AARHUS UNIVERSITY

Marfan syndrome
Diagnostics, epidemiology, and aortic events

PhD dissertation

Kristian Ketill Ambjørn Groth

Health

Aarhus University

2016

Marfan syndrome
Diagnostics, epidemiology, and aortic events

PhD dissertation

Kristian Ketill Ambjørn Groth

Health

Aarhus University

Department of Cardiology

Department of Molecular Medicine

Department of Endocrinology and Internal Medicine

Department of Clinical Medicine

Supervisors

Professor Claus H. Gravholt, Ph.d, dr.med.

Department of Molecular Medicine (MOMA), Aarhus University Hospital, SKS

Department of Endocrinology MEA, Aarhus University Hospital, NBG

Niels Holmark Andersen, Ph d, dr.med.

Department of Cardiology, Aarhus University Hospital, SKS

Professor John Østergaard, dr.med.

Center for Rare Diseases, Department of Pediatrics, Aarhus University Hospital, SKS

Opponents

Svend Rand-Hendriksen, Ph.d

TRS, Sunnaas sykehus, 1450 Nesoddtangen

Associate Professor Jacob Tfelt-Hansen, Ph.d

Department of Cardiology, Rigshospitalet

Clinical Associate Professor Kent Lodberg Christensen, Ph.d, dr. med.

Department of Cardiology, Aarhus University Hospital

The thesis is based on the following manuscripts:

1. Difficulties in diagnosing Marfan Syndrome relying on existing *FBN1* databases.

   Kristian A Groth, Mette Gaustadnes, Kasper Thorsen, John R. Østergaard, Uffe Birk Jensen, Claus H. Gravholt, Niels H. Andersen.

   Genet Med. 2016 Jan;18(1):98-102.

2. Evaluating Marfan genotype-phenotype correlations in existing *FBN1* databases.

   Kristian A Groth, Yskert Von Kodolitsch, Kerstin Kutsche, Mette Gaustadnes, Kasper Thorsen, Niels H. Andersen, Claus H. Gravholt

   Accepted for publication in Genetics in Medicine.

3. Prevalence, incidence, and age at diagnosis in Marfan Syndrome.

   Kristian A Groth, Hanne Hove, Kasper Kyhl, Lars Folkestad, Mette Gaustadnes, Niels Vejlstrup, Kirstine Stochholm, John R. Østergaard, Niels H. Andersen, Claus H. Gravholt

   Orphanet J Rare Dis. 2015 Dec 2;10:153.

4. Aortic events in a nationwide Marfan Syndrome cohort

   Kristian A Groth, Kirstine Stochholm, Hanne Hove, Kasper Kyhl, Pernille Axelsen Gregersen, Niels Vejlstrup, John R. Østergaard, Claus H. Gravholt, Niels H. Andersen

   Clin Res Cardiol. 2016 Aug 22. [Epub ahead of print]

Preface

The work in this thesis is done in cooperation between the Department of Cardiology and the Department of Molecular Medicine at Aarhus University Hospital, Aarhus Denmark. The studies were carried out during my PhD fellowship in the period 1st of May 2013 to 30th of April 2016.

Acknowledgements

I owe a special and deep-felt gratitude to my closest supervisors Niels Holmark Andersen, prof. Claus Højbjerg Gravholt and prof. John Østergaard for unique guidance, inspiration and motivation. I am grateful to have benefitted from their support both academically and personally. I could not have asked for better supervisors.

During the last three years I have been in contact and cooperated with so many helpful and inspiring people who I all owe gratitude.

Special thanks go to Prof Torben Falck Ørentoft, Mie Gade Farsinsen and Connie Juhler and the rest of Department of Molecular Medicine (MOMA) for providing the vital physical and academic condition in which I have done my research. The people at MOMA have given me new knowledge on genetics and I have learned the basis of variant analysis. For this I owe a special thanks to the analyst group on the "second floor" Mette Gaustadnes, Friedrik Wikman and Lisbeth Nørum Pedersen. Also the "NGS people" on the 4th floor have been helpful in enlightening me in the area of sequencing DNA. Special thanks go to Kasper Thorsen for his always happy mind and inspiring cooperation. Katja Adolf is the sole reason that I did not experience computer failures and she have been very helpful in providing support in computer and software issues as well as suggestions on database handling.

At the Department of Cardiology I owe a special gratitude to Mie Bruun who provided a unique help in handling everything from patients records to blood samples. Whenever Mie is involved things just work! Also the nurses at the GUCH outpatient clinic have been helpful when consulting Marfan syndrome patients. Thanks to Helena Friis Jensen, Tina Isager, Hanne Kildahl Mathiasen, and Connie Willer Albertsen for having me around.

Center for Rare diseases have been most welcoming and especially the Marfan conferences have been highly interesting and I have learned a lot about Marfan syndrome from Stense Farholt and Pernille Axel Gregersen.

I have a special connection to Department of Endocrinology and Internal Medicine (MEA) established by Claus. I owe Sine Knorr, Anne Skakkebæk, Agnethe Berglund, Christian Høst, Anders Bojesen and the rest of the research group around Claus great appreciation. Very special thanks go to Kirstine Stochholm who has provided a very special and skilled statistical effort in my work and Kirstine “you are my statistical guru”.

I appreciate the close cooperation I have with Department of Clinical Genetics and especially Prof. Uffe Birk by whom I have gained important genetic knowledge. I owe thanks to Marianne Geilswijk and Maria Rasmussen for their interesting cooperation.

I also owe gratitude to national partners. At Rigshospitalet, my gratitude goes to Department of Cardiology especially Niels Vejlstrup and Kasper Kyhl, and the Department of Pediatrics’ Center for rare diseases with a special gratitude to Hanne Hove. Also the employees at the hospital archive placed a TV-byen in Søborg have provided vital support and I owe a special thanks to head of the archive Bjarne Rødtjer. At Odense University Hospital, Department of Cardiology Jens Mogensen has been highly inspiring as an expert in cardiology and genetics.

I also owe gratitude to prof. Ulrik T. Baandrup for coorporation and kind hospitality in Hjørring and I owe a speciel gratitude to Ulrik for a quick response in providing microscopic pictures of cystic media necrosis.

Without the support in providing access to patient files from other hospitals and departments around the country this thesis would not have been possible. I owe thanks to Odense University Hospital, Sydvestjysk Sygehus, Aalborg University Hospital, Regionshospital Herning, and Regionshospital Randers.

To my international collaborators in Germany I owe a special gratitude to Yskert Von Kodolitsch, Kerstin Kutsche.

I also owe my wife, friends and family great gratitude for invaluable support during the last three years of my Ph.d work.

Table of contents

List of abbreviations and explanations

ACMG	The American College of Medical Genetics and Genomics.
Berlin criteria	A list of phenotypical features distinctive for a set of connective tissue conditions including Marfan syndrome. The criteria was published in 1988 and included a set of major manifestation and a demand of affection of more than one organ system[1].
CI	Confidence Interval of 95%
ClinVar	Variant database based on an initiative of the National Center for Biotechnology Information (NCBI).
CPR-number	A personal identification number for Danish citizens. The number is registered in the Central Personal register. The CPR number can be used for record linkage across Danish registries.
Database-MFS	Variants reported to be associated with Marfan syndrome by one of the *FBN1* databases.
DNPR	Danish National Patient Registry.
DRCD	Danish Register of Cause of Death.
DST	Statistical Denmark (www.dst.dk)
E-journal	A Danish electronic patient record system gathering patients record from Danish hospitals in one system.
ESP	NHLBI GO Exome Sequencing Project driven by the National Heart, Lung, and Blood Institute (NHLBI)
ESP6500	A version of the ESP containing data on around 6500 exomes.
FBN1	Gene coding for the protein Fibrillin-1.
Ghent I criteria	A revision of the Berlin criteria but still the first set of diagnostic criteria with a sole focus on Marfan syndrome. The criterion was published in 1996. The criteria came short after the discovery of variants in the *FBN1* gene as causal for the condition.[2] The criteria include major and minor criteria as well as affection of more than organ system.
Ghent II criteria	A revision of the Ghent I criteria abandoning the major and minor criteria but introducing a new systemic point scoring system. The criteria highlight aorta dilatation and *FBN1* variants. The Ghent II criteria is somehow easier to use by the clinician as unspecific phenotypical criteria was abandoned.
HGDM	Human Gene Mutation Database. A general variant database also including the *FBN1* gene.
HR	Hazard Ratio
ICD	International Classification of Disease. In this thesis is used references to the version 8 (ICD-8) and version 10 (ICD-10).

Marfan-score	A score based on the Ghent II systemic scoring system and aorta dilatation to stratify phenotype in associations with *FBN1* variants.
MFS	Marfan syndrome
NCBI	National Center for Biotechnology Information.
NGS	Next Generation Sequencing.
Non-database-MFS	Variant reported in at least one *FBN1* database but none of the databases associated the variant to Marfan syndrome. Most of these variant are simply mentioned in the *FBN1* databases but without an association to phenotype og disease.
Non-score-MFS	Variant scoring low (under 7 points) in the Marfan-score.
Phenotype	From the Greek words “phainen” meaning “to show” and “typos” meaning “type”. The phrase means observable characters. In this thesis the phrase is used in the meaning of special characteristics that can be observed in the patient.
Polymorphism	Traditionally regarded as a variant more common than 1% a population. The phrase is often associated to “benign” variants. As the term is not very specific defined and can have several understandings organizations like ACMG do not recommend the use of the term. In this thesis the term is used in the meaning of “not disease causing”. The term is used as a reference to older literature using the term.
Raredis	A Nordic database developed in Denmark containing clinical data on patients with a number of rare diseases. www.raredis.eu
RR	Relative Risk
Score-MFS	Variant scoring high (at least 7 point) in the Marfan-score.
UMD-*FBN1*	The Universal Mutation Database for the *FBN1* gene.
UniProt	The Universal Protein Resource. A European initiative focusing on protein sequence and annotation.
Variant	A neutral indication of a change in the DNA in comparison with the reference genome. The term in itself does not indicate effect. The variant effect is indicated by an attached modifier ex. “Variant, Pathogenic”.
VUS	Variant of uncertain significance.

Introduction

Diagnosing Marfan syndrome

Marfan syndrome (MFS) was first described in 1896 by Antoine-Bernard Marfan in a case report that described a five-year old girl with distinctive long and thin extremities[3]. The girl died in early puberty probably due to tuberculosis. Whether the girl in fact did have MFS or maybe another condition has been discussed to such an extent that the possibility that she had congenital contractural arachnodactyly is mentioned as late as in the diagnostic criteria from 1996[2].

Since the first description of MFS, decades of research in the syndrome[4-7] have contributed to the knowledge about the phenotypical presentation and the genetic background. In 1986, the first definition of MFS was described by the Berlin criteria[1] and was solely based on the clinical phenotype. Later on, Dietz et al. discovered the connection between MFS and the gene coding for the fibrillin protein named *FBN1*[4]. The revised Ghent criteria from 1996 (Ghent-I)[2] were a revision of the Berlin criteria. These new criteria included the newly discovered *FBN1* gene as a component in the diagnostic criteria. The Ghent-I criteria operated with major and minor criteria in seven "organ systems" (skeletal, ocular, cardiovascular, pulmonary, dura, skin and family/genetic) on which the diagnosis could be based in an index patient by so called major criteria involvement in 2 systems and involvement in a third system. Major criteria are understood as a criterion that "carries high diagnostic specificity, because it is relatively infrequent in other conditions and in the general population"[2]. Major criteria are found in the skeletal, ocular and cardiovascular systems supplemented with dura ectasia and family/genetic also counting as major criteria[2], there are no major criteria in the pulmonary and skin systems. There is no definition of minor criteria but it is obvious that these criteria are less specific for MFS. Minor criteria are found in the skeletal, ocular cardiovascular, pulmonary and skin system. The Ghent II criteria include most phenotypical features from the Ghent I criteria but some criteria "have not been sufficiently validated" and "are not applicable in children or necessitate expensive and specialized investigation"[8]. In the Ghent II criteria the following phenotypical features was removed: joint hypermobility, highly arched palate, flat cornea, hypoplastic iris/ciliary, dilatation of the pulmonary artery, calcification of

the mitral annulus, dilatation/dissection of aorta descendent, apical blebs on lung X-ray and recurrent or incisional hernia. In theory it is possible to fulfill Ghent I and not Ghent II and vice versa but the majority of patients will fulfill both set of criteria[9]. In 2010, the revised second Ghent criteria (Ghent-II)[8] highlighted *FBN1* mutations, aortic dilatation and ectopia lentis as the three major cornerstones when diagnosing MFS[8]. Nevertheless, the MFS phenotype[10] remained in focus in the Ghent II criteria advising the physician to look for specific characteristic features such as; long and slender arms and legs with skewed upper/lower segment ratio[5] (Figure 1), arachnodactyly (long fingers (Figure 2)) resulting in positive thumb sign[11]and wrist sign[12] (Figure 3), mitral valve prolapse[13] (Figure 4), thorax deformities [5] (Figure 5), scoliosis [5] (Figure 6), dural ectasia[14](Figure 7), and pes planus[15] (Figure 8) etc.. Other features like highly arched palate with crowding of teeth[16] (Figure 9), hypermobile joints[5] (Figure 10) and dilatation of the pulmonary artery[17] (Figure 11C) are also r elated to MFS even though they are not included in the recent diagnostic criteria.

