requiring surgery. One patient had markedly tortuous internal carotid arteries and possible evidence of a femoral artery rupture. Additional findings include joint laxity and increased skin elasticity.

**Conclusions:** A subset of individuals with Sotos syndrome appear to be at risk of aortopathy and connective tissue dysfunction. As such, consideration of continued cardiovascular screening into adulthood may be warranted, as is further study to assess both prevalence and natural history.



**P.24****ALTERED MYOGENIC DIFFERENTIATION IN CELLS FROM PATIENTS WITH A *TGFBR1* MUTATION FROM A FAMILY WITH THORACIC AORTIC ANEURYSMS AND DISSECTIONS**

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**Objective:** Investigation of pathogenicity of a mutation in *TGFBR1* in a large family with familial thoracic aortic aneurysms and dissections (TAAD).

**Methods:** Sanger sequencing was used to confirm the mutation. Pathogenicity was determined using the Alamut® Visual 2.10 program. A novel method of growth factor-based myogenic transdifferentiation was used to examine the myogenic potential of patient-derived fibroblasts. The expression pattern of phosphorylated SMAD3 in patient aortic sections was analysed by immunohistochemistry whereas western blotting was used to analyse SMAD3 phosphorylation in cultured fibroblasts.

**Results:** The c.1043G>A (p. Cys348Tyr) mutation in the *TGFBR1* gene was identified in seven affected members of the family and was predicted to have a damaging effect in the highly conserved kinase domain. Myogenic transdifferentiation was found to be increased in fibroblasts from two affected family members as shown by the higher expression of *CNN1* (p<0.05), *ACTA2* and *SM22* compared to healthy control fibroblasts. Increased phosphorylated SMAD3 was observed in smooth muscle cells in the media section of aortic tissue in two patients. Stimulation of serum-starved fibroblasts with TGF-β1 from two patients did not show a gain-of-function effect indicating that increased phosphorylated SMAD3 in aorta is potentially due to additional factors.

**Conclusions:** We report the c.1043G>A *TGFBR1* mutation as causative for TAAD. Given the fundamental role of excessive SMAD2/3 signalling in aneurysm pathology and the key function of TGF-β/SMAD2/3 signaling as driver of smooth muscle cell differentiation, our findings indicate that the mutation may exert its pathogenic effect by perturbation of myogenic differentiation.

**P.25****THE CARDIOVASCULAR ASPECT OF PATIENTS WITH MARFAN SYNDROME IN MANITOBA, CANADA**

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**Background:** Marfan syndrome (MFS) is a heritable, multisystem connective tissue disorder with autosomal dominant transmission, resulting from a mutation of FBN1. We review our patients with confirmed or probable MFS diagnosis based on available genetic testing, clinical findings and family history (FH).

**Methods:** Retrospective chart review for children diagnosed with MFS, followed at the Variety Heart Center, Children's Hospital of Winnipeg MB, Canada.

**Results:** 7 patients (5 males) aged between 0-18 years (median age 10.5 years) with genetically confirmed (2, 1 de novo mutation) or probable (5) MFS are enrolled. Aortic root dilation (Z-score > 2) is found in 5 and Mitral Valve Prolapse in 4 (isolated finding in 2). 4 out of 5 patients with documented aortopathy are covered by medical treatment. One patient with probable MFS (skeletal and cardiovascular but not ocular criteria, FH including sudden death) and aortopathy detected in utero, required prophylactic surgery for severe aortic root dilation. Bicuspid aortic valve was identified in him and his affected mother. Extensive mutation analysis for connective tissue disorders was negative for both. Two families refused genetic testing for their affected members.

**Conclusion:** Age depended penetrance, phenotypical variability observed even among affected members of the same family[1], and life-threatening cardiovascular complications for the MFS patients, justify an accurate diagnosis based on clinical criteria[2] and/or molecular analysis. Properly managed MFS patients with adequate investigations, detailed FH and regular follow up in combination with prophylactic medical and surgical treatment can deliver an excellent prognosis.

1. Loeys, B., et al., Genotype and phenotype analysis of 171 patients referred for molecular study of the fibrillin-1 gene FBN1 because of suspected Marfan syndrome. Arch Intern Med, 2001. 161(20): p. 2447-54.

2. Loeys, B.L., et al., The revised Ghent nosology for the Marfan syndrome. J Med Genet, 2010. 47(7): p. 476-85.

**P.26****CASE REPORT OF MISTAKEN IDENTITY: A FAMILY WITH A COL1A1 VARIANT MASQUERADING**

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We report a family with skeletal anomalies and aortic dissection. They were ascertained after the proband had an acute dissection at age 50. Family screening identified 2/3 children with skeletal involvement: severe scoliosis and Marfanoid habitus. Cardiac features range from sudden death at age 51 secondary to type A dissection with minimal dilation, survivable dissections and stable dilation. Genetic testing performed in 1994 identified a COL1A1 c.3331C>T (p.Arg1111Cys) variant. The connective tissue and cardiac features were attributed to COL1A1 cysteine substitution in the triple helix domain of the proa(I) chain. The proband's affected grandson was tested for the COL1A1 variant and it did not segregate with the phenotype. In 2015 a pathogenic mutation was identified in TGFB3 c.899G>A (p.Arg300Gln) that did segregate with the phenotype. We compare the clinical features in family members with both COL1A1 variant and TGFB3 mutation; TGFB3 mutation alone and in those with neither. Overall the phenotype fits in the spectrum of Loeys Dietz syndrome. There are no reported features consistent with osteogenesis imperfecta, nor Ehlers Danlos syndrome.

**P.27****MUTATION SPECTRUM IN THE FBN1 GENE IN THE RUSSIAN PATIENTS WITH MARFAN SYNDROME**

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**Objectives:** The assessment of the genetic polymorphism in the *FBN1* gene in patients with Marfan syndrome (MS) in the surgical center.

**Materials and methods:** DNA analysis was performed for 60 patients with the MS: 39 patients were fulfilled the Ghent criterias, for 17 patients the only inclusion criterion was an aneurysm or an aortic dissection, and 4 children diagnosed with neonatal form. Analysis of the coding exons and adjacent intron regions of the *FBN1* gene was performed by high-throughput sequencing by Ion Torrent PGM. The all clinically important findings was verified by Sanger sequencing.

**Results:** We found 43 genetic variants with the pathogenic or potentially pathogenic clinical significance. There were 24 missense variants, 14 nonsense variants and 5 frameshift or splicing changes. In the group of patients with the classic form of MS was founded 35 variants (89,7%), in the neonatal group – 3 described missense variants in the 25-27 exons. Among the patients who were not fulfilled the Ghent criteria's, the pathogenic substitutions detected in 5 patients (29,4%). In our research, only two variants were repeated in several unrelated families (p.N2144S in 2 probands and p.R2776\* in 3 patients).

**Discussion:** The results of our studies in Russian patients corresponded with the international data.

**P.28****CASE REPORT: A COMPLEX DELETION INSERTION IN *FLNA* CAUSES A LOEYS-DIETZ-LIKE PHENOTYPE**

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**Objectives:** Loeys-Dietz syndrome (LDS) is characterized by vascular and skeletal manifestations resembling Marfan syndrome. Its most typical presentations consist of thoracic aortic aneurysms (TAA), tortuosity and aneurysms throughout the arterial tree, bifid uvula, hypertelorism and a high risk of aortic dissection. Here we present a one-year old girl with LDS-like manifestations including: TAA (z-score 3), bifid uvula, hypertelorism, a narrow high arched palate, blue sclera, translucent skin, easy bruising, scoliosis, hypotonia, extreme laxity of the joints, gross-motor developmental delay and severe feeding problems. Her mother and her sister have TAA, while the maternal aunt had a dissected ascending aorta. The pedigree shows a paucity of males.

**Methods:** DNA from the proband and her mother was analyzed with a next generation sequencing panel containing 30 TAA genes. Both sequence and copy number variant analysis was applied. A breakpoint overspanning PCR product was analyzed by Sanger sequencing.

**Results:** We found a heterozygous c.1567+16\_2280+186delins317 complex mutation in the *FLNA* gene in both mother and daughter, expected to lead to nonsense-mediated mRNA decay. In addition, we identified a variant of uncertain significance in *TGFB3* (c.454C>T; p.(Arg152Trp)) in the mother. An MRI head scan showed areas of cortical heterotopia in both mother and daughter. Segregation analysis for both variants is ongoing.

**Conclusion:** Clinical manifestations of *FLNA* mutation carriers show a significant overlap with those of LDS patients. *FLNA* has been associated with TAA but, so far, not with dissection. Segregation analysis will determine whether the *TGFB3* or the *FLNA* variant is responsible for dissection.

**P.29****AORTIC ANEURYSM: AN UNDERESTIMATED SERIOUS FINDING IN THE *EP300* MUTATION PHENOTYPICAL SPECTRUM**

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**Objectives:** A 12.5-years old boy presented with failure to thrive, intellectual disability, autism, psychiatric disease, Rubinstein-Taybi facial dysmorphism, unilateral iris coloboma and 3-4 syndactyly of hands and feet. He has a bicuspid aortic valve (BAV) and underwent a valve-sparing Hemashield graft surgery because of progressive ascending aortic dilatation (Z-score 9, just prior to surgery). We aimed to identify his genetic disease cause and, subsequently, determine if BAV and aortic aneurysm are frequent findings in *EP300*-related disease.

**Methods:** Exome sequencing was performed on gDNA of the proband and unaffected parents. The resulting data were filtered for protein-altering *de novo* variants with a minor allele frequency below 0.1% in the ExAC population. Selected variants were validated using Sanger sequencing. Next, the incidence of BAV and aortic aneurysm in *EP300* mutation carriers was determined through a literature search.

**Results:** A novel *de novo EP300* frameshift mutation (c.3092\_3093delinsG; p.Ser1031Argfs\*28) was identified in the proband. Combination of this finding with the *EP300* cases reported by Costain et al and Fergelot et al revealed an incidence for (most commonly ascending) aortic aneurysm of 8% (4/51). Two patients presented aortopathy at birth, while aneurysm was only detected later in life in the two others. No prior evidence for BAV was found.

**Conclusions:** Aortic aneurysm, but not BAV, is a recurrent finding in *EP300* mutation carriers. Continued surveillance of the aorta throughout life in *EP300* cases is warranted. Moreover, CT or MRI imaging might be necessary if echocardiography does not allow proper visualization of the ascending aorta.

**P.30*****FBN1* mutation type did not affect aortic events in Marfan syndrome patients**

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**Objectives:** Marfan syndrome (MFS) is a systemic connective tissue disorder, which exhibits high clinical heterogeneity. Baudhuin et al. observed an increased frequency of *FBN1* truncating and splicing variants in MFS patients with aortic events, but the number of patients with aortic events in their cohort was limited. Here, we tried to explore the correlation between *FBN1* mutation type and aortic events in a larger MFS cohort with aortic events.

**Methods:** Genotype and phenotype information from 227 patients with a rare *FBN1* mutation were assessed. The phenotype information of aortic events mainly included the aortic disease type and severity, such as mild aortic dilation, aortic aneurysm, aortic dissection, and severe valvular disease.

**Results:** There were comparable proportion of patients who suffered an aortic event (n=173) between patients with a *FBN1* truncating/splicing variant and those with a missense one (50.3% vs. 49.7%). It was similar in the patient cohort without an aortic event (n=54, 55.6% vs. 44.4%). There was only a trend toward a higher frequency of life-threatening aortic dissection and severe valvular disease (51.2% vs. 40.0%, not significant) in patients with a truncating/splicing variant (n=117) versus those with a missense variant (n=110).

**Conclusions:** *FBN1* mutation type did not affect aortic events in MFS patients, which was not consistent with Baudhuin's observation, probably because of an incoordinated proportion of patients with or without aortic events, which needed further study to definite the correlation between *FBN1* mutation type and aortic events.

## P.31

## THE 101 GENOMES MARFAN PROJECT OF THE 101 GENOMES FOUNDATION

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**OBJECTIVES:** The 101 Genome Foundation (F101G) aims to create a **bioinformatics tool** containing the **genomic and phenotypic cross data** of patients with **rare diseases** (like Heritable Thoracic Aortic Disease (HTAD)). This tool, accessible to scientists through a **secure computer platform**, will allow researchers to reach a better comprehension of variable phenotypic expressivity in rare diseases. Moreover, it provides the opportunity to identify the existence of possible modifier genes that protect against (or aggravate) the major damage caused by the primary mutation in rare diseases. Identification of possible **protective modifier genes** could allow the development of treatments that replicate their protective effects in patients whose genes are not activated in the same way.

**METHOD:** The 101 Marfan Genome Project (P101GM) is the pilot project of the F101G and is dedicated to **Marfan syndrome (MFS)**. It is built on an **expandable starting cohort of 101 patients** with MFS. The P101GM will initially focus on **cardiovascular manifestations**, but is extendable in a later stage to other MFS related manifestations. Individuals harboring the same recurrent *FBN1* mutations but with variation in their cardiovascular phenotype are chosen for the composition of the initial cohort, after which the patients at the extreme ends of the phenotypical spectrum will be selected for further analysis. **WGS** data will be generated from the selected participants and stored in a secure computer platform.

**CONCLUSION:** The platform will allow researchers to better understand the clinical variability in MFS and identifying possible protective modifier genes that prevent cardiovascular manifestations caused by MFS.



**P.32****DETECTION OF CARDIOMYOPATHY IN CHILDREN WITH MARFAN SYNDROME WITH 2D STRAIN ECHOCARDIOGRAPHY**

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**Introduction:** Studies have found cardiomyopathy in adult patients with marfan syndrome (MS) with systolic dysfunction. Sophisticated techniques such as 2D strain echocardiography or "speckle tracking imaging" (STI) have been reported to detect early cardiac dysfunction and to be more accurately. The aim of our study was to evaluate the validity of STI for the study of myocardial function in children with MS, and to assess the interest for cardiomyopathy detection in this population.

**Methods and Results:** Echocardiographic parameters of left ventricular systolic function were compared between two populations in standard 2D, STI and cardiac MRI. We included 39 MS (clinically confirmed from the 2010 modified criteria of Ghent or genetically with mutations on the FBN1 gene) and 41 healthy children, aged 4 to 18 years old respectively on average  $12.07 \pm 4.81$  and  $11.82 \pm 3.64$ . They were paired on the body surface. The values of global strain longitudinal peak (SLG) of MS were compared with values of ejection fraction of the left ventricle (LVEF) assessed by cardiac MRI (gold standard). Relations between the two types of mutations (PTC and inframe) in SM and altered strain were studied. Impaired SLG was significant for 7 segments : basal inferoseptal ( $p = 0.0001$ ), mid anterolateral ( $p = 0.0083$ ), basal anterolateral ( $p < 0.0001$ ), basal inferior ( $p = 0.0057$ ), apical lateral ( $p = 0.0084$ ), basal anterior ( $p < 0.0001$ ) and basal inferolateral ( $p = 0.0103$ ). This segmental impairment predominated the basal level with apex-to-base gradient. Patients with more altered SLG appeared to have a greater dilatation of the ascending aorta ( $p = 0.03$ ).

**Conclusion:** This study is the largest pediatric cohort who compare 2D strain echocardiography and MRI in children with SM. The interest for primary or secondary cardiomyopathy detection remains to be validated and consolidated with larger studies and could justify not only to focus on the aorta.

**P.33****INVESTIGATION OF ASSOCIATION BETWEEN AORTIC DIMENSIONS, BIOPHYSICAL PROPERTIES, AND PLASMA BIOMARKERS IN CHILDREN AND ADULTS WITH MARFAN SYNDROME**

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**Objectives:** In this study we have investigated the association between aortic biophysical properties and/or biomarkers such as circulating transforming growth factor beta-1 (TGF-β1) and matrix metalloproteinase (MMP)-2/-9 with aortic anatomical changes in children and adults with MFS that could impact clinical care.

**Methods:** We randomized 43 MFS and 86 age/sex-matched controls, and subjected them to echocardiographic assessment of the biophysical properties of the aorta including pulse wave velocity (PWV), arterial stiffness, total compliance, and aortic root size. Plasma biomarkers including TGF-β1, MMP-2/-9 were measured. Flow-mediated dilation (FMD) was measured to determine the endothelial function.

**Results:** Aortic diameters were larger in MFS; PWV was increased in children with MFS compared to controls (443±86 vs. 379±69cm/s), but not in adult patients. PWV correlated with age in both controls and patients with MFS, but correlated with aortic root dimensions (r=0.42) only in controls. Only MMP-2 measurements were higher in MFS compared to controls (202.2±54.5 vs 174.4±24.7). There was no difference in aortic root dimensions/central aortic function in MFS patients on medical therapy (either ARB or Beta blocker) compared to non-treated subjects. An age related decline in FMD in the young patients under 40 was observed.

**Conclusions:** In MFS, central aortic function was stiffer, independent of aortic root size. Our study confirms elevation of MMP-2, with no difference in MMP-9 or TGF-β1. These findings suggest that i) evaluation of aortic vascular function does not provide additional insight to assist clinical management than aortic size, and ii) therapies that inhibit MMP-2 warrant further evaluation in MFS.