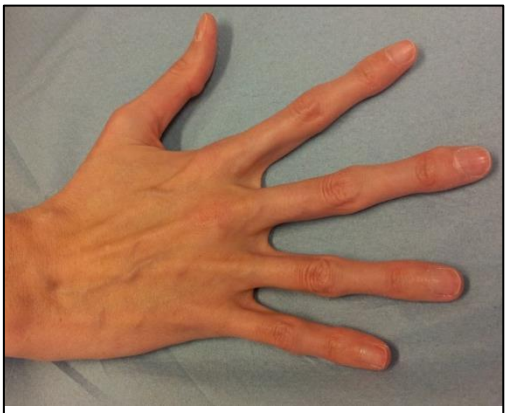

Figure 2: Arachnodactyly, long and slender finger (spider-fingers). Courtesy of Niels Holmark Andersen

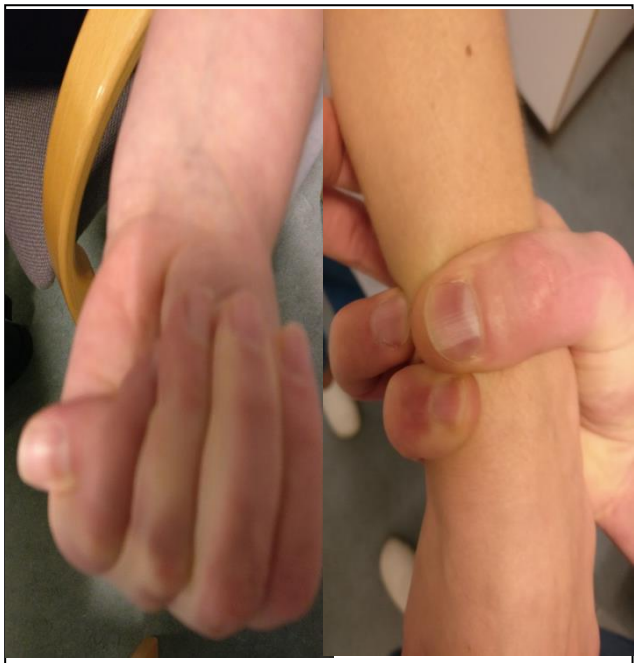

Figure 3: Thumb- (left) and wrist- sign (right).

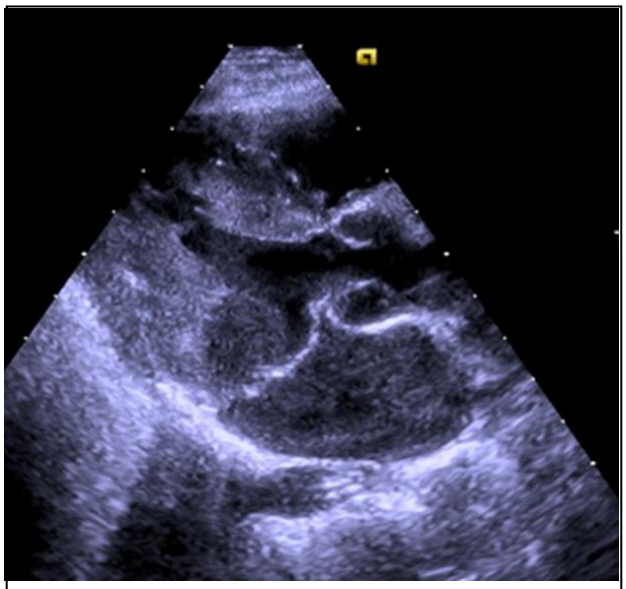

Figure 4: Echocardiography substernal view showing the mitral valve prolapsing into the left atrium. Picture from internet.
Courtesy of Niels Holmark Andersen

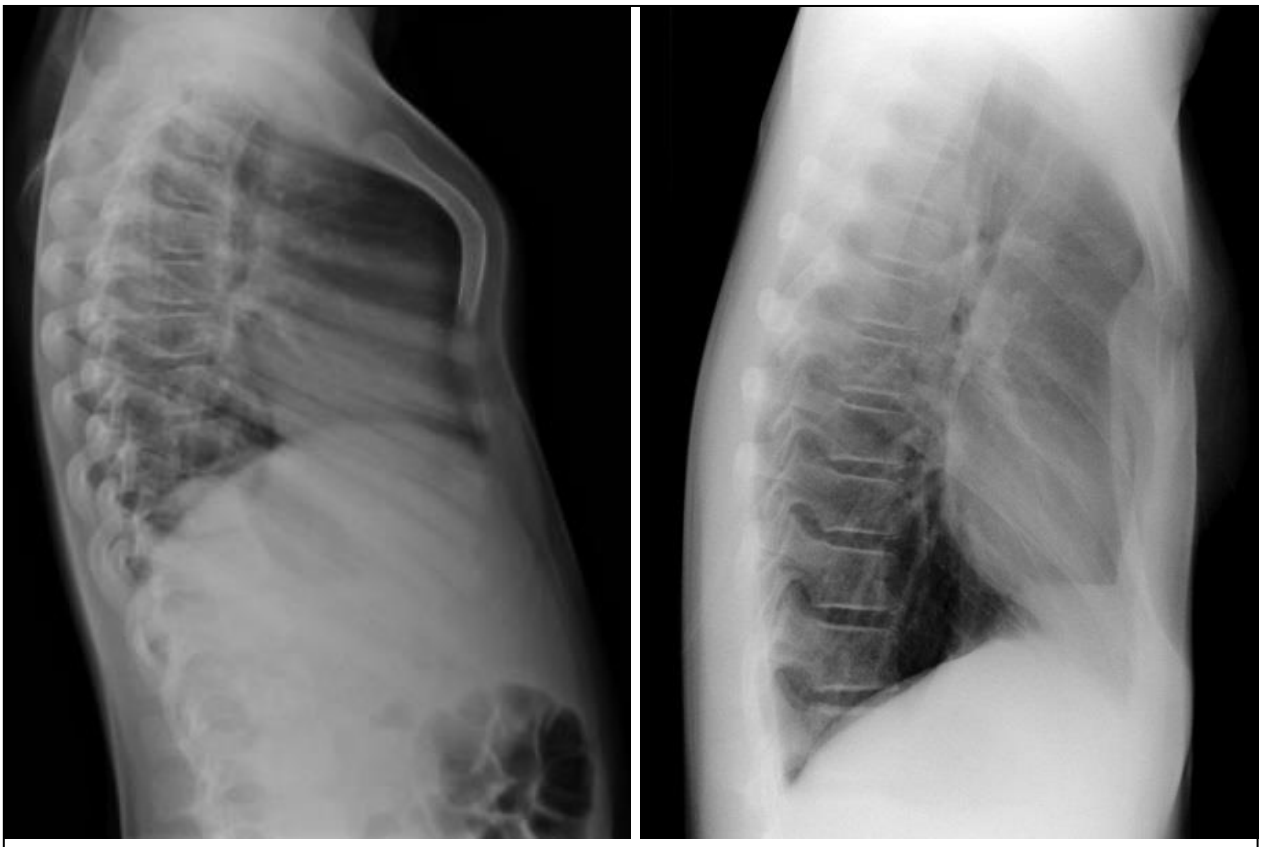

Figure 5: Anterior thorax deformity. Pectus carinatum (left (Case courtesy of Dr Maulik S Patel, Radiopaedia.org, rID: 13668)) and pectus excavatum (right (Case courtesy of A.Prof Frank Gaillard, Radiopaedia.org, rID: 8284)). Case courtesy of Dr Maulik S Patel, Radiopaedia.org, rID: 13668

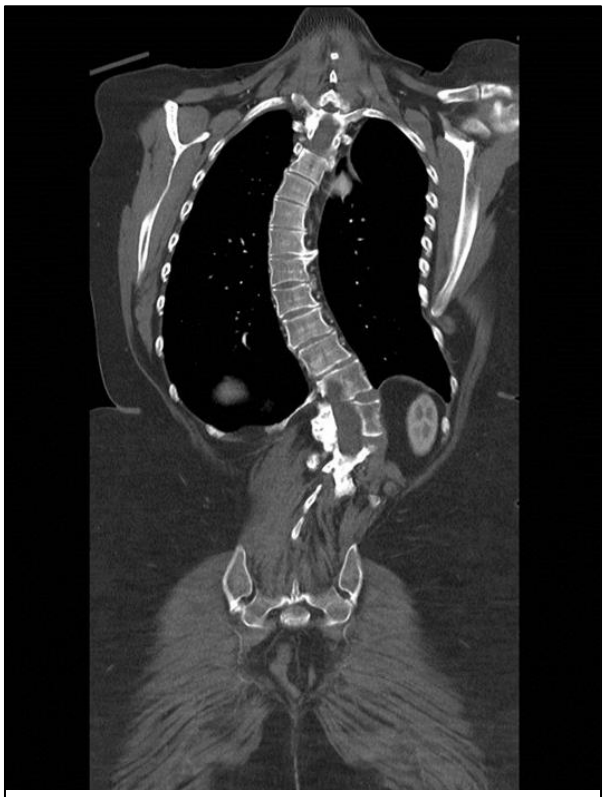

Figure 6: CT scan of a severe scoliosis. Courtesy of Niels Holmark Andersen.

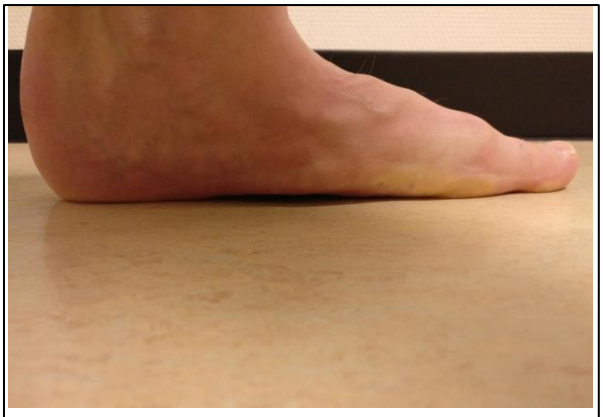

Figure 8: Pes planus (plain flat foot). The arch or instep of the foot collapses and comes in contact with ground.

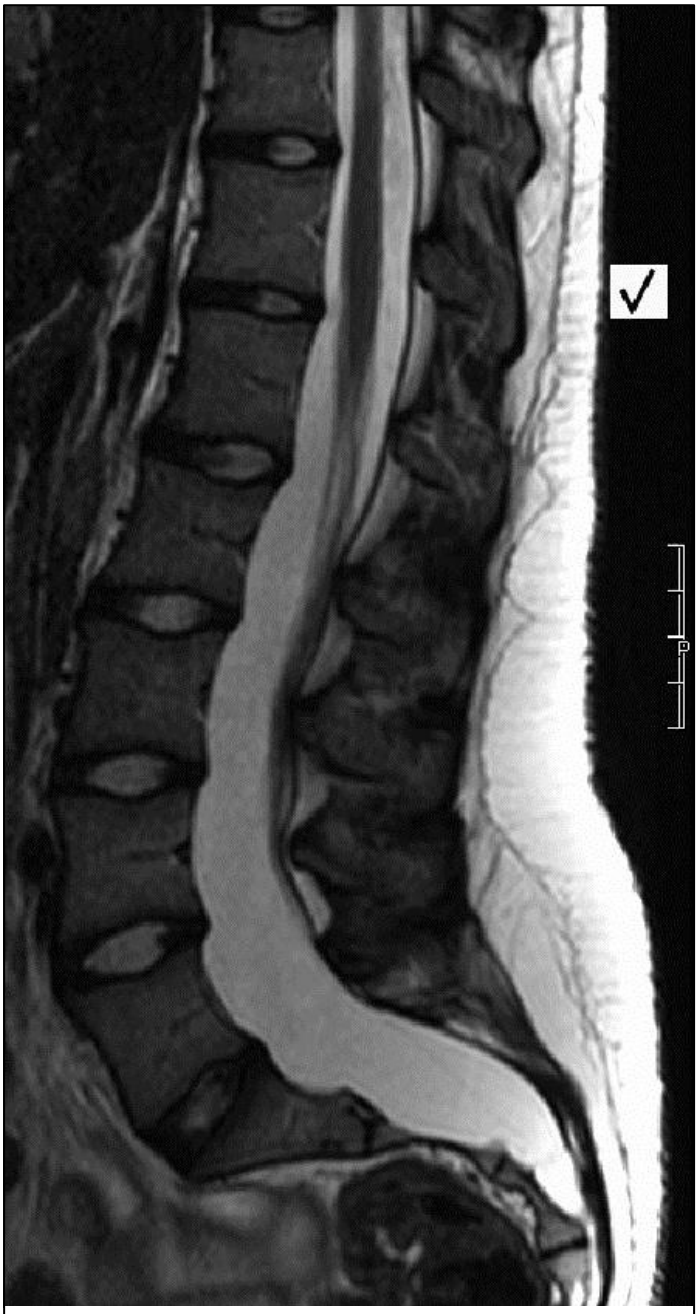

Figure 7: Dural ectasia. MR T2 sagittal scan. Dural showing bollooning or widening of the dura sac. Case courtesy of Dr Franco Ruales, Radiopaedia.org, rID: 16114

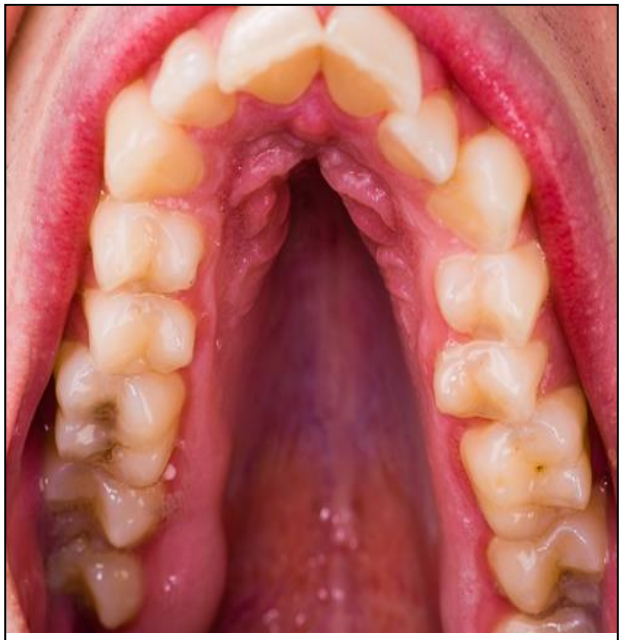

Figure 9: Highly arched palate and dental crowding.

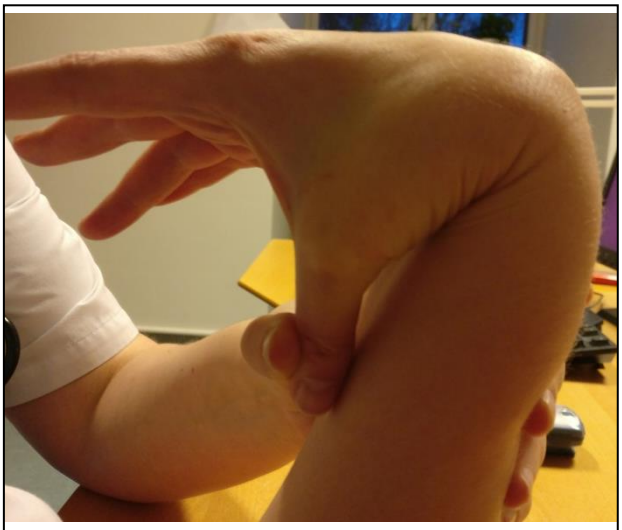

Figure 10: Hypermobile joints. Hyperextension of the thumb where the thumb can reach the forearm when bent backwards.

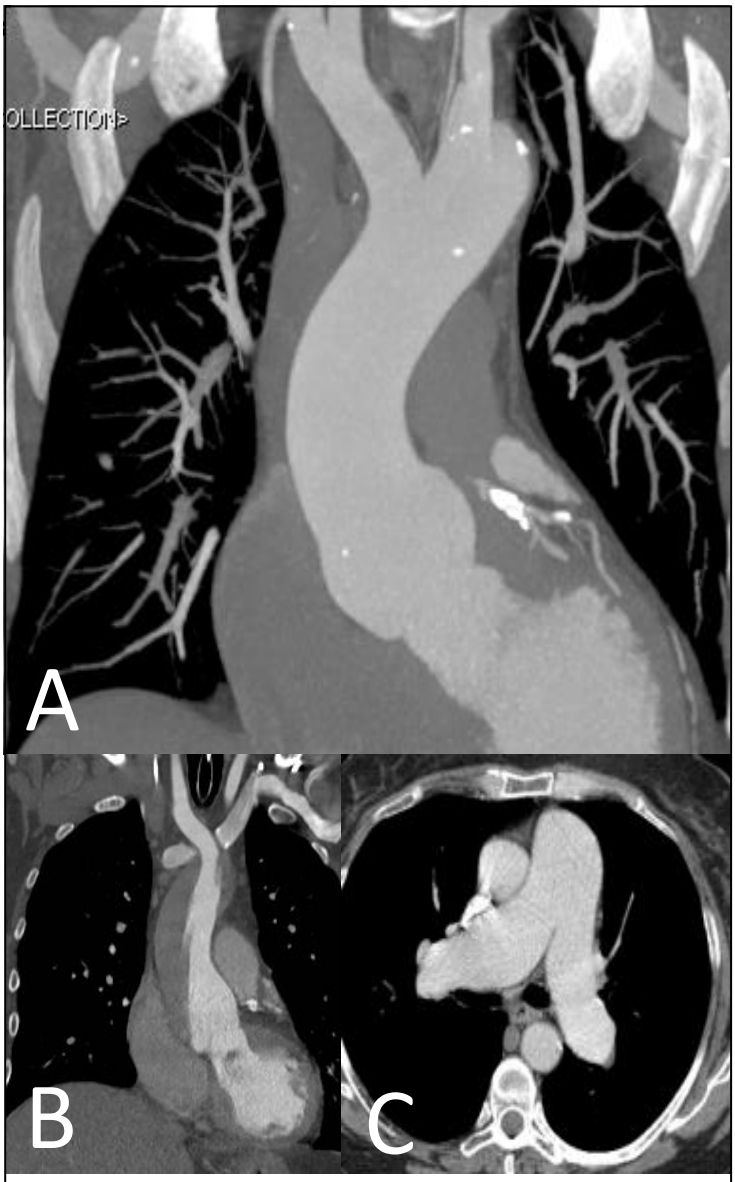

Figure 11 A-C: A: CT scan image of dilatation of ascending aorta in a 67 year old male wit MFS. B: CT scan of ascending aorta dissection in the same patient. C: CT scan of a 70 years old woman with severe dilatation of the pulmonary artery trunk and main right and left branches. Courtesy of Niels Holmark Andersen.

Marfan Syndome is probably much more complex[18, 19] than previously thought, since studies have shown an increased incidence of more unspecific features such as migraines [20], sleep apnea, and cholelithiasis [21].

As mentioned, a selected group of phenotypical features associated to MFS, are incorporated in the Ghent II criteria in the so called systemic scoring system (Figure 12).

The systemic scoring is used to clinically asses the patient for MFS by scoring points according to phenotypical features presented in the patient (Figure 12). Aortic dilatation (Figure 11A) or dissection, ectopia lentis, causal *FBN1* variants and a family history of MFS are independent indicators of MFS and "count" significantly more than the features mentioned in the systemic score. Aortic dilatation or dissection is regarded as the most serious of these features as they are associated with considerable morbidity and mortality [22, 23]. Aortic disease is also an important key in the diagnosis of MFS since the diagnosis is unobtainable without aorta dilatation/dissection either in the patient, present in the close family, or associated to a causal *FBN1* variant in the patient.

- Wrist AND thumb sign — 3 (wrist OR thumb sign — 1)
- Pectus carinatum deformity — 2 (pectus excavatum or chest asymmetry — 1)
- Hindfoot deformity — 2 (plain pes planus — 1)
- Pneumothorax — 2
- Dural ectasia — 2
- Protrusio acetabuli — 2
- Reduced US/LS AND increased arm/height AND no severe scoliosis — 1
- Scoliosis or thoracolumbar kyphosis — 1
- Reduced elbow extension — 1
- Facial features (3/5) — 1 (dolichocephaly, enophthalmos, downslanting palpebral fissures, malar hypoplasia, retrognathia)
- Skin striae — 1
- Myopia > 3 diopters - 1
- Mitral valve prolapse (all types) — 1

Maximum total: 20 points; score ≥7 indicates systemic involvement; US/LS, upper segment/lower segment ratio.

Figure 12: Phenotypical features of MFS and points given in the systemic scoring system.

MFS patients represent a wide variability in the phenotypical presentation even within families and also among persons carrying the same causal FBN1 variant[24-26]. The existence of very mild or atypical MFS presentation can be a challenge in diagnosing MFS[26]. Several phenotypical signs included in MFS diagnostic criteria, are very common in the general public often resulting challenges in defining specific MFS phenotype from common and general phenotype. Phenotypical signs like ex. pes planus, myopia or long and slender arms and legs are a common variation in the general public and are not specific for MFS as standalone

phenotypical sign .As MFS can manifest in a variety[10] of ways it is often difficult differentiate between other conditions like Loeys Dietz syndrome[27], vascular Ehlers Danlos or other connective tissue conditions. For the clinician diagnosing children it is even more challenging as the phenotype in children is not as clear as the grown up patients[28]. A study from 2012 showed that almost all patients fulfilling Ghent II also fulfilled the earlier Ghent I criteria[9], so it is reasonable to expect a high correlation between studies using either diagnostic criteria.

In general, the Ghent II criteria are complex and difficult to use for a non-expert. Ghent II operates with two scenarios, one where the patient has a family history of MFS and one without (or only sporadic MFS in the family). In this sense a family history of MFS means that a first degree family member (e.i. siblings, parents, or children) fulfill the MFS diagnosis. So in the case of a family history of MFS, a patient can obtain the diagnosis by having either one or more of the following features:

1. Aorta dilatation/dissection
2. Ectopia lentis
3. Seven or more systemic points

In cases of sporadic MFS in the family (i.e. no family history) the diagnosis can be obtained by one of the following:

1. Aortic dilatation/dissection and ectopia lentis
2. Aortic dilatation/dissection and seven or more systemic points
3. Aortic dilatation/dissection and a causal *FBN1* variant
4. Ectopia lentis and a causal *FBN1* variant associated to aortic dilatation.

Other genetic conditions can feature differential diagnostic difficulties. A range of genetic conditions are associated with aortic disease in the thoracic aorta and therefor referred to a Thoracic Aortic Aneurysm and Dissection condition (TAAD) and is divided in a syndromic form and a non- syndromic form[29, 30]. The syndromic form is associated with diseases like MFS, Loeys Dietz, Turner or Ehlers-Danlos syndrome in which there are also seen abnormalities in other organs. The non-syndromic form is not associated with any special phenotype and the causality is often unknown or multifactorial including the presence of hypertension[31]. However, in about 20% of cases of non-syndromic TAAD[32, 33] there is also aortic disease in the closest family.

These subgroups are often referred to as *familial thoracic aortic aneurysm and dissection* (FTAAD). During recent years an increasing number of genes have been associated with FTAAD[34] and with the introduction of Next Generation Sequencing (NGS) the opportunities to identify new genes causing FTAAD have improved.

The need for an *FBN1* gene test when diagnosing Marfan syndrome

According to the Ghent II criteria, a patient with aortic dilatation as a sole clinical manifestation should be evaluated for causal *FBN1* variants since the MFS diagnosis can be obtained simply by having aortic dilatation and a causal *FBN1* variant. No other phenotypical features are actually necessary. Therefore, genetic testing in diagnosing MFS has proved to be increasingly important[35]. Studies show that up to 91% of patients that fulfill the Ghent I criteria have mutations in the *FBN1* gene[36] and it is debatable whether the remaining patients in reality do have MFS or could suffer from another (not yet discovered) condition. So the question is whether testing for *FBN1* mutation is the way forward when suspecting MFS in a patient? This issue will be dealt with below.