**P.34****8-COLOUR MULTIPLEX IMMUNOHISTOCHEMISTRY FOR DEEP PHENOTYPING OF THE IMMUNOLOGICAL RESPONSE IN HUMAN AORTOPATHIES**

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**Objectives:** *In situ* phenotyping of cells to gain insight into pathophysiological mechanisms, and to enable development of new diagnostic, prognostic, and medical strategies.

**Methods:** Human thoracic ascending aortic aneurysm (TAAA) and Stanford type-A aortic dissection (AD) tissue samples and clinical data were collected from patients with a preventive ascending aortic replacement or acute AD respectively. To identify cells of the immune system within tissue samples, two 8-color multiplex panels staining relevant players of the adaptive and innate immune system on a single slice per panel, were developed. Multispectral images of the samples were acquired with the Vectra automated multispectral imaging system and were subsequently unmixed and processed with inForm software. With in-house developed processing software, cells can be quantified (excess of 100.000 cells); automation of the total system enabled analysis of specific locations within the tissue. Additionally, since tissue integrity is maintained with this technique, spatial analysis, including localization, penetration, clustering, and nearest-neighbour analysis, is possible.

**Results:** With the adaptive immune cell panel, B and T-lymphocytes, and their interaction with dendritic cells, could be identified. The panel designed to classify the innate immune cells could assess MMP9 production by cells and macrophages polarisation (M1/M2). Preliminary data indicates the accumulation of immune cells, polarised towards and inflammatory phenotype, in dissected specimens.

**Conclusion:** Here we show a powerful immunohistochemistry (IHC) method, capable of deep phenotyping a large number of cells numbers, while preserving the integrity of the original tissue. Detection of specific immune infiltrates could lead to a hypothesis-driven diagnostic marker for aortic disorders.

**P.35****VASCULAR DEFECTS OF THE RETINAL VASCULATURE IN A MOUSE MODEL OF MARFAN SYNDROME**

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**Objectives:** Marfan syndrome (MFS) is mostly caused by mutations in FBN1 gene, and associated with severe cardiovascular, skeletal and ocular defects. The gene encodes fibrillin1, an extracellular matrix protein that assemble into microfibrils which provide a scaffold for elastin deposition. The defective fibrillin microfibrils affects the integrity of elastic tissue such as the aortic wall, resulting in a progressive enlargement of the aortic diameter (aneurysm) and aortic dissection. Moreover, in the eye, microfibrils also hold the lens in dynamic suspension and lens dislocation is thus a common manifestation of the disease. Patients with MFS may also experience retinal detachment that lead to impaired vision. Because microfibrils are also present in microvessels, FBN1 mutation may affect the retinal vasculature as well. In the present study, we have explored the consequences of FBN1 mutation in the developing and in the mature retinal vasculature.

**Methods:** Retinal vascular alterations were investigated in the Fbn1C1039G/+ mouse model of MFS by confocal imaging performed on whole mounted retinas. Sprouting angiogenesis was analysed during the first week after birth. The adult retinal vasculature was studied in 1 year-old animals.

**Results:** Quantification of radial expansion of the vascular plexus revealed reduced developmental angiogenesis in MFS mice compared to WT littermates. In 1 year-old animals, we observed a significant enlargement of the retinal arterioles. Ongoing experiments aim at a better characterization of the retinal vascular phenotype of MFS mice, focusing on vessel mural cell coverage and extracellular matrix protein deposition.

**Conclusions:** Our data suggest that fibrillin1 is indispensable for normal retinal vasculature architecture. The alterations detected in MFS mouse retinas may influence microvascular functions or their response to changes in intraocular pressure, leading to further ocular complications.

## P.36

**“NOVEL HOMOZYGOUS *ADAMTSL4* MUTATION  
IN A LARGE CONSANGUINEOUS BRITISH ECTOPIA LENTIS (EL) FAMILY”**

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**Objectives:** Ectopia Lentis (EL) is clinically and genetically heterogeneous. The dominant form can arise through mutations in *FBN1* (Comeglio et al. 2007) and the recessive form in *ADAMTSL4* (Aragon et al. 2010). EL is characterized by the disruption of the zonular fibers. In time, the lens moves out of place causing lens subluxation.

The aim of this project is to study the genetic significance of a large British EL family.

**Methods:** The proband of a large consanguineous British family (9 members, 3 siblings affected) with isolated EL was bidirectional Sanger sequenced for mutations in the coding exons including intron/exon boundaries of *ADAMTSL4* gene. The proband did not fulfil the Ghent criteria for Marfan syndrome (MFS). Genome databases and *in-silico* tools were used to help with variant interpretation.

**Results:** The novel mutation [ENST00000369039: c.1234G>A, p.(V412I)] was found to be of great significance for family segregation. This family was comprised of the proband (Sample3 [homozygous]), father (Sample1 [genetic carrier], mother (Sample2 [genetic carrier]), two sisters (Sample4 and Sample5 [both genetic carriers]) and 4 brothers (Sample6 [wild type], Sample7, Sample8 and Sample9 [last 3 homozygous]). All family members with the homozygous mutation c.1234G>A were diagnosed with EL except sample8 who was diagnosed with eye problems but was never screened for EL by the ophthalmologist.

**Conclusions:** This study supports the evidence that homozygous mutations in *ADAMTSL4* cause autosomal recessive EL. Based on the location of this novel mutation it is imperative to rule out the possible causative interference with the splice site.

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## P.37

### DISRUPTION OF THE ELASTIC FIBERS IN THE OCULAR SYSTEM OF MOUSE MODEL FOR MARFAN SYNDROME

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Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder that affects 1/3200 individuals. Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder that affects 1/3200 individuals. The disease is caused by mutations in the *fbn1* gene, which encodes fibrillin-1, one of the essential proteins of the elastic fibers. MFS patients present intra and inter familial clinical variability and the most frequent features include ocular, skeletal and cardiovascular manifestations. These abnormalities are caused by structural alterations that affect the biomechanical properties of the elastic fibers. In the ocular phenotype, the major manifestation is ectopia lentis. The current explanation of this phenotype is that mutations in fibrillin1 cause biomechanical weakness of ciliary zonules, which changes the suspension pattern of the lens. Given the complexity of MFS, mouse models are essential to the understanding of these manifestations. However, the current models do not describe ocular phenotype. In this study, our objective was to characterize the ocular morphology in C57BL/6 and 129/Sv mice from the  $mg\Delta^{LoxPneo}$  MFS model. Twenty male mice were used 5  $mg\Delta^{LoxPneo}$  and 5 wild type animals from each genetic background. Eyes were fixed in 4% paraformaldehyde in 0.1 M PBS solution, pH 7.4. Eye sections were analyzed by histologic techniques. In the MFS group, we observed lower density and disruption of the elastic fibres in the ciliary body. These results suggest that the  $mg\Delta^{LoxPneo}$  is the first mice model that shows an ocular phenotype of MFS and it may contribute for pathophysiological studies and research of new treatments.

**P.38****MUTATION IN FIBRILLIN-1 RESULTS IN JOINT DEGENERATION IN TIGHT SKIN (TSK) MICE**

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**Objective:** Skeletal manifestations of Marfan syndrome includes disproportionately long limbs, joint laxity, scoliosis and early onset osteoarthritis (OA; joint degeneration). Despite the prevalence of OA worldwide, there are currently no therapies to slow or prevent joint degeneration. TSK mice harbour a mutated Fibrillin-1 gene and show Marfan characteristics (skeletal overgrowth, lung emphysema, myocardial hypertrophy and kyphosis). The aim of this project is to define the level of joint degeneration in TSK mice for future use as a model.

**Methods:** Immunohistochemistry for Fibrillin-1 was performed in normal and OA mouse knees (spontaneous OA in Str/ort mouse model; trauma-induced OA). Knees and Spine from 35wk-old TSK and littermate control male mice were used for microCT and histology. Non-invasive mechanical trauma was induced at 7 and 9N loads in 10wk-old male TSK and WT knees and joints analysed by histology after 1 week.

**Results:** Fibrillin-1 was found in the pericellular matrix of chondrocytes in the growth plate and in the articular cartilage in healthy CBA mice. During OA development, Fibrillin-1 immunolabelling was decreased (spontaneous and trauma-induced OA). Increased OA severity (histology and microCT analyses) was seen in 35wk-old male TSK mice knees and spine. Trauma at both magnitudes of mechanical loads induced articular cartilage lesions in both WT and TSK mice, however these were more severe in TSK mice (preliminary data).

**Conclusion:** These data suggest that normal Fibrillin-1 expression and function may play an important role in joint homeostasis and that abnormal expression or mutations promote joint degeneration.