The *FBN1* gene and MFS

The *FBN1* gene is a large gene, placed on chromosome 15[37]. It consists of 2871 amino acids divided into 65 coding exons[38]. The gene is coding for the extracellular protein Fibrillin-1 that is the backbone component in microfibrils widely distributed in both elastic and non-elastic connective tissue. Microfibrils act as a scaffold for the deposition of tropoelastin which is a keys regulatory mechanism in elastogenisis and the formation of elastin in large arteries, lungs and skin[39, 40]. Microfilbrils also play a major role in regulating the growth factors of the transforming growth factor beta (TGF-β) and by disorder in this mechanism somehow results in the development of MFS[41].

In theory, discovery of the *FBN1* gene as causal of MFS[4] provides a new tool to diagnose MFS. However, when using older techniques like Sanger sequencing (se later), the sequencing was expensive and time-consuming mainly because of the big size of the *FBN1* gene. Only a few years ago it was quite normal that the clinician had to wait up to two years before receiving an answer on a *FBN1* test. Since MFS has been (and still is) a clinical diagnosis independent of finding a causal *FBN1* variant and because of the pricing on

sequencing, clinicians often did not prescribe a genetic test, unless it was essential for establishing a diagnosis.

In 1977 Sanger et al described a method to sequence DNA[42] that since have evolved to a kind of "gold standard of DNA sequencing" that through innovation has led to a variety of techniques that use this methodology in sequencing. This basic methodology is often described as the "Sanger method". The Human Genome Project (1990-2003) was a turning point in genetic sequencing both because the project mapped the entire genome but also because the project pushed high scale sequencing techniques forward[43]. In 2008 a new technique emerged and sequencing changed from long final reads to small reads that matched to a reference genome could be merged to a final long sequence. This new way of sequencing was regarded as a new generation of sequencing and named thereafter: "Next Generation Sequencing" (NGS). NGS has since shown to be an innovative explosion outpacing the so-called "Moore's law"[*] on cost and speed of sequencing, resulting in a distinct reduction in resources used for sequencing in genetic testing.

It is our experience that the use of genetic testing has significantly changed after the introduction of NGS. Patients with only a few phenotypical points in systemic score are now being tested, resulting in more tests on patients without a clear MFS association. However the evolution of variant analysis and interpretation has in no way followed the rapid innovation in sequencing that came with NGS. Therefore, a significant problem remains on how to analyze the increasing number of sequenced variants that is a result of the high output from the NGS technology.

Analysing *FBN1* variants

In this thesis we use the term *variant* as a neutral indication of a change in the DNA compared to a reference genome as recommended by The American College of Medical Genetics and Genomics (ACMG)[44]. A variant is

[*] Moore law: The observation is named after Gordon E. Moore, the co-founder of Intel and Fairchild Semiconductor, whose 1965 paper described a doubling every year in the number of components per integrated circuit. The simplified version of this law states that processor speeds, or overall processing power for computers will double every two years Source: en.wikipedia.org and mooreslaw.org

simply a change in the DNA strand in comparison with the reference genome, but the term variant do not state if the DNA change is causing a disease or causing another effect in the patient or having no effect.

In the Ghent II criteria, a set of criteria for defining causality of *FBN1* variants is described. Parts of the variant causality criteria stated in Ghent II are widely accepted while other parts of the criteria are specific for Ghent II and MFS.

First of all, it is widely accepted, that variants that are shown to segregate in MFS families are causal of MFS[8]. To show segregation of a variant it is needed to test several family members as well as characterizing their phenotype. This process is time consuming and is often impossible due to family members being deceased or unwilling to participate or the index patient is adopted.

For de novo variants, the Ghent II criteria define causal variants in the *FBN1* gene as (simplified):

1. Nonsense mutation (variants creating a premature termination codon)
2. Inframe and out of frame deletion/insertion
3. Variants affecting splice sites
4. Missense variants affecting cysteine (either substitute or create cysteine residues)
5. Missense variants affecting conserved EGF sequence[†].

Furthermore, Ghent II defined "other missense variants" as causal if they were absent in 400 ethnically matched control chromosomes. Ghent II also accepted linkage to a predisposing *FBN1* haplotype as evidence of a causal *FBN1* variant. It is not clear to what extend the *Ghent II criteria for causality of variants* were based on scientific evidence even though some of the statements are generally accepted in the genetic society[44, 45]. In reality, laboratories seldom report results referring to the Ghent II variant criteria and it seems that each laboratory has its own set of criteria for evaluating variant causality and even the nomenclature can be troublesome for some laboratories[46].

ACMG have made a set for recommendations on how to evaluate variants[44]. First of all, ACMG state that terms like mutation or polymorphism should not be used and also declare that a variant should be classified

[†] EGF sequence: The *FBN1* protein contains 47 tandem domains with homology to a module found in human epidermal growth factor (EGF) precursors (source: www.umd.be/FBN1).

with one of the following modifiers: 1) Pathogenic, 2) Likely pathogenic, 3) Uncertain significance, 4) Likely benign, or 5) Benign. According to ACMG the term “likely” indicate that a variant is “benign” or “pathogenic” with a certainty of at least 90% and even stronger when not using the “likely” term. Variants of Uncertain Significance (VUS) are variants with insufficient evidence of either being disease causing or not. The demand for 90% certainty is very high, resulting in a high proportion of sequenced variants termed VUS which in clinical practice is of little diagnostic value[47]. To provide a certainty of variant effect over 90%, the laboratories need to gather sufficient evidence in the direction of either “benign” or “pathogenic”. In general, there is no genotype-phenotype relation to specific areas of the *FBN1* gene, except for variants in exon 24 to exon 32 which are associated with variants of a severe form of MFS often called “neonatal MFS” causing early onset of severe MFS manifestations and death in the neonatal period[48]. However, other variants in the same neonatal exons have shown milder forms of MFS, so variants cannot be defined solely on location in the gene[25, 48, 49].

The vast majority of the genetic variants is found to be benign and represents a part of our genetic variation. When analyzing for a disease causing variant in sequenced variant data, the laboratories need to find the one (or maybe more than one) disease causing variant among several possible variants. When evaluating variants, the analyst normally uses a filtering system to exclude variants that are unlikely to cause disease or include variants that are likely to cause disease. For a rare disease as MFS it is reasonable to expect a disease causing variant to be rare and therefore the variants may not be common in the general population. So the first step would be to only evaluate the rare variant and sort out the common variants. Different laboratories use different setoff points to when a variant is rare. In reality, most disease causing *FBN1* variants have not been seen in the current reference databases so the setoff point is probably not really important as long as it is set lower than 5%. If any of the resulting rare variants are of a nature where they are obviously pathogenic like nonsense, frameshift or large deletion variants, they are interpreted as being “pathogenic”. Other less obvious variants are more difficult to analyze and unfortunately they are also the variants that are found in a large number of patients[39].

Variant databases

There is a set of different tools that can be used to evaluate variants, but none of them are able to fully determine if a variant is pathogenic or benign and can only provide guidance or support of a final conclusion. Laboratories often try to establish an association between the disease and the variant by finding the same disease in another patient with the same variant. Variant databases are often used for this association. The ACMG and the Ghent II criteria actually do not accept this as a sole indication of causality but our experience is that laboratories often use this kind of association as proof of causality. Several variant databases exist with different characteristics. We identified four variant databases that were publicly available. The Human Gene Mutation Database (HGMD)[50] which is based on published variants throughout the human genome. It is our impression that the HGMD database is widely used by many laboratories as a general reference resource. The Universal Mutation Database for *FBN1* (UMD-*FBN1)*[51] is a database reserved for only *FBN1* variants and consist of published variants as well as unpublished variants provided as "personal communication" by laboratories related to the database. The ClinVar database (National Center for Biotechnology Information(NCBI))[52] is an initiative from NCBI and is recommended by the ACMG as the variant database to use. ClinVar consist of reported variants from laboratories (mostly unpublished) and to some extend also published variants. The Universal Protein Resource (UniProt) database[53] is a European initiative focusing on protein sequencing and annotation data. UniProt provide a so called "Knowledgebase" were they have published variants and their association to diseases such as MFS.

While working with variant analysis, it is quite clear that some of the data in the databases are not very valid but it is not clear to what extend that data is unreliable. A study by Yang et al.[54] showed that some variants associated with MFS by the HGMD database are very common in the reference database ESP6500 (ESP) (Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/)) that is a set of 6503 sequenced exomes. The study by Yang et al showed that there are in fact variants associated with MFS in HGMD database that are highly common in the background population which does not correlate with the conception that MFS is a rare disease. The

different problems listed above, show that the present databases and the current approach has different pitfalls, which will be dealt with below. The objective of study 2 was to evaluate the quality of the large quanta of data in FBN1 variant databases. In trying to handle the large amount of data provided by the databases we developed a scoring system intended to summarize available phenotypical features associated to each variant in a single figure.

MFS prevalence

The MFS prevalence 20/100,000[55, 56] is the far most frequently cited. It seems that an early version of the textbook of Emery and Rimoins: Principles and practice of Medical Genetics is the source for this estimate[57]. The latest version of the book only refers to a rough number based on patients found in the catchment area around Johns Hopkins Hospital in Baltimore, Maryland, USA which results in a prevalence of 4-6/100,000. We found additional five prevalence studies, published over the last 70 years. All prevalence studies except tweo were based on the Berlin criteria. Lynas et al. in 1958 published a study based on the population from Northern Ireland and reported a prevalence of 1.5/100,000[58]. Sun et al. (1990) published a study based on data from China and reported a prevalence of 17.2/100,000 [59]. Gray et al[60] (1994) published data from the north-east Scottish population and reported a prevalence of 6.8/100,000. Fuchs et al. (1997) reported a prevalence of 4.6/100,000[61] based on a Danish population. In the study of Fuchs et al, all cases were diagnosed before 1993 and the diagnosis was drawn from medical records.

In 2014, Chiu et al. published a study with a considerably higher prevalence of 10.2/100,000 based on data from Taiwan collected from 2000 to 2012. The diagnosis in this study was collected solely on diagnosis registration in registries and without any regard to diagnostic criteria or clinical presentation[62]. None of the published prevalence studies were based on the Ghent-I or the Ghent-II criteria or are clinically verified MFS and no prevalence studies reported on genetic *FBN1* mutation data.

Mortality in MFS

Mortality data in MFS has only been reported by very few studies. In 1993 an American/Scottish study reported an increase in survival with a median cumulative probability of survival from 48 years in 1972 to 72

years in 1993[63]. Others have also reported mortality data and the general tendency is an increased survival for MFS patients over time[22, 64, 65]. This increase in survival over time is probably caused by better treatment strategies. Many of the studies were performed on selected MFS patient groups so results cannot be extrapolated into a general MFS population.

Systemic disease MFS

MFS is regarded as a systemic disease affecting the connective tissue resulting in a variety of symptoms from several organs. Symptoms can vary even within the same family (and with the same *FBN1* variant) but a main feature in the diagnostic criteria is that the disease affects several organs. Patients are often disabled if they are affected by entities such severe scoliosis or ectopia lentis, which is also part of the syndrome. The most lethal in the condition is still the effect on the aortic vessel with aortic dilatation and eventually aortic dissection.

Aortic events in MFS

Aortic dilatation and dissection in MFS are mostly seen in the ascending aorta. The histopathological appearances are cystic media degeneration(Figure 13)[66], characterized by disruption and loss of elastic fibers and increased deposition of proteoglycans[67]. Also areas with loss of smooth muscle cells in the aortic media are seen and some evidence also indicates involvement of inflammatory cells[67].

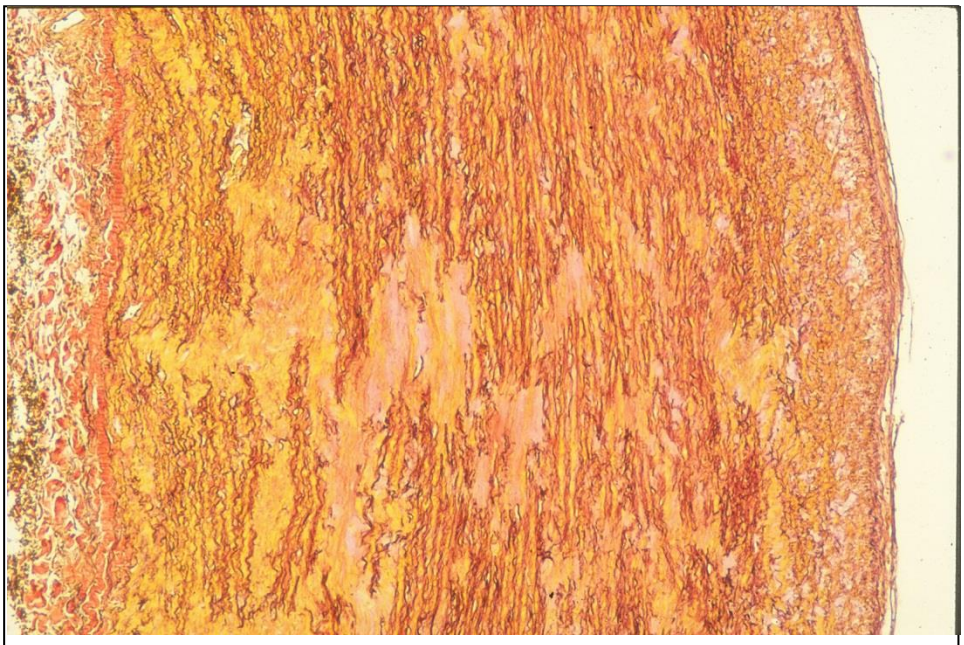

Figure 13: Gieson colored tissue of aortic vessel wall with cystic media necrosis from aMFS patient. Showing media degeneration, increase of extracellular translamellar mucoid material and minor reduction the number of smooth musclecell nuclei.
Courtesy og prof. dr. med Ulrik T. Baandrup

For MFS patients with aortic dilatation, prophylactic surgery with aortic root replacement has been the standard treatment[68-70]. Originally, the procedure was a composite aortic valve and root replacement with a mechanical valve, but aortic valve-sparing surgery has become an attractive surgical procedure during the latter years, for MFS patients with a competent aortic valve[68, 71, 72].

There seems to be a good long term prognosis after prophylactic surgery of the ascending aorta [73] and the mortality rate is below 10% over 15 years[73]. MFS patients with aortic dilatation, that for some reason do not have prophylactic aortic surgery, have a high risk of aortic dissection[74]. MFS patients with an acute aortic dissection have a great risk of reoperation or death[75], which emphasizes the need of standardized control programs for MFS patients and prophylactic surgery as recommended by the current guidelines[69]. In some cases the MFS diagnosis may only follow subsequent to the acute aortic event which can be detrimental for the patient and emphasizes the effort for an early diagnosis.

In a highly specialized and centralized outpatient clinic, the risk of death or aortic dissection in patients with known MFS is reported to be 0.17% events per year [74]. No reports outside such specialized clinical settings have been published and no data exist on a nationwide MFS cohort. There is also very little data on the morbidity and mortality of patients who suffer from aortic dissection before they are diagnosed with MFS.

Aim of the thesis

1. The aim of study 1 was to evaluate if *FBN1* databases associate *FBN1* variants with Marfan syndrome without evidence of the association.
2. The aim of study 2 was to evaluate the quality of *FBN1* databases and the variant association of *FBN1* variants to Marfan syndrome and build a combined curated FBN1 variant database.
3. The aim of study 3 was to establish a cohort of all Danish Marfan syndrome patients and based on this cohort to calculate a prevalence, incidence and estimate age at diagnosis of Marfan syndrome in Denmark.
4. The aim of study 4 was to evaluate the number of aortic events in the Danish Marfan syndrome population.

Study 1 and 2 originate from data accessed in publicized literature and *FBN1* databases. Study 1 only deal with a small set of variants not likely to cause MFS, but associated with MFS in the HGMD database. Study 2 evaluates all public available *FBN1* variants.

Study 3 originates from data received from Danish registries and subsequent evaluation of patient medical files to evaluate wither each patients did in fact have Marfan syndrome or not. Study 4 is based on the cohort established in study 3. Additional data received from The Danish National Patient Registry (DNPR) on aortic events like aortic dissection or aortic surgery was used to evaluate aortic events in the Danish Marfan syndrome population. The cohort was also updated so mortality in the cohort during 2014 was recorded.

Methods

Background study 1

Yang et al. [54] presented an evaluation of common variants in the NHLBI GO Exome Sequencing Project (ESP) classified as "disease causing" in the widely used Human Gene Mutation Database (HGMD)[50]. According to Yang et al. the prevalence of MFS in the literature is estimated to be 1 per 5,000 individuals[76, 77]. Based on this prevalence Yang et al. expected to find a maximum of two patients with disease causing *FBN1* variants in the ESP database. Yang et al. found 100 individuals with a *FBN1* variant that in the HGMD database were stated to be disease causing. These 100 individuals carried 23 different variants indicating that the HGMD database provides misinterpretation of common variants as disease causing.

Methods study 1

Since the aim of the study was to evaluate if FBN1 databases associates FBN1 with MFS without evidence of association we evaluated these 23 variants in four databases and reviewed the according literature, we searched the HGMD professional database[78] , Universal Mutation Database for *FBN1* (UMD-*FBN1*)[51], the ClinVar database (National Center for Biotechnology Information)[52] and the Universal Protein Resource (UniProt) database[53] for the 23 variants. In each database we identified reference material as detailed as possible. Published peer reviewed articles were all identified via PubMed searches and all of the material was accessible. The UMD-*FBN1* database also contained data classified as "personal communication" which is not published in the literature and therefore only accessible via the UMD-*FBN1* database homepage (www.umd.be/*FBN1*/).

Each variant was evaluated according to published information. The accessible data was evaluated according to the Ghent II nosology and each variant was evaluated as "Not MFS", "Maybe MFS" or "Inconclusive". "Not MFS" stating that the variant does probably not cause MFS based on a majority of reported phenotypes that do not fulfill the Ghent II nosology. "Maybe MF" stating that the variant could cause MFS but there is not full documentation for genotype-phenotype association. "Inconclusive" indicates that the data was insufficient to evaluate the variants effect on the phenotype. The term "MFS" was also intended to be used to describe

variants that cause an evident MFS phenotype but none of the variants had a clear genotype-phenotype association with a MFS phenotype fulfilling the Ghent II nosology. The manual evaluation of the variants was then compared with the database conclusions.

Methods study 2

We created a database with all publicly available *FBN1* variants and all known associated case-based MFS phenotype data. All data was manually evaluated on a case-based level and relevant phenotype data according to the Ghent II nosology [8] was extracted and entered into our database. Each record was linked to a reference that represents the source of the record. We searched the same four databases as in study 1 (UMD-*FBN1*, HGMD, ClinVar, UniProt) for all known *FBN1* variants. In each database, we identified the reference articles and other materials as detailed as possible. Published peer reviewed articles were all identified via PubMed searches. All of the scientific papers written in English were evaluated and nine references in Chinese were discarded. It was not possible to get access to 12 references recorded in the data base PubMed representing 15 of 4,904 variant entries in the database. The "personal communication" option accessible via the UMD-*FBN1* database homepage (www.umd.be/*FBN1*/) was also recorded.

While evaluating papers for variants referred in the databases, we found additional variants (n=168) that for unknown reasons were not registered in any of the databases. These variants were also registered in our database and evaluated in the current setup.

Each record on a variant was regarded as a specific individual representing a specific phenotype. Only records where it was possible to identify a specified individual, a specific phenotype was classified. In cases of possible multiple reports on the same individual, only one record was evaluated. For all individuals reported more than once in the literature, the scientific report with the most detailed phenotype was used and all other records representing the same individual were discarded.

Each record was classified in one of seven groups:

1. “Non-Classified” representing records where no phenotypic data was available or the recorded individual was already registered in the database.
2. “Polymorphism” representing records stating that the variant was found in individuals not having MFS or stated as a polymorphism.
3. “MFS Berlin” representing records without detailed phenotypical data but describing the individual as fulfilling the Berlin criteria of MFS[1].
4. “MFS Ghent I” representing records without detailed phenotypical data but describing the individual as fulfilling the first revised Ghent criteria of MFS[2].
5. “MFS Ghent II” representing records without detailed phenotypical data but describing the individual as fulfilling the second revised Ghent criteria of MFS[8].
6. “Incomplete MFS” representing records without detailed phenotypical data but describing the individual as incomplete MFS, with MFS habitus, MFS-like phenotype etc.
7. “Clinical classification” representing records with phenotypic data.

During evaluation of *FBN1* databases we registered if the database associated the variant with MFS. If the variant at least once was associated to MFS we defined the variant as a database MFS diagnosis (database-MFS).

Marfan-score

To provide phenotypical data with a numeric value, the Marfan-score was established.

For all cases classified as "clinical classification" the Marfan-score (Table 1) was based on the "systemic criteria" in the Ghent II nosology[8], based on the provided clinical data. Since aortic dilatation/dissection is not a part of the systemic criteria in the Ghent II nosology, but nevertheless a very important clinical feature, we chose to score aortic dilatation/dissection with 10 points. Moreover, a causal *FBN1* variant and aortic dilatation is sufficient for diagnosing MFS, according to Ghent II nosology [8].

		Point
Polymorphism		-10
Non classified		0
MFS Berlin		5
MFS Ghent I		8
MFS Ghent II		10
Incomplete MFS		2
Clinical classification	Handsign + thumbsign	3
	Only handsign	1
	Only thumbsign	1
	Spontaneous pneumothorax	2
	Pectus carinatum	2
	Pectus excavatum	1
	Hindfoot deformity	2
	Plain flat foot	1
	Dural ectasia	2
	Protucio acetabulae	2
	Upper/lower segment & arm/height ratio	1
	Scoliosis or thoracolumbar kyphosis	1
	Reduced elbow extension	1
	3 of 5 facial features	1
	Skin striae	1
	Severe myopia	1
	Mitral valve prolapse	1
	Aorta dilatation/dissection	10
	Fulfilling Ghent II criteria	20 + systemic points

Table 1: Points in the Marfa-score

We also regarded a clinical presentation of a patient with a clear MFS phenotype as the best marker for the genotype-phenotype association of a variant and MFS. To highlight the effect of high quality phenotype records and secure that these records had impact on the mean score of a given

genetic variant's Marfan-score, cases fulfilling the Ghent II criteria solely based on phenotypical data (ex. lens luxation and aorta dilatation) were added additional 20 points.

We regarded a Marfan-score equal to or larger than seven as associated with MFS (score-MFS) but it is obvious that a higher Marfan-score had a greater significance of association than a lower score. In theory, it is possible to score more than seven points in the Marfan-score without fulfilling the Ghent II criteria, but a score of seven points would only be found in patients with at least some phenotypical characteristics typical for MFS. Therefore, a cutoff point of seven was a conservative estimate when evaluating database diagnoses.

For all cases defined as a "polymorphism", "MFS Berlin", "MFS Ghent I", "MFS Ghent II" or "Incomplete MFS" the Marfan-score was defined as in Table 1. All "Non-classified" records were not scored.

Methods study 3 and study 4

Since 1968, all Danish citizens have a unique personal identification number (CPR-number) in the Danish Central Person Register (www.cpr.dk) which is used in a number of Danish registers, thus providing a unique opportunity for record linkage, including The Danish National Patient Registry (DNPR)[79, 80] and The Danish Register of Cause of Death (DRCD)[81]. From 1977 and onwards, the DNPR registered all in-patient contacts with the Danish healthcare system and from 1995, also registered all outpatient contacts. All contacts were given an International Classification of Diseases (ICD) code (ICD-8 until 1993 and ICD-10 from 1994 and onwards). DRCD record all death certificates since 1973 according to the ICD system, and used ICD-8 in 1973-1983, and ICD-10 from 1984 and onwards. DRCD was updated through 2013 in study 3 and through 2014 in study 4.

We retrieved CPR-numbers from all persons recorded in at least one of the two registries with the ICD-10 diagnosis Q87.4 "Marfan Syndrome" or ICD-8 759.80 "Arachnodactylia (syndroma Marfan)".

As several persons were noted with an ICD-8 or ICD-10 diagnosis of MFS in the DNPR register only based on the suspicion of suffering from MFS, all medical records were evaluated manually, to confirm or reject the

diagnosis. When a patient had positive signs fulfilling the diagnostics criteria according to Ghent II, we accepted the diagnosis did not continue to search for further phenotype data in the patient file. As the MFS diagnosis has evolved considerably during the years with the changing criteria, Berlin[1], Ghent-I[2] and Ghent-II[8], we chose to perform the medical record evaluation according to the Ghent-II criteria[8]. Medical records were accessed via a central electronic patient journal system (E-journal) provided by the Danish Healthcare System. If the E-journal material was insufficient to determine whether the person had MFS or not, the original medical file was obtained.