### SUCCESSFUL AORTIC ROOT RECONSTRUCTION WITH SEVERE LUNG DISEASE DUE TO CHEST DEFORMITY – CASE PRESENTATION

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Marfan syndrome comes often with chest deformities (pectus carinatum, excavatum, severe scoliosis). Due to these deformities - even after surgical correction - some patients suffer from depressed lung function.

Hereby we report a case of a 34-year-old Marfan patient with dilatation of the sinus Valsalva (49mm). Her pulmonary function tests showed severe lung function disturbances (vital capacity of 1l, FEV 1 of 23%). Patient lives with mobile oxygen since 13 years. In general such values of lung function would contraindicate an open heart operation. With the help of our pulmonary department we prepared the young lady for aortic root reconstruction. She trained herself with BIRD and pulmotrainer for two months before the operation. After surgery patient was extubated on the day of surgery, received non invasive pulmonary support for 4 weeks. Now patient is doing well at home.

In selected cases intensive pulmonary training can help Marfan patients with severe lung disease to survive open heart surgery. Of course team approach is inevitable in such cases to prepare the team and the patient for the operation.



**P.40****FROM AORTIC MORPHOLOGY TO DIAGNOSIS AND INDICATION FOR SURGERY**

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**Objectives:** Lack of evident phenotypic manifestations limits correct diagnosis of genetic diseases, thus timely adjustment of the threshold diameter for prophylactic aortic surgery is frequently missed. Aortic morphology could lead to a specific diagnosis and appropriate surgical indication.

**Methods:** Retrospective blinded review of prospectively collected data at the only cardiac surgery department of a geographically closed region of Switzerland.

**Results:** From 01/2001 to 12/2016, 117 patients, 87/117 (74%) males and 30 (26%) females, aged  $64 \pm 13$  years, were operated for spontaneous acute aortic dissection type Stanford A (AADA), of which 94 were analyzed. Risk factors were: dilatation of the ascending aorta in 91/94 (97%), arterial hypertension in 58/94 (62%), obesity 64/94 (68%), bicuspid aortic valve in 6/94 (6%). Mean diameter of the ascending aorta (AD) was  $51 \pm 8$  mm, in 70% of patients AD was  $< 55$  mm. Prevalently dilated was the post-junctional ascending aorta in 74/94 (78%) and aortic root in 20/94 (22%) patients. Obesity was present in 58/74 (78%) of prevalent post-junctional vs 6/20 (30%) of aortic root dilation. Hypertension in 49/74 (66%) and 9/20 (45%) respectively. Two patients with Marfan syndrome and 3 with previously unknown related disorders were identified due to prevalent aortic root dilation and no other risk factors.

**Conclusions:** Morphological criteria, such as root dilatation in Marfan syndrome, could identify occult genetic aortic disease. This could lead to preciser indication for prophylactic surgery and avoid aortic catastrophies. Larger multicenter studies are needed to confirm the validity of this approach.

**P.41****AORTIC CONTRACTION VERSUS THE WINDKESSEL MODEL**

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**Objective:** Genetic findings in familial aortic aneurysms suggest a role for aortic contraction. Causative mutations have been found in genes encoding proteins involved in smooth muscle cell (SMC) contraction or differentiation. However, interpretation of these findings is hampered by the fact that the aorta is still generally believed to act according to the “Windkessel model”, as described by Frank in 1899. This model assumes a narrow rest state of the aorta, which expands due to a pulse of blood from the heart, subsequently elastically resuming its narrow rest state. In this model, a causative role for errors in aortic contraction is impossible. The windkessel model completely ignores the fact that the aorta consists mainly of circularly organized SMC's, whose rest state is expanded. It has already been shown in 1982, in an elegant *in vivo* study by Mangel et al, that aortic contraction is neurologically and not mechanically coupled to the heartbeat. We propose that disturbed aortic contraction leads to reduced blood pressure, leading to activation of angiotensin II, which will lead to increased smad2/3 phosphorylation, disturbed SMC differentiation and eventually aneurysm formation.

**Methods:** We have used mathematical modelling to describe aortic function in terms of contraction, rather than elasticity.

**Results:** Our model predicts blood pressure profiles similar to the windkessel model.

**Conclusion:** The blood pressure in the aorta can be described by a model that involves contraction rather than elasticity. This may explain the role of mutations in genes involved in SMC contraction or differentiation.

**P.42****PREVALENCE OF DILATED CARDIOMYOPATHY IN THE RUSSIAN PATIENTS WITH MARFAN SYNDROME (MFS)**

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**Objective:** To evaluate the risk of surgical intervention for patients with MFS and cardiomegaly. Changes in fibrillin-1 leads to cardiomegaly due to a combination of a structural component of the ECM and volume overloading due to aortic or mitral valve regurgitation, and progressive aortic root dilatation

**Materials and methods:** We have performed clinical (Standard and 24-hours Holter ECG, EchoCG, Dopplerography, chest radiography) and genealogical examination of the 70 probands with MFS who planned an operation on the aorta, its branches or heart valves. All probands had met the Ghent's criteria for MFS. Affected relatives were found in 21 families. Mutational screening in the *FBN1*, *TGFBR1* genes was performed by PGM Ion Torrent followed Sanger sequencing.

**Results:** Cardiomegaly was found in 69 adult probands. All heart chambers enlargement, increased end-diastolic volume of the LV and reduced ejection fraction were found in 15 (21,4%) index cases. Three patients who had an ejection fraction of less than 30% and size of LV was greater than 8 cm were denied with surgical treatment due to a high risk of mortality during the operation. In 46 patients was detected 37 mutations in the *FBN1* gene, 1 mutation *TGFBR1* gene index cases.

**Conclusion:** The prevalence of DCM is very high in probands with MFS. The influence of this complication for the long-term prognosis in this clinical group might be underestimated. We suggest that mutations in the *FBN1* gene may play a direct causative role in cardiac remodeling in MFS patients. This work was supported by grant RNF №16-15-10421

**P.43****EPIDEMIOLOGY OF MARFAN SYNDROME IN A LARGE, INTEGRATED HEALTH CARE DELIVERY SYSTEM, 2006-2017**

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**OBJECTIVE:** To describe the incidence and prevalence of the Marfan syndrome (MFS) in a large, non-referral-based, integrated health care system.

**METHODS:** Using administrative databases that combine data from electronic health records and other health system source files into standardized data tables, we identified patients with a diagnosis of MFS in Kaiser Permanente Northern California, USA, an integrated healthcare delivery system caring for >4 million persons. Annual incidence was calculated by confirming the first year of MFS diagnosis based upon an encounter with a valid MFS ICD-10-CM code (ICD-10-CM codes Q87.4, Q87.40, Q87.41, Q87.410, Q87.418, Q87.42 and Q87.43, and older ICD-9-CM and internal codes mapped to these ICD-10-CM codes), and dividing the number of MFS patients by the total enrolled health plan membership for that year. Prevalence was calculated by dividing the total number of current health plan members with MFS on December 31, 2017 by the total health plan membership at that time.

**RESULTS:** For the 12 study years from 2006-2017, incidence of MFS averaged 1.73 cases per 100,000 [range from 0.81 to 2.32 cases per 100,000; median 1.61 cases per 100,000]. Prevalence of MFS in December 2017 was 11.4 cases per 100,000.

**CONCLUSIONS:** In a large, integrated health care system, identification of MFS patients via diagnosis codes yielded prevalence estimates similar to previously published reports. Future research will describe the baseline characteristics and clinical outcomes for the MFS population, validate the accuracy of diagnostic codes for MFS, and explore natural language processing methods for better identification of MFS patients.

**P.44****A HEART FOR FIBRILLIN:  
DISTRIBUTION- AND EXPRESSION-PATTERN IN ADULT WILD-TYPE MURINE MYOCARDIAL  
TISSUE**

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Fibrillins are major constituents of microfibrils, which are essential components of the extracellular matrix of connective tissues where they contribute to the tissue homeostasis. Although it is known that microfibrils are abundantly expressed in the left ventricle of the heart, limited data are available about the presence of microfibrils in the other parts of the myocardial tissue and whether there are age- or sex-related differences in the microfibrillar expression- and distribution-pattern. This basic knowledge is essential to better understand the impact of fibrillin-1 pathogenic variants on the myocardial tissue as seen in Marfan-related cardiomyopathy.

We performed histological analyses on wild-type male and female murine myocardial tissue collected at different time-points (1, 3 and 6 months). Fibrillin-1 and -2 immunofluorescent stainings were performed on serial cross-sections at the level of the apex, the mid-ventricles (left and right) and the atria. In addition, other myocardial matrix components such as collagen and elastin were also investigated.