If we, during the evaluation, found other persons such as family members that could also have MFS, we evaluated their MFS status as well (*Figure 14*).

There are seven ways a person can meet the Ghent-II criteria (described in the introduction). All patients who fulfilled at least one of the seven principal diagnostic features were classified as "MFS", whereas all who did not were classified as "not MFS".

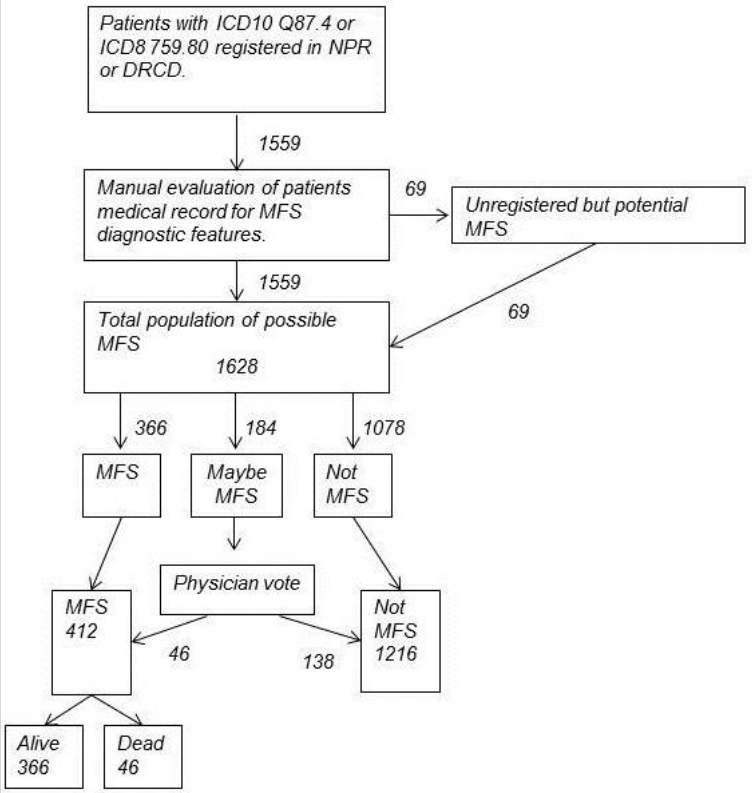

Figure 14: The total cohort and evaluation process defining patients with MFS.

If medical records were insufficient (or non-existing) in both electronic and non-electronic versions, or if for some reason (ex. deceased or emigrated) it was not possible to fully determine the persons MFS status, a committee of three MFS specialist physicians evaluated the available data and determined the MFS status by consensus. All persons with no clinical data were classified as "not MFS".

For all patients defined as "MFS" we retrieved codes of diagnosis and operations associated with aortic events from DNPR and DRCD (for details, see Table 1S in supplemental material), as well as the relevant

dates. We discriminated between two types of aortic events: 1) prophylactic aortic surgery i.e. aortic surgery before aortic dissection, or 2) acute aortic dissection without prior prophylactic surgery. We were not able to discriminate between types of aortic dissection as most surgery codes were without specified anatomical location. We also registered aortic dissection subsequent to prophylactic surgery as a secondary aortic event. We defined the date of the aortic event as the first date of a registration of the surgical code of aortic intervention or the first date of a registration of dissection. If the MFS diagnosis was registered up to 15 days before the aortic event, it was still considered an event prior to the MFS diagnosis.

Statistical analysis

Age at diagnosis was studied by median age at diagnosis with range interval and time trends were studied with quantile regression including 95% confidence intervals (CI). Time trends in incidence including 95% confidence intervals (CI) were analyzed using Poisson regression. To graphically illustrate time trends in incidence we used linear regression lines. Gender difference and difference between the cohort with MFS and without MFS were studied using Mann-Whitney's nonparametric test. $P< 0.05$ was considered significant.

Since MFS is a genetic disorder the risk time started at birth and exposure-time was calculated from date of birth to date of first registered aortic event, emigration or death, whichever came first. Kaplan-Meier estimates were calculated for the first aortic event. Hazard ratios (HR) and p-values were calculated using Cox regression analyses.

Stata 12.1 for Windows (StataCorp LP, College Station, TX, USA) was used for all calculations.

Results

Study 1 - Evaluation of 23 common variants

The HGMD was the only database to report all 23 variants (Table 2). The UMD-*FBN1* reported 22 variants, ClinVar reported eight variants and UniProt reported five variants (Table 3). Most references were reported by several of the four databases but none of the four databases covered all identified references and all databases reported references not included in any of the other databases (supplemental table S2).

The study was based on variants associated with MFS by the HGMD database, therefore we expected all 23 variants reported as "Disease Causing Mutations" and we confirmed all variants to be associated to MFS. In the UMD-*FBN1* database, 22 of the 23 variants were obtained in the database and 21 variants were reported as "Mutations". One variant was in a single sub-record classified as a "polymorphism" but the overall classification of this specific variant was still "Mutation". All 22 registered variants could therefore be interpreted as "Mutations". The sub-record stating "polymorphism" was based on a patient with the X-linked Lujan-Fryns syndrome. Historically the term "polymorphism" is defined as a common variant with a frequency of more than 1% in the background population. However, in modern literature the term is seldom used. While most references reported by UMD-*FBN1* database were based on patients with MFS or MFS-like phenotypes some references were based on other conditions and syndromes as well. This resulted in an association between variants and a variety of phenotypes and syndromes. We registered up to seven different conditions/phenotypes associated to one variant. This may not be a problem since the *FBN1* gene is associated to other conditions than MFS, but in some cases the database stated syndromes like Lujan-Fryns syndrome[‡] which is not associated with the *FBN1* gene.

[‡] Lujan-Fryns syndrome is an X-linked genetic disorder with mental retardation and a Marfanoid habitus. The condition is by OMIN.org classified as X-linked recessive but the condition has also been reported in female patients.

Table 2: Summary of reference articles reviewed and the diagnostic conclusion. The conclusion is based on published data and according to the Ghent II criteria. Not MFS: Variant does probably not cause MFS as the majority of reported phenotypes with this variant do not fulfill the Ghent II criteria. Maybe MFS: Indicates that the variant could result in the MFS phenotype but the full documentation is not provided on genotype-phenotype association. Inconclusive: Assessable data on the variant is inconclusive to assess the variant effect on phenotype.

Nr	Variant	Patients with MFS	Patient with unknown MFS status	Patients without MFS	Diagnostic conclusion
1	c.59A>G	0	0	1	Inconclusive
2	c.1027G>A	0	1	0	Inconclusive
3	c.1345G>A	(1)	0	0	Maybe MFS
4	c.2056G>A	0	0	1	Inconclusive
5	c.2927G>A	(1)	1	0	Maybe MFS
6	c.3058A>G	0	0	3	Not MFS
7	c.3422C>T	0	2	0	Inconclusive
8	c.3509G>A	0	8	12	Not MFS
9	c.3797A>T	0	1	0	Inconclusive
10	c.3845A>G	0	3	0	Inconclusive
11	c.4270C>G	3	4	5	Maybe MFS
12	c.6055G>A	0	0	2	Not MFS
13	c.6700G>A	0	3	2	Not MFS
14	c.7241G>A	0	2	0	Inconclusive
15	c.7379A>G	0	3	0	Inconclusive
16	c.7660C>T	0	1	0	Inconclusive
17	c.7661G>A	1	0	1	Inconclusive
18	c.7702G>A	0	2	0	Inconclusive
19	c.7846A>G	0	2	0	Inconclusive
20	c.7852G>A	0	2	3	Not MFS
21	c.8081G>A	0	1	0	Inconclusive
22	c.8176C>T	0	11	15	Not MFS
23	c.8494A>G	0	2	0	Inconclusive

Table 3: Summary of conclusions in databases and conclusions of the manual evaluation of background material in this study. ESP count is the number of alleles registered in ESP6500 with the specific variant. UMD-FBN1, ClinVar and UniProt stated as classification conclusion and disease association. All variant in HGMD as expected classified as disease causing and associated with MFS.

Nr	ESP count	UMD-*FBN1*	ClinVar	UniProt	This study conclusion
1	3	Mutation: Incomplete MFS	NR	MFS	Inconclusive
2	2	Mutation	NR	NR	Inconclusive
3	2	Mutation: Incomplete MFS	NR	NR	Maybe MFS
4	1	Mutation	NR	NR	Inconclusive
5	2	Mutation: Classical MFS	Uncertain significance: AllHighlyPenetrant	NR	Maybe MFS
6	3	(2)Mutation/ (1)Polymophism: Classical MFS/ Lujan-Fryns syndrome/ isolated skeletal features	Pathogenic/ Likely pathogenic: MFS	NR	Not MFS
7	14	Mutation: Classical MFS/ Isolated skeletal features	Uncertain significance: AllHighlyPenetrant	NR	Inconclusive
8	25	Mutation: Classical MFS/ Isolated skeletal features/ incomplete MFS	Pathogenic/ Likely pathogenic: MFS, subdiagnostic variant of	MFS	Not MFS
9	4	Mutation	NR	NR	Inconclusive
10	3	Mutation: MFS	NR	NR	Inconclusive
11	4	Mutation: Incompletes MFS/ Classical MFS/ Shprintzen-Goldberg syndrome/ Unknown/ Marfanoid syndrome	Pathogenic/ Likely pathogenic: MFS	MFS	Maybe MFS
12	1	Mutation: Probable MFS	NR	NR	Not MFS
13	8	Mutation: Incomplete MFS	conflicting data from submitters: MFS, AllHighlyPenetrant	NR	Not MFS
14	1	Mutation	NR	NR	Inconclusive
15	2	Mutation: MFS	NR	NR	Inconclusive
16	1	Mutation: Incomplete MFS	NR	NR	Inconclusive
17	1	Mutation: Classical MFS	NR	NR	Inconclusive
18	1	Mutation	NR	NR	Inconclusive
19	4	Mutation	NR	NR	Inconclusive
20	2	Mutation: Incomplete MFS	Pathogenic/ Likely pathogenic: MFS	MFS	Not MFS
21	1	Mutation	NR	NR	Inconclusive
22	14	Mutation: Isolated skeletal features /classic MFS/ Incomplete MFS/ MASS/ Mafanoid syndrome/ Lujan-Fryns syndrome/ AAA	conflicting data from submitters: MFS	MFS	Not MFS
23	1	Mutation	NR	NR	Inconclusive

Eight of the 23 variants were registered in the ClinVar database. Four of these variants were classified with the term “Pathogenic/Likely pathogenic” under the heading “Clinical significance”. Two variants were classified as being of “Uncertain significance” and two were classified as “conflicting data from submitters” which somehow also indicates an uncertain conclusion. Of the eight registered variants, six were associated with MFS. These six variants were all classified as either “Pathogenic/Likely pathogenic” or “conflicting data from submitters”. Variants labeled “Uncertain significance” was stated to be “All Highly Penetrant”. The meaning of the “All Highly Penetrant” statement is unclear.

Of the 23 variants the UniProt database recorded five variants. All variants in the UniProt database were associated with MFS.

In the material referred to by the databases, we did not find any clear evidence of a phenotypical association to MFS in any of the 23 variants (Table 2 and 3). Three variants were evaluated to be “Maybe MFS” as we did not find documentation of variant association to MFS. The term “Maybe MFS” was used as the leak of documentation do not exclude that the variants could cause MFS. Fourteen variants were found “Inconclusive” during our evaluation due to very little information on genotype-phenotype correlation. We classified six variants as “Not MFS” because the majority of the reported patients described with these variants did not fulfill the Ghent II criteria.

FBN1 database (study 2)

In our dataset, we identified 4,904 single records in 307 references distributed on 2,250 *FBN1* variants. In 2,303 records we were able to identify a specific individual and in the remaining 2,601 records we could not identify a specific individual or the records were representing an already published individual. During evaluation of the databases we identified additional 168 variants not registered in any of the databases resulting in a total of 2,082 variants for database evaluation.

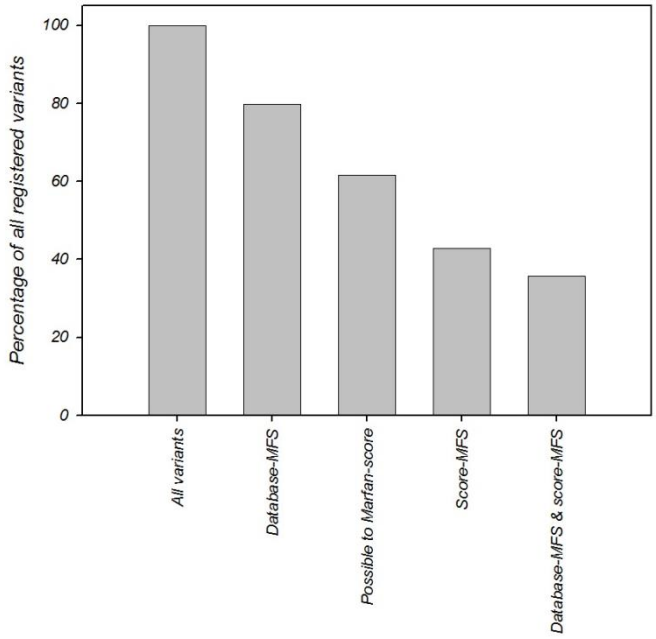

Figure 15: Percentages of all variants in different subgroups. Database-MFS represents variants associated with MFS at least in one *FBN1* database. Possible to Marfan-score represents all variants classifiable with a Marfan-score. Score-MFS represents variants with a Marfan-score ≥7. Database-MFS & score-MFS represent all variants associated with MFS in at least one *FBN1* database and with a Marfan—score ≥7.

Database-MFS and score-MFS

We were able to calculate a Marfan-score in 1,283 of the 2,082 variants (61.6%). In 893 variants representing 69.8% of all scoreable variants the score exceeded a Marfan-score ≥7 which we regard as likely associated with clinical MFS in the following classified as "*score-MFS*".

We found 1,661 variants (79.8%) (Figure 15, Table 4) associated with MFS by at least one database in the following stated as "*database-MFS*". Comparing variants in the databases associated with MFS (database –MFS) and variants not associated with MFS (non-database-MFS) we found a generally higher Marfan-score for database-MFS than non-database-MFS. The mean Marfan-score for database-MFS ranged from high 13.64 in UMD-*FBN1* to low 10.51 in ClinVar. For non-database-MFS variants, the mean Marfan-score ranged from high 8.67 in UMB-*FBN1* to low -7.16 in ClinVar.

Comparing score-MFS with database-MFS, we could only confirm the correlation between database-MFS and score-MFS in 746 variants representing 35.8% of all registered variants (Figure 15) or 77.1% of all scoreable variants. Evaluating each database individually, we found correlations between database-MFS and score-MFS in 612 variants (81.5%) in UMD-*FBN1*, 516 (76.7 %) in HGMD, 71 (68.3%) in ClinVar and 169 (76.8%) in Uniprot (Table 4).

As a marker for no phenotypical association between the variant and MFS (non-score-MFS), we defined a variant with a Marfan-score <7 as being without a phenotypical association i.e. the term “non-score-MFS”. Comparing database-MFS with non-score-MFS showed that the UMD-*FBN1 had* 18.5% of variants classified as database-MFS that were non-score-MFS. For HGMD the number was 33.3%, ClinVar 31.7%, and UniProt 23.2% (Table 4). When evaluating score-MFS with non-database-MFS we found score-MFS in 54.4% of all scoreable non-database-MFS. This indicates that databases do contain incorrect conclusions on variants.

Table 4: Overview of database characteristics. Score-MFS represents variants with a Marfan-score ≥7. Non score-MFS represents variants with a Marfan-score <7. Database-MFS represents variants associated with MFS in the *FBN1* database. Non-database-MFS represents variants not associated with MFS by an *FBN1* database.

	UMD-*FBN1*	HGMD	ClinVar	Uniprot	Total
Total number of variants (%)[1]	1,840 (88.0%)	994 (47.5%)	329 (15.7%)	252 (12.0%)	2,082
Unique variants only registered in the specific database (%)[2]	857 (46.6%)	50 (5.0%)	198 (60.2%)	2 (0.8%)	1107 (53.2%)
Database-MFS (%)[2]	1254 (68.2%)	894 (89.9%)	240 (72.9%)	226 (89.7%)	1661 (79.8%)
Possible to Marfan-score (%)[2]	1,113 (60.5%)	739 (74.3%)	126 (38.3%)	230 (91.3%)	1,283 (61.6%)
Score-MFS (%)[3]	830 (74.6%)	542 (73.3%)	73 (57.9%)	171 (74.1%)	893 (69.7%)
Database-MFS and possible to Marfan-score (%)[3]	751 (67.5%)	673 (91.1%)	104 (82.5%)	220 (95.7%)	967 (75.4%)
Mean Marfan-score for database-MFS (range)	13.64 (-10 to 33)	12.22 (0 to 34)	10.51 (-10 to 30)	12.99 (0 to 29)	
Score-MFS and database-MFS (%)[4]	612 (81.5%)	516 (76.7%)	71 (68.3%)	169 (76.8%)	746 (77.1%)
Non score-MFS and database-MFS (%)[4]	139 (18.5%)	157 (33.3%)	33 (31.7%)	51 (23.2%)	221 (22.9%)
Non database-MFS and Possible to Marfan-score (%)[3]	180 (16.2%)	43 (5.8%)	17 (13.5%)	4 (1.7%)	195 (20.2%)
Mean Marfan-score non database-MFS (range)	8.67 (-10 to 32.5)	4.4 (-10 to 28)	-7.16 (-10 to 0)	0.25 (0 to 1)	
Score-MFS and non database-MFS (%)[5]	106 (58.9%)	13 (30.2%)	0 (0%)	0 (0%)	106 (54.4%)
Non score-MFS and non database-MFS (%)[5]	74 (41.1%)	30 (69.8%)	17 (100%)	4 (100%)	89 (46.6%)

[1]% of all variants registered in all four databases. [2]% of total registered variants in the specific database.

[3]% of variants possible to Marfan-score in the database. [4]% of possible to Marfan-score & database-MFS.

[5]% of possible to Marfan-score & non database-MFS.

Forming the Danish MFS cohort

For all persons registered in the DNPR and DRCD with a relevant ICD-8 or ICD-10 diagnosis (supplemental Table 1S) we extracted 1559 unique CPR-numbers (*Figure 14*). In addition, we found 69 extra potential MFS patients during the evaluation of medical records forming a final total cohort of 1628 patients.

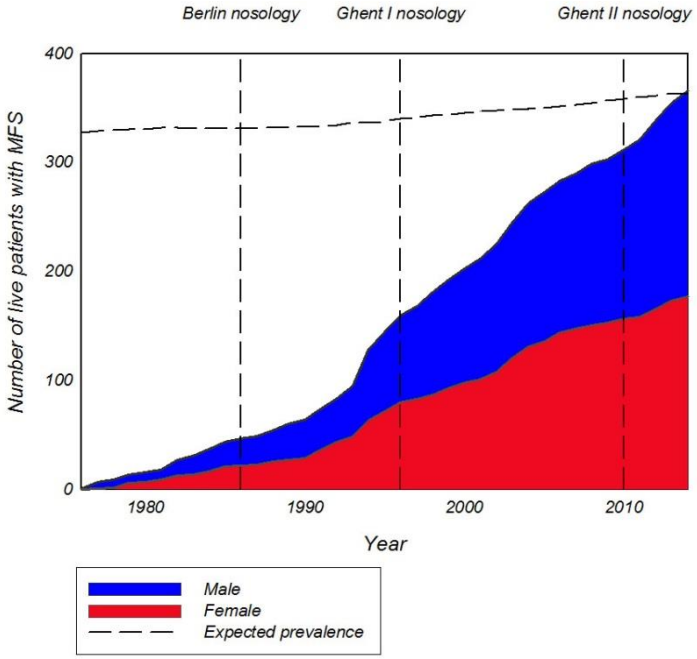

Figure 16: Observed cumulated absolute number of Marfan syndrome patients alive per year during the study period from 1977 to 2014. The dashed line (expected prevalence) indicates the expected number of Marfan syndrome patients assuming a prevalence of 6.5 per 100,000 Danish inhabitants. The year of change of nosology is indicated by a horizontal line and marked with the nosology name.

Of the initial 1628 patients we found 366 (22.5%) fulfilling one of the seven ways of obtaining the MFS diagnosis according to Ghent II. We rejected the MFS diagnosis in 1078 (66.2%) persons since none of these fulfilled the Ghent II criteria. Due to insufficient clinical data, it was impossible to determine MFS status in 184 cases (11.3%) while we were able to obtain sufficient data on the 1444 (88.7%) to determine the MFS status.

Of the 184 undetermined cases, 73 (4.5%) were classified as “not MFS” because we found no clinical data. Out of these patients, 69 were deceased and four had emigrated. The remaining 111 cases were determined by a committee (KAG, NHA and CHG) resulting in 46 “MFS” and 65 “not MFS”. The final number of MFS was 412 (25.3%) (Male n=215) and 1216 (74.7%) not MFS.

By the end of 2013, 366 (male n=189) of the MFS cohort of 412 MFS patients were still alive (Figure 16).

There was no difference in persons classified with or without MFS in gender (p=0.3) or in birth year (p=0.2).

For data used in study 4, we updated the registry data with deaths until the end of 2014 and identified that 364 (88.3%) were alive and 48 (11.7%, men n=27) dead. The exposure time was 13,932 follow-up years.

Prevalence and incidence

We calculated a point prevalence of MFS of 6.5/100,000 by January 1st 2015 based on a Danish population of 5,659,715 citizens (www.dst.dk) at the same time.

During the study period we found an averagely increasing prevalence of 0.17/100,000 per year.

We found a significantly increasing incidence with an average of 11.1 newly diagnosed MFS patients per year (*Figure 17 and 18)*.

The median annual incidence was 0.19/100,000 (0.0 - 0.7) (*Table 5*). The absolute number of patients diagnosed with MFS increased significantly during the study period with an incidence rate ratio (IRR) of 1.03 (95%CI: 1.02 - 1.04, p<0.001) (*Figure 18*).

In an effort not to be biased by an insufficient access to patient records, in the first part of the study period we also calculated the IRR for the last 10 years of the study period (2004-2014).

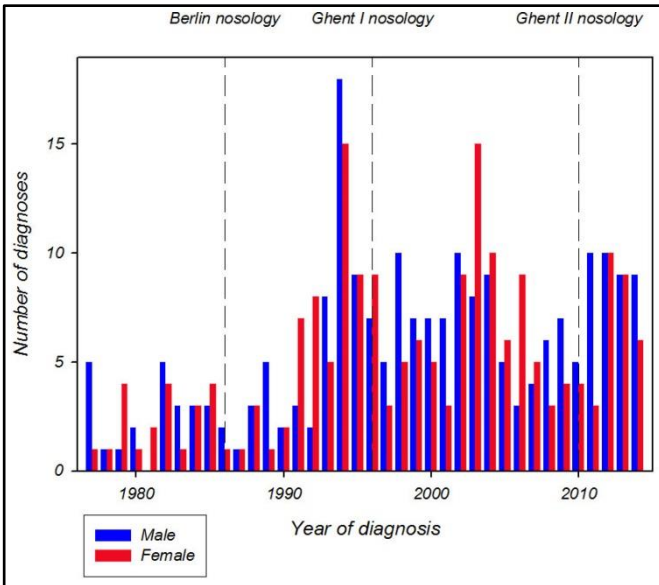

Figure 17: Number of MFS patients diagnosed per year during the study period from 1977 to 2014. Bars divided by sex. The year when the MFS nosology was changed, is indicated by a horizontal line and marked with the nosology name.

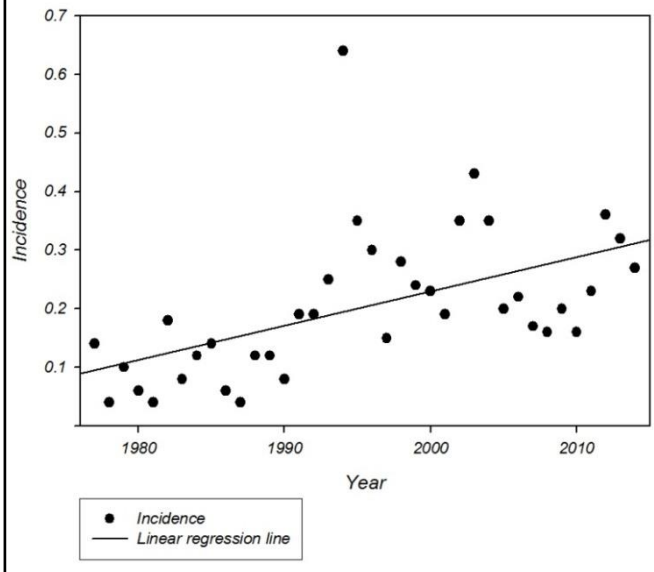

Figure 18: Yearly incidence of MFS in Denmark during the study period 1977 to 2014. For clarity, the significant increase in incidence during the study period is visualized by linear regression.