Fibrillin-1 presented as thread-like fibrils in the apex, mid-ventricles and atria. The expression- and distribution-pattern differed between the investigated regions, but not between age groups or sexes. Collagen had a similar broad distribution pattern to that of fibrillin-1, whereas elastic fibres were primarily present in the atria and the vessels. In contrast to fibrillin-1, limited amounts of fibrillin-2 were observed.

Fibrillin-rich microfibrils contribute to the architecture of the myocardial tissue in a region-dependent manner in wild-type murine hearts. This knowledge is helpful in future studies evaluating the impact of fibrillin-1 pathogenic variants on the myocardial tissue.

**P.45****HOLISTIC CARE OF PAIN IN MARFAN SYNDROME**

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Objectives

To assemble advice regarding pain management from a team of Marfan syndrome care specialists.

Methods

Opinions and up to date management were contributed to textbook "Diagnosis and Management of Marfan Syndrome" ed. Child AH, Springer, 2016

Results

Specific pains associated with MFS include migraine headache, musculoskeletal pain, dural ectasia headache and back pain, and early arthritic pain such as in protrusio acetabuli. Usual pain medication should be supplemented by specific migraine preventative medications, NSAIDs, gabapentin, TENS machines, joint supports and back and chest braces, and exercises for joint pain, surgery for dural ectasia leakage, joint subluxations and dislocations, and for early arthritis of the hip.

Conclusions

An expert team of physicians and surgeons should be assembled to care for each Marfan syndrome patient's pain, as required. Pain may limit daily activities, sports choice, career choice, and age of retirement. Disability due to pain can severely impair the quality of life.

**P.46****ADULTS WITH VASCULAR EHLER-DANLOS, LOEYS DIETZ SYNDROME AND OTHER GENETIC AORTIC DISORDERS IN NORWAY: PSYCHOSOCIAL ASPECTS AND CHALLENGES IN PHYSICAL ACTIVITY**

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**Objectives**

To present an ongoing study on adults with genetic aortic disorders. There is a lack of knowledge on psychosocial aspects and everyday life in these conditions. One of the authors (GV) has previously conducted a similar quantitative study on adults with Marfan syndrome.

**Methods.**

Mixed methods design. 1. Focus group interviews with adult ( $\geq 18$ y) with verified vEDS, LDS and MFS and their adult family members. 2. A study specific questionnaire with several standardized instruments, measuring life satisfaction (SWLS, LISAT11), chronic pain (BPI, SNQ), fatigue (FFS) and psychological distress (HADS) will be sent to adults ( $\geq 18$ y) with these verified genetic aortic disorders (except MFS) registered at TRS National Resource Center for Rare Disorders in January 2018. The study is planned in collaboration with the Marfan association and the EDS association. A reference group is established.

**Results**

Some preliminary results from the quantitative part will be presented. Otherwise, the results are planned to be published in international journals, conferences and will also be implemented in follow up programs for persons with the conditions in Norway. Preliminary results from the qualitative part are presented in another abstract here.

**Conclusions**

Knowledge based on experiences from persons with these rare potentially life threatening conditions is important for the persons themselves, their families, the professionals they meet and those planning medical follow-up and rehabilitation programs. It is important to explore differences and similarities between the study's diagnostic groups.

**P.47****SYSTEMATIC REVIEW OF THE LITERATURE OF PAIN IN PATIENTS WITH MARFAN SYNDROME; PREVALENCE, LOCATIONS, IMPACT AND TREATMENT**

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**Objectives:** To explore the literature on pain in adults with MFS, to critically appraise and synthesize relevant literature.

**Methods:** A Systematic Review with systematic search in all relevant databases was published in 2016. A new search was done 15.01.2018. Only peer-reviewed papers are included. Specific validated criteria are used to critically appraise each paper. The reviewed studies were categorized in three groups depending on how much they addressed pain in MFS, and the results were synthesized by aggregating the themes.

**Results:** Of 360 search results, 22 articles satisfied the eligibility criteria. Only one small study was an intervention study, the rest were cross-sectional. Most studies were of small sample sizes, many had low response rate or dealt with other aspects of the diagnosis than pain. Four studies dealt mainly with pain, all published from 2015 or later. A total of 2300 persons with MFS had been examined. The prevalence of pain varied from 47 to 92 %, and affected several anatomic sites. Studies indicated that back pain is the most common location, and several studies found high incidence of headache. Several studies found that chronic pain limits daily function. Few studies describe treatment options for pain in patients with MFS.

**Conclusions:** Despite that there have been more focus on pain in MFS the last 3 years; the research on chronic pain in MFS is still limited in size and quality. Research is needed to obtain more evidence-based knowledge for developing more appropriate rehabilitation programs for people with MFS.



**P.48****HEALTH PROBLEMS, CHRONIC PAIN AND FATIGUE. ASSOCIATIONS WITH WORK PARTICIPATION AND LIFE SATISFACTION IN ADULTS WITH MARFAN SYNDROME**

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**Objectives:** Discuss the possible associations between health problems, fatigue and pain may have on work participation and life satisfaction in adults with Marfan syndrome (MFS).

**Methods:** A cross sectional questionnaire based study on psychosocial issues in adults with MFS in Norway was conducted. Method described in previous presentation.

**Results:** 73 adults with verified MFS participated. Chronic pain and severe fatigue was significantly higher than in the general Norwegian population (GNP). Adults with MFS had significantly lower work participation than the GNP. Most young adults (under 40) worked full time despite comprehensive health problems, but they left work earlier than the GNP. In multiple logistic regression analyses only low educational level, older age and severe fatigue were significantly associated with not working. Having Marfan- related health problems and chronic pain was not associated with being employed or not. Satisfaction with life was significantly lower than in the GNP. Multivariate regression analyses found significant associations with fatigue, aortic dissection and having regular contact with psychologist. Marfan- related health problems and chronic pain was not associated with degree of life satisfaction.

**Conclusions:** Persons with MFS may have debilitating fatigue and pain, reduced work participation and satisfaction with life. This study highlight that fatigue is a little researched and addressed problem closely linked to chronic pain, that gives more restrictions in daily life and work participation than Marfan-related health problems. Clinicians meeting persons with MFS need to address these issues. Further research on possible causes and treatment and rehabilitation programs for both fatigue and pain is needed.

## P.49

# HOW TO GET KNOWLEDGE INTO PRACTICE? CHALLENGES IN THE KNOWLEDGE TRANSLATION PROCESS OF RESULTS FROM A STUDY OF PSYCHOSOCIAL ASPECTS IN MARFAN SYNDROME

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2. Norway Faculty of Social Sciences, Department of Social Work, Child Welfare and Social Policy, Oslo Metropolitan University, Norway,

**Objectives:** The presentation will focus on our experiences and challenges in *the dissemination and implementation process of the results from our study of psychosocial aspects of MFS*. To find strategies for how knowledge from the project can be conveyed to benefit the users.

**Methods:** *Integrated Knowledge Translation (IKT) was used. An important ideal with IKT is that scientific research needs to include the perspectives of those being studied.* Our research project was initiated and conducted in cooperation with the Norwegian Marfan Association, who has been involved in all phases of the research process. We are now especially concerned with finding methods for implementation of knowledge to clinical practice in order to benefit the users.

**Results:** Despite that our study of psychosocial aspects of MFS has resulted in a number of international and Norwegian articles, we experience difficulties in spreading the information and get an integration between research and practice. Research on the implementation process shows that implementation of knowledge is often difficult to accomplish. Our experience is that user involvement in the knowledge- translation process is central for effective knowledge implantation.

**Conclusion:** An increased focus of the “know to do” gap and the lack of interaction between research and practice has promoting growing interest for strategies and model for the implementation process. Integrated Knowledge Translation may be appropriate for implementing knowledge in practice for devolving more evidence- based decision making.

**P.50****PSYCHOSOCIAL ASPECTS AND PHYSICAL FUNCTION IN ADULTS WITH LOEYS DIETZ SYNDROME (LDS), VASCULAR EHLERS DANLOS (V EDS) AND MARFAN SYNDROME (MFS)**

**Authors:** Velvin Gry, PhD <sup>1</sup>, Heidi Johansen Msc<sup>1</sup>

1. TRS National Resource Centre for Rare Disorders, Sunnaas Rehabilitation Hospital, Nesoddtangen, Akershus, Norway,

**Objective:** The objective is to present some preliminary qualitative data from a comprehensive study dealing with psychosocial aspects and physical function in adults with LDS, vEDS and MFS. Systematic searches in relevant databases revealed a lack of literature on these issues in these diagnoses.

**Methods:** Focus groups interviews with 22 patients with verified LDS (11) and vEDS (11), aged 22 to 67 year. In addition similar focus groups will be conducted of approximately 14 patients with verified MFS in April 2018. The focus groups emphasized issues as: physical activity and psychosocial aspects (family life, social network, economy, partnership and sexuality, physical and psychological health). Analysis applied Systematic Text Condensation, searching for issues and synthesizing the participants' experiences and management of living with these diagnoses. Similarity and differences between the diagnoses will also be analyzed.

**Results:** The preliminary results revealed that challenges adults with LDS and vEDS meet are multifold, ranging from social, to personal and medical impact. Many reported that chronic pain, fatigue and psychological distress had great impact on family life and daily function. The unpredictability of body function, contradictory advises from professionals about physical limitations and lack of tailored rehabilitation programs may cause inactivity and passivity. Many required psychosocial support, particularly related to thoracic surgery.

**Conclusion:** The results indicate that there is a need for more research in order to establish more consistent recommendations on physical restrictions and activity for these patient groups. Multidisciplinary rehabilitation programs are needed in order to meet their multifactorial needs.

# GENERAL INFORMATION

## **Location**

Hilton Amsterdam  
Apollolaan 138, Amsterdam, 1077 BG, The Netherlands  
Tel +31-20-7106005

The centrally located Hilton Amsterdam is a chic canal side hotel with easy access to numerous boutique shops, restaurants, and tourist attractions. The hotel is just a 15-minute drive from Amsterdam Schiphol Airport and within a short walking distance of the old city center.

The symposium and poster session will take place in the convention center of the hotel on the first level of the hotel. The symposium will be held in Ballroom A-C. The poster session will take place in the Diamond/Staten/Nassau rooms. For those staying at the Hilton, the breakfast (included in your room rate), will be served in Rolands beginning at 7:00 AM.

## **Speaker Presentations**

All speakers are requested to tailor their talks between around 8-10 minutes. Please remember that Q&A are expected after each presentation. Presentation timers will be used. All speakers will be required to load their presentations onto a single computer between 7:00 and 8:15 AM or during the breaks. Please bring your presentation on a USB.

## **Poster Presentations**

Poster Session I — Posters 1-25  
Thursday, May 3, 3:45-5:45 PM

Poster Session II & Late-Breaking Abstracts — Posters 26-50  
Friday, May 4, 5:00-7:00 PM

Posters have been assigned a number and a poster presentation session. This information can be found on the Poster List included in this booklet. Set up must be conducted prior to plenary sessions or during lunch or breaks. Please place your poster on the appropriate numbered board. Please remove your board after the poster session ends. Posterboards can be found in the Staten/Nassau Room.

## AUTHOR INDEX

Aalders, J	P.11	Benarroch, L	S.11, S.22, S.38
Abraham, D	P.38	Benke, K	P.19, P.39, S.39, S.67
Agg, B	P.19, P.39, S.39	Bernatchez, P	S.2
Akazawa, H	S.10	Beroud, C	S.54
Al-Amodi, H	S.15	Berraondo, P	S.3
Al-Essa, M	S.23	Beyens, A	S.23
Al-Manea, W	S.23	Bharj, J	S.38
Alaerts, M	S.28, S.29	Bjorck, E	S.67
Albuisson, J	S.23, S.29	Björck, H	S.29
Alderweireldt, R	P.31	Birtwell, D	S.34
Aldred, M	S.26	Blackstone, E	S.1
Alexander, T	S.62	Blinder, K	P.20
Almarzooq, Z	S.42	Boel, A	S.23
Alonso, F	P.35	Bogunovic, N	P.24
Alvarez, N	P.23	Boileau, C	S.11, S.22, S.27, P.31, S.35, S.37, S.38, S.54
Amudhavalli, S	S.25	Boland, A	S.11
Andelfinger, G	S.29	Bolar, N	P.29, S.29
Anselmo, M	S.21	Bonnet, D	S.23
Antolik, C	S.37	Bonorino, F	S.3, P.7
Apte, S	S.1, S.49	Bors, A	P.19
Aragon-Martin, J	P.6, P.36, S.38	Bostan, O	S.23
Arai, Y	P.9, P.16	Bou-Chaaya, C	S.50
Arbustini, E	S.67	Bou-Gharios, G	P.38
Ardinger, H	S.25	Bouleti, C	S.24, S.54
Ardinger, R	S.25	Bouma, B	S.36
Arnaud, P	S.11, S.22, S.27, S.37	Bourgeron, T	S.11
Arnoult, F	S.24, S.54	Boute, O	S.23, S.30
Asch, F	S.40, S.56	Bowen, C	S.18
Aubart, M	S.11	Boye, Sanford	S.46
Baars, M	S.33	Boye, Shannon	S.46
Baas, A	S.33	Braverman, A	P.20, S.35
Bagirzadeh, R	S.4, S.5, S.12, S.13, S.17	Broderick, T	S.62
Bakker, E	P.13	Buffo, I	P.25
Baldus, S	S.9	Bukaeva, A	P.27, P.42
Balm, R	S.20	Burgos, R	S.60
Bamshad, M	S.26	Buratti, J	S.11
Barbera, L	S.3, P.7	Busa, T	S.23
Barge-Schaapveld, D	P.24	Calderon, J	S.4
Bartha, E	P.19, P.39, S.39	Cale, J	P.12
Bassnett, S	S.45	Callewaert, B	S.23, S.27, S.30
Bathen, T	P.47, P.48, P.49, S.65	Cameron, E	S.62
Bauters, F	S.59	Campanero, M	P.2, S.7
Beattie, D	P.21	Canelas, L	P.2
Beauchesne, L	S.28	Canham, N	S.23
Bedja, D	S.4, S.5, S.12, S.13, S.16, S.17	Cannaerts, E	P.28, S.28, S.29
Beelen, A	S.61, S.66	Carlberg, B	S.37
Behr, E	S.38	Carlson, E	S.40
Bekkers, B	S.36	Caspar, S	P.5, P.15
Bellen, H	S.26		

Catroxo, M	P.37	Deleuze, J	S.11
Cavan, J	S.21	Delhon, L	S.50
CCA Clinical Care Group	S.30	Delorme, G	S.54
Celauro, K	S.21	Demertzis, S	P.40
Challman, M	S.40	Desguerre, I	S.11
Charchyan, E	P.27, P.42	Devereux, R	P.8, S.40, S.42, S.56
Chen, A	S.64	Devriendt, K	S.23
Chen, J	S.26	Dewandele, I	S.23
Chen, Y	S.4, S.12	Deyle, D	S.23
Child, A	P.6, P.36, S.38, P.45	Diallo, K	S.22
Chiodo, V	S.46	Dietz, H	S.4, S.5, S.12, S.13, S.16, S.17, S.18, S.23, S.29, S.31
Chu, M	S.15	Diéz, N	S.23
Cikach, F	S.1	Diness, B	P.29
Cil, E	S.23	Dobbbs, M	P.14
Clark, F	S.48	Dodd, N	S.40
Claudio, R	P. 31	Doyle, A	S.16
Collins II, R	S.23	Doyle, J	S.16, S.17
Collinson, B	S.21	Dubacher, N	P.13, P.15
Colombo, R	S.37	Duffelen, A	P.34
Cooke, S	S.17	Duijnhouwer, T	S.29, S.36
Cormier-Daire, V	S.50	Dulac, Y	P.32
Couccke, P	P.18, S.23, S.30, P.31	Dulfer, E	S.33
Cousin, M	S.23	Dupuis-Girod, S	S.23
Cozijnsen, L	P.24, S.36	Dux-Santoy, L	S.43
Crabb, J	P.13	Dyack, S	P.26
Creamer, T	S.4	Eagle, K	S.40
Cui, J	P.33	Eagleton, M	S.1
Dakhli, T	P.35	Edouard, T	P.32
Dalton, S	S.21	Egea, G	S.3, P.7, S.63
Damrauer, S	S.34	Elting, M	P.1
Dantas, A	P.7	Emerton, K	S.1
Daradics, N	P.19, S.39	Engelbert, R	S.61, S.66
Dasouki, M	S.23	Eriksson, P	S.29
Davis, A	P.43	Esfandiarei, M	S.2, P.33, S.62, S.64
Davis, E	S.23	Essers, J	S.44
De Backer, J	S.8, P.11, S.16, P.18, S.23, S.27, S.30, P.31, S.35, S.38, P.44, S.58, S.59, S.67	Evangelista, A	S.35, S.43, S.57
De Boer, R	S.61	Falcon, M	P.43
De Coninck, S	S.30	Famaey, N	S.55
De Paepe, A	S.16, P.18, S.23, S.30	Farinha-Arcieri, L	P.37
De Saffel, H	P.18	Fehervary, H	S.55
De Schepper, S	S.23	Fernandez, R	S.43
De Silva, D	S.23	Filip, R	S.55
De Souza, A	P.33	Fischer-Zirnsak, B	S.23
De Vries, C	S.44	Fisher, E	S.38
De Vries, J	P.3, P.34, S.61	Fletcher, S	P.12
De Waard, V	P.3, S.20, S.44	Fontenot, E	S.23
De Wilde, H	S.58, S.59	Forteza, A	S.57, S.60
De Wolf, D	S.30, S.58, S.59	Franco-Cereceda, A	S.29
Dean, J	S.38	Franken, R	P.3, S.44
Dekker, S	S.66	Franssen, E	S.31
		Fukushima, Y	S.32

Furlan, A	S.9	Haller, G	P.14
Galatioto, J	S.1	Hanna, N	S.11, S.22, S.27, S.37
Galian, L	S.43	Hannuksela, M	S.37
Gallo MacFarlane, E	S.4, S.12, S.17	Hardie, K	S.34
Gandhi, M	P.10	Hardin, J	S.23
Garcia, M	P.6, P.36, S.38	Harman, C	S.46
Garcia-Dorado, D	S.43	Harris, K	S.2, P.33
Garcia-Montero, C	S.57, S.60	Hascoet, S	P.32
Gardeitchik, T	S.28	Hassan, A	P.4
Gattas, M	P.28	Hauswirth, W	S.46
Gazal, S	S.11, S.22	Hecht, J	S.41
Geirdal, A	P.47, P.48, P.49, S.65	Hegarty, S	P.21
Génin, E	S.11	Heggarty, S	P.21
Génot, E	P.35	Helmers, M	S.18
GenTAC Investigators	S.40	Hertegonne, K	S.59
Geuzebroek, G	P.34	Hibender, S	S.44
Gezdirici, A	S.23	Hilhorst-Hofstee, Y	S.33, S.36
Ghoumid, J	S.23	Hollander, Z	P.33
Giadrosic, J	S.18	Holmes, K	S.40, S.41
Gibson, C	S.62, S.64	Hunt, I	P.45
Gillis, E	S.29	Hostetler, E	S.26, S.27, S.37
Giménez-Llort, L	S.63	Houweling, A	S.33
Giuliano, F	S.23	Hove, H	P.29
Go, A	P.43	Hoxha, B	S.62
Gong, L	S.37	Hubmacher, D	S.49
Goobie, S	S.15	Hurard, M	S.67
Gorris, M	P.34	Huso, D	S.12
Goudin, N	S.50	Huso, N	S.12
Goudot, G	S.29	Indrakusuma, R	S.20
Gould, R	S.29	Isekame, Y	S.38
Goumans, MJ	P.24	Jacob, M	S.11
Gouya, L	S.11, S.22	Jadhav, S	S.40
Grahame, R	P.45	Jahangiri, M	S.38
Granata, A	S.19	Jalalzadeh, H	S.20
Granato, C	S.43	Janda, A	S.37
Greer, K	P.12	Janssen, L	P.31
Grego, S	P.40	Jenkins, J	S.25
Groeneveld, M	P.1	Jeremy, R	S.35
Groenink, M	P.3, S.20, S.33, S.44, S.67	Jeunemaitre, X	S.23, S.29
Grote, L	S.25	Jiménez-Altayo, F	S.3, P.7, S.63
Gross, M	S.11	Johansen, H	P.46, P.50
Gruhl, S	P.6	Jondeau, G	S.11, S.22, S.24, S.27, S.35, S.37, p.31, S.38, S.54, S.67
Guala, A	S.43		
Guien, C	S.54	Jones, W	S.45
Guo, D	S.26, S.27, S.37, S.38	Johnston, T	S.37
Gurnett, C	P.14	Kancherla, K	S.40
Gutierrez, L	S.43	Kang, B	S.4, S.5, S.13
Gyuricza, I	P.37	Karlién, D	S.59
Habashi, J	S.4, S.12	Kautzner, J	P.22
Hadeed, K	P.32	Keene, D	S.8
Haelterman, N	S.26	Kember, R	S.34
Haider, M	S.23	Kempers, M	S.28, S.29, S.33, S.36, S.67

Kerkhof, J	S.15	Lyonnet, S	S.23
Kerstjens-Frederikse, M	S.36	Ma, Y	P.30
Klee, E	S.23	Macek Jr., M	P.22
Koch, C	S.1	Macek Sr., M	P.22
Kočková, R	P.22, S.53	MacKay, S	P.26
Koehl, K	S.46	Macrae, R	S.19
Koh, I	P.37	Madronero Waitman, L	S.25
Komáromy, A	S.46	Malý, J	P.22, S.53
Komarova, S	S.6, S.51	Manalo, E	S.8
Komuro, I	S.10	Manning, M	S.12
Koolbergen, DR	P.3	Marcadier, J	P.23
Kopps, A	P.15	Marcelis, C	S.28
Kornak, U	S.23	Marshall, L	S.41
Kosho, T	S.32	Marsili, L	S.33
Kövy, P	P.19	Martín Lopez, C	S.57, S.60
Khanna, S	P.20	Martínez, D	S.60
Knadler, J	S.52	Martins, A	P.37
Krapels, I	S.33	Masand, P	S.40
Krebsova, A	P.22, S.29, S.53	Maskatia, S	S.40
Krishnamurthy, R	S.40	Maslen, C	S.40
Kuchtey, J	S.47, S.48	Masoha, A	P.6, P.36
Kuchtey, R	S.47, S.48	Masuno, M	S.32
Kulmacz, R	S.37	Mathur, V	P.10
Kumar, A	S.29	Matsumoto, M	P.16
Kumra, H	P.4, S.14	Matsunari, H	P.9, P.16, P.17
Kurosumi, A	S.10	Matthys, A	S.30
Kusters, B	P.34	Matyas, G	P.5, P.13, P.15
Laberge, A	S.15	Maugeri, A	P.1, S.28, S.33
Laboš, M	S.53	Mayr, M	P.3
Lacro, R	S.40	McCallion, A	S.29
Lahidji, S	P.43	McConnell, V	P.21
Lambermon, E	S.36	McKee, T	S.6
Landecho, M	S.23, S.54	McManus, B	P.33
Langeois, M	S.22	McNamara, M	S.19
Lautatzis, M-E	P.25	Mead, T	S.1
Leal, S	S.26, S.27, S.37	Meerschaut, I	S.30
Lee, K	S.26, S.27, S.37	Meester, J	S.29, S.31
Le Goff, C	S.50	Mehrrens, D	S.9
Legrand, A	S.23	Meienberg, J	P.5, P.13, P.15
LeMaire, S	S.35, S.40, S.52	Meirelles, T	P.7
Leong, T	P.43	Melenovský, V	P.22
Levins, A	P.36	Menke, L	S.33, S.61, S.66
Li, W	P.30	Merkely, B	P.19, P.39, S.39
Liang, D	P.43	Mertens, L	S.29
Liberatore, A	P.37	Messas, E	S.29
Lindsay, M	S.4	Meyer, V	S.11
Liu, Q	S.10	Meyten, N	S.28
Loeys, B	S.15, S.16, P.22, S.23, P.28, S.28, P.29, S.29, p.31, S.31, S.36, S.38, S.67	Mibava Leducq Consortium	S.17, S.29
		Micha, D	P.1, P.24, S.28
Luo, M	P.30	Michael, H	S.23
Luyckx, I	S.15, P.22, P.29, S.29, S.38	Michel, P	P.15
		Milewicz, D	S.26, S.27, S.35,



	S.37, S.38, S.40	Pals, G	P.1, P.24, P.41
Miller, DC	P.43	Pals, T	P.41
Milleron, O	S.11, S.24, S.54	Papadopoulos, M	P.45
Mingo, S	S.57	Parkash, S	P.26
Mital, S	S.29	Parker, S	S.4
Mocerri, P	S.23	Pasi, N	S.24
Moffett, B	S.52	Peeters, N	P.28, S.28
Mohamed, S	S.29	Pediatric Heart Network	S.31
Mohammed, S	S.23	Pepe, G	S.67
Moñivas, V	S.57	Pepelassis, D	P.25
Montalcino Aortic Consortium	S.26, S.35, S.37	Pedroza, C	S.40
Morisaki, H	S.32	Pênzes, I	P.39
Morisaki, T	S.32	Pereira, L	P.37
Morooka, A	P.17	Perlman, G	S.6
Morris, S	P.10, S.35, S.40, S.52	Perrier, R	P.23
Morshuis, W	P.34	Petit, F	S.30
Muiño-Mosquera, L	S.23, S.27, S.58, S.59	Pichler, K	S.23
Mulder, B	P.3, S.20, S.44, S.67	Pinard, A	S.26, S.38
Münger, J	P.13	Pini, A	S.67
Murphy, D	S.64	Pirk, J	P.22, S.53
Murphy, L	S.67	Pitsillides, A	P.38
Muthu, M	S.50	Plaisancié, J	P.32
Nagashima, H	P.9, P.16, P.17	Plasschaert, F	S.30
Nagaya, M	P.16	Plomp, A	P.24
Najafi, A	P.5	Pólos, M	P.19, P.39, S.39
Nakano, K	P.9, P.16, P.17	Post, J	P.24, S.36
Nampoothiri, S	S.23	Potts, J	P.33
Narula, N	P.8	Poulet, B	P.38
Nashabi, A	S.47	Prakash, S.	S.26
National Working Group on BAV & TAA	S.36	Preiss, L	S.40
Natowicz, M	S.26	Prescott, K	S.23
Nelea, V	S.14	Preuss, C	S.29
Nemcikova, M	S.29	Pyeritz, R	S.34, S.35, S.40
Németh, E	P.39	Quiggle, A	P.14
Nickerson, D	S.26	Rader, D	S.34
Nicot, F	S.24	Raffin, L	P.33
Niessen, H	P.24	Rajeb, A	S.23
Noel, C	S.40	Ramanayake, W	P.38
Nollet, F	S.66	Ramirez, F	S.1
Noordeen, M	P.45	Ramos-Arroyo, M	S.23
Norambuena, P	P.22	Rand-Hendriksen, S	P.47, P.48, P.49, S.65
Nupoor, N	S.56	Ratsimandresy, M	P.32
Óber, A	P.39	Ravekes, W	S.40
Oda, S	S.40	Redondo, J	P.2, S.7
Oh, A	S.46	Regalado, E	S.26, S.27, S.35, S.37, S.38
Ohata, T	S.32	Reinhardt, D	P.3, P.4, S.6, S.14, S.51
Ohgane, J	P.9, P.16	Renard, M	S.8, P.31, P.44
Okazaki, N	P.9	Reocreux, M	S.22
Osborn, D	S.38	Richer, J	S.15, P.23, S.28
Ou, P	S.24	Rideout, A	P.26
Overwater, E	S.33	Ridwan, Y	S.44
Paine, H	P.33	Rifkin, D.	S.26

Rigelsky, C	S.26	Sips, P	P.18
Rivas, J	S.60	Skroblin, P	P.3
Robert, L	S.67	Smith, A	S.38
Roberts, A	P.17	Smith, D	S.19
Rode Diness, B	P.29	Smith, H	P.38
Rodríguez, I	S.3	Smith, R	P.37
Rodríguez, J	S.45	Smits, G	P.31
Rodríguez-Palomares, J	S.43	Sneddon, J	S.38
Rogozhina, Y	P.27, P.42	Solomon, M	P.43
Roman, M	P.8, S.40, S.42, S.56	Soni, R	P.25
Rombaut, L	S.61	Souza, R	P.37
Roos-Hesselink, J	S.15, S.36, S.67	Srinivas, M	P.34
Ropers, J	S.54	Stańczyk, E	P.6, P.36
Roselli, E	S.1	Staal, A	P.34
Rossi, M	S.23	Steichen-Gersdorf, E	S.23
Ruiz-Muñoz, A	S.43	Steijns, F	P.44
Ruiz-Rodríguez, M	P.2	Stengl, R	P.19, S.39
Rumyantseva, V	P.27, P.42	Steyaert, W	S.30
Russo, C	S.37	Stheneur, C	S.11
Russo, M	P.10	Sung, S	P.43
Rykiel, G	S.12, S.18	Symoens, S	S.30
Sadikovic, B	S.15	Szabolcs, Z	P.19, P.39, S.39, S.67
Sagggar, A	P.36, S.38	Szilveszter, B	S.39
Saito, M	P.16	Takahashi, K	S.32
Sakai, L	P.2, S.8, S.41	Takeda, N	S.10
Salemink, S	S.29	Takeuchi, K	P.9
Salih, M	S.23	Talley, N	S.62
Sandor, G	S.2, P.33, S.64	Tan, K	S.26
Saya, H	P.16	Taylor, A	S.23
Schaefer, E	S.23	Tchitchinadze, M	S.24, S.54
Schantz, D	P.25	Tedesco, R	P.37
Scheper, M	S.61	Tehrani, A	S.2
Schepers, D	S.28	Teixido-Tura, G	S.43
Schlingmann, T	S.40	Temel, S	S.23
Schmitt, S	S.24	Tence, N	S.24
Schneider, L	S.64	Textor, J	P.34
Scholte, A	S.20	Tiedemann, K	S.6, S.51
Schweitzer, R	S.49	Tierney, S	S.62, S.64
Sellers, S	S.2	Timmermans, J	S.20, S.28, P.34
Seidahmed, M	S.23	Tome-Esteban, M	S.38
Sengle, G	S.9	Treasure, T	S.55
Serrano, F	S.19	Tufa, S	S.8
Serrano-Fiz, S	S.57, S.60	Tuytens, B	S.30
Sherman, A	S.25	Tyson, J	S.40
Shin, J	S.4	Uchikura, A	P.16
Shittu, T	S.40	Umeyama, K	P.9, P.16, P.17
Shohe, R	S.40	University of Washington Center for Mendelian Genomics	S.26
Shoumo, B	S.19	Uysal, F	S.23
Siegert, AM	P.7	Valente, F	S.43
Simpson, M	S.38	Valero, I	S.60
Singh, P	S.42, S.56	Vallejo-Elias, J	S.62
Sinha, S	S.19		

Van Bavel, E	P.13	Wanga, S	P.3, S.44
Van Brakel, T	S.36	Wardlow, R	S.16
Van Breemen, C	S.2, P.33	Warner, D	S.23
Van de Beek, G	S.28, S.29	Warnink-Kavelaars, J	S.61, S.66
Van de Beek, I	S.33	Watabe, T	S.32
Van de Berg, M	S.20	Watanabe, M	P.9, P.16, P.17
Van de Laar, I	S.15, S.29, S.36, S.67	Watanabe, Y	S.32
Van den Vreken, N	P.11	Weinsaft, J	S.42, S.56
Van der Made, I	S.44	Weiss, M	S.33
Van Hagen, J	S.33	Wessels, M	S.15, S.29, S.36
Van Hengel, J	P.11, P.44	White, Z	S.2
Van Kimmenade, R	P.34	Whitener, A	S.48
Van Laer, L	S.23, P.28, S.28, S.29, S.31, S.38	Wieschendorf, B	S.29
Van Maldergem, L	S.23	Willaert, A	S.23, P.18
Van Riel, L	S.44	Willard, B	S.1
Van Rijn, P	S.33	Williams, H	S.34
Van Roomen, C	S.44	Willing, M	P.20
Van Tintelen, J.	S.33	Wilson, N	S.16, S.17
Van Vliet, N	S.44	Wilton, S	P.12
Vandersteen, A	P.26, P.28	Wohlner, B	P.6, P.36
Vanderveken, E	S.55	Wortel, I	P.34
Vandeweyer, G	S.29, S.31	Wright, W	P.21
Vanhomwegen, M	S.23	Wu, H	S.47
Velvin, G	P.46, P.47, P.48, P.49, P.50, S.65	Wünnemann, F	S.29
Vastmans, J	S.55	Yabumoto, C	S.10
Vattay, B	S.39	Yagi, H	S.10
Vera, Y	S.60	Yamanaka, I	S.32
Verbeken, E	S.55	Yang, H	P.30
Verbelen, T	S.55	Yazdanifar, G	S.17
Verboogen, L	P.31	Yetman, A	S.41
Verbrugghe, P	S.55	Yeung, K	P.1, S.33
Verhagen, J	S.15, S.29, S.33, S.36	Yin, X	P.3
Vermassen, P	P.18	Young, L	S.41
Vermeersch, P	S.28	Youssef, B	P.31
Verstraeten, A	S.15, P.22, S.28, P.29, S.29, p.31, S.31, S.38	Zaklyazminskaya, E	P.27, P.42
Vila, E	P.7	Zarate, Y	S.23
Villalva, N	S.43	Zeisberger, S	P.13
Villar, S	S.57, S.60	Zerbino, D	S.29
Von Kodolitsch, Y	S.67	Zeyer, K	P.4
Voorhoeve, E	S.33	Zhang, R	P.4
Vos, M	S.44	Zhang, Y	P.30
Votypka, P	P.22	Zhou, Z	P.30
Wagner, J	S.25	Zhu, G	P.30
Wakeling, E	P.36	Zhurayev, R	S.29
Wakui, K	S.32	Ziegler, S	S.4, P.13
Wallace, S	S.26, S.27, S.37	Zigrino, P	S.9
Wan, Y	P.6, P.36, S.38	Zilberberg, L	S.26
		Zwijnenburg, P	S.33
		Zwinderman, A	P.3, S.20, S.44

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