The latest 10 years of the study period the IRR increased significantly with 1.11 (95% CI 1.01 - 1.21 p=0.018). We found no IRR (p=0.47) difference in gender.

We explored different scenarios based the prevalence of MFS, calculated on the current data and visualized a forecast in the development of MFS in the general Danish population with different relative risks of mortality of 1.1, 1.25, 1.5, 2.0 or 3.0 (Figure 19). These calculations nicely show that the future number of MFS heavily depends on the prevailing rates of mortality and that better healthcare for MFS patients will increase the number in the future.

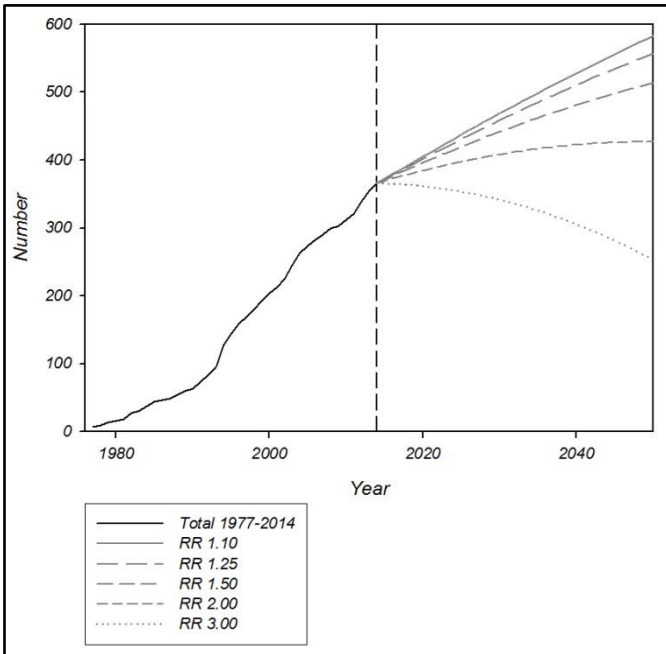

Figure 19: Absolute numbers of MFS in Denmark during the study period 1977 to 2014 and the absolute theoretical numbers extrapolated onwards to 2050. Extrapolation is based on the expected Danish population according to Statistical Denmark (www.dst.dk). Incidence is set to 0.19 per 100,000 as found in this study. Since there has been no studies reporting mortality ratios in comparison with general population, we have for illustration plotted five different relative risks (RR) of mortality compared to the general Danish population.

Table 5: Yearly incidence per 100,000 of MFS in Denmark. Data on gender only, with an accuracy of 1000 individuals for the years 1977-1979. For the line 1977-2014 the presented data are the summed number of patients diagnosed with Marfan syndrome, mean values for the Danish population and mean values for Marfan syndrome incidence.

Year of diagnosis	Male			Female			Total		
	Diagnosed	Population	Incidence	Diagnosed	Population	Incidence	Diagnosed	population	Incidence
1977	6	2,513,000	0.24	1	2,567,000	0.04	7	5,079,879	0.12
1978	1	2,520,000	0.04	1	2,577,000	0.04	2	5,096,959	0.04
1979	1	2,526,000	0.04	4	2,586,000	0.15	5	5,111,537	0.10
1980	2	2,529,053	0.08	1	2,593,012	0.04	3	5,122,065	0.06
1981	0	2,528,225	0.00	2	2,595,764	0.08	2	5,123,989	0.04
1982	5	2,523,825	0.20	4	2,595,330	0.15	9	5,119,155	0.18
1983	3	2,521,220	0.12	1	2,595,244	0.04	4	5,116,464	0.08
1984	3	2,517,942	0.12	3	2,594,188	0.12	6	5,112,130	0.12
1985	3	2,517,072	0.12	4	2,594,036	0.15	7	5,111,108	0.14
1986	2	2,520,563	0.08	1	2,595,710	0.04	3	5,116,273	0.06
1987	1	2,526,020	0.04	1	2,598,774	0.04	2	5,124,794	0.04
1988	3	2,527,996	0.12	3	2,601,258	0.12	6	5,129,254	0.12
1989	5	2,528,165	0.20	1	2,601,613	0.04	6	5,129,778	0.12
1990	2	2,530,597	0.08	2	2,604,812	0.08	4	5,135,409	0.08
1991	3	2,536,391	0.12	7	2,610,078	0.27	10	5,146,469	0.19
1992	2	2,544,454	0.08	8	2,617,672	0.31	10	5,162,126	0.19
1993	8	2,554,594	0.31	5	2,626,020	0.19	13	5,180,614	0.25
1994	18	2,563,442	0.70	15	2,633,200	0.57	33	5,196,642	0.64
1995	9	2,573,324	0.35	9	2,642,394	0.34	18	5,215,718	0.35
1996	7	2,592,222	0.27	9	2,658,805	0.34	16	5,251,027	0.30
1997	5	2,604,937	0.19	3	2,670,184	0.11	8	5,275,121	0.15
1998	10	2,615,669	0.38	5	2,679,191	0.19	15	5,294,860	0.28
1999	7	2,625,421	0.27	6	2,688,156	0.22	13	5,313,577	0.24
2000	7	2,634,122	0.27	5	2,695,898	0.19	12	5,330,020	0.23
2001	7	2,644,319	0.26	3	2,704,893	0.11	10	5,349,212	0.19
2002	10	2,654,146	0.38	9	2,714,208	0.33	19	5,368,354	0.35
2003	8	2,662,423	0.30	15	2,721,084	0.55	23	5,383,507	0.43
2004	9	2,670,135	0.34	10	2,727,505	0.37	19	5,397,640	0.35
2005	5	2,677,292	0.19	6	2,734,113	0.22	11	5,411,405	0.20
2006	3	2,685,846	0.11	9	2,741,613	0.33	12	5,427,459	0.22
2007	4	2,696,662	0.15	5	2,750,422	0.18	9	5,447,084	0.17
2008	6	2,712,666	0.22	3	2,763,125	0.11	9	5,475,791	0.16
2009	7	2,732,020	0.26	4	2,779,431	0.14	11	5,511,451	0.20
2010	5	2,743,286	0.18	4	2,791,452	0.14	9	5,534,738	0.16
2011	10	2,756,582	0.36	3	2,804,046	0.11	13	5,560,628	0.23
2012	10	2,766,776	0.36	10	2,813,740	0.36	20	5,580,516	0.36
2013	9	2,778,852	0.32	9	2,823,776	0.32	18	5,602,628	0.32
2014	9	2,792,279	0.32	6	2,834,956	0.21	15	5,627,235	0.27
1977-2014	215	2,609,146	0.22	197	2,671,729	0.19	412	5,281,280	0.20

Age at diagnosis

For the entire MFS cohort, the median age at diagnosis was 19.0 (0.0-74.5) years. We found no gender difference (median age at diagnosis: males 18.3 years (0.0-74.5) and females 19.9 years (0.0- 72.1) (p=0.3)).

Ten percent were diagnosed at the age of 1.5 years, 25% at the age of 6.5 years, and 75% at the age of 38.8 years. The remaining 25% were diagnosed throughout life and some patients were diagnosed in their seventies (*Figure 20*). During the study period there was a non-significant increasing age at diagnosis with an increase of 0.29 (95% CI -0.03-0.60, p=0.075) years per year of age at diagnosis (*Figure 21*).

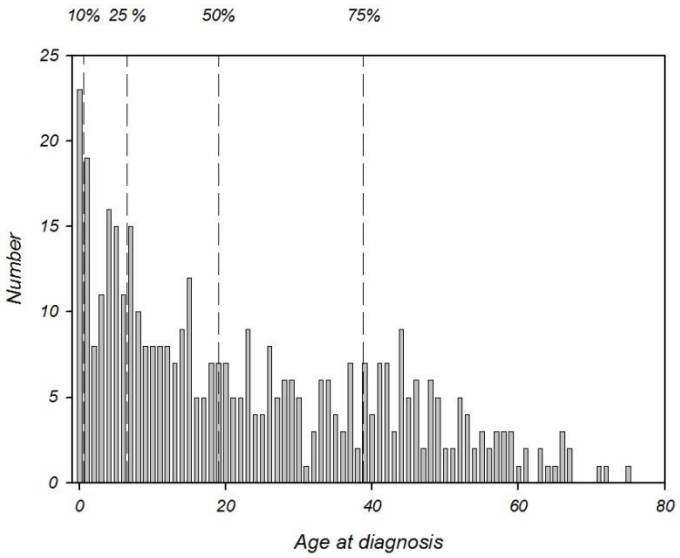

Figure 20: Number of MFS patients by age at diagnosis. Patients diagnosed during the study period 1977 to 2014. Dashed lines indicating the age when 10%, 25%, 50% and 75% of MFS patients are diagnosed.

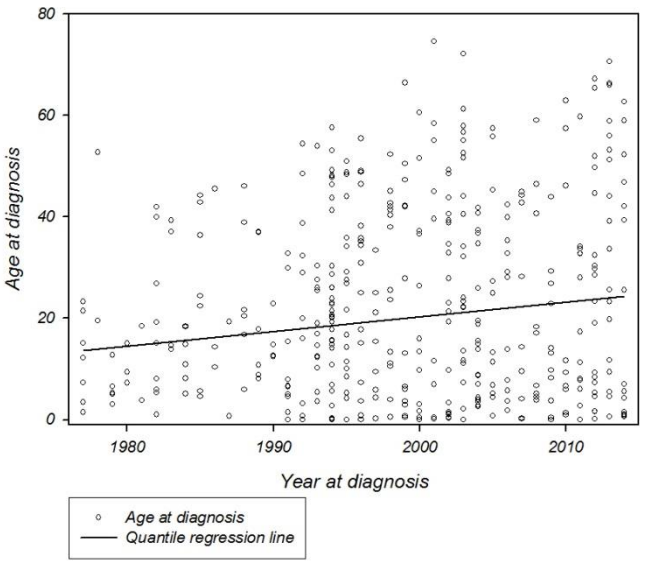

Figure 21: Age at diagnosis versus year of diagnosis during the study period 1977 to 2014. The non-significant increase in age at diagnosis is visualized by quantile regression.

FBN1 evaluation

One-hundred-ninety-six MFS patients of the total cohort of 412 patients had been genetically tested for *FBN1* mutations of which 193 did have mutations in the gene. The three cases without *FBN1* mutations did however fulfill the Ghent II criteria (dilatation of the ascending aorta and minimum seven systemic points (n=2) or by a family history of MFS and aorta ascendens

dilatation (n=1)) and was therefore still included in the MFS cohort. The three patients could in fact suffer from a MFS related disorder. One patient had only *FBN1* tested another patient had *FBN1* and a panel of collagen genes tested while the last patient was evaluated with a wide genetic panel spanning all MFS related disorders including all known genes causing aorta dilatation.

Preimplantation and prenatal diagnostics

The introduction of preimplantation and prenatal diagnostics could in theory have influenced the prevalence and incidence in our dataset. According to unpublished data from the Central Danish Cytogenetic Register extremely few patients chose preimplantation diagnostics during the last 15 years. This was primarily due to limited service. In total, 24 MFS patient had prenatal diagnostics. Out of these, 10 fetuses carried an *FBN1* mutation and in only three of these cases the parents chose an abortion before the 12th gestation week. It is unlikely that such low numbers had a significant effect on the prevalence and incidence in our dataset.

Aortic events (study 4)

Of the total MFS cohort, 150 patients (36.4%) had an aortic event during the study period (Table 6, Figure 22). At an age of 20, less than five percent had experienced an aortic event and at age 49.6 years, 50% were still free of an aortic event (Figure 23). The annual aortic event rate for the period 1994 to 2014 was 0.02 events/ year / patient. Among those who had an aortic event, 80 (53.3%) had prophylactic aortic surgery as their first event, while 70 patients (46.7%) (Figure 22, Table 6) experienced aortic dissection as their first event.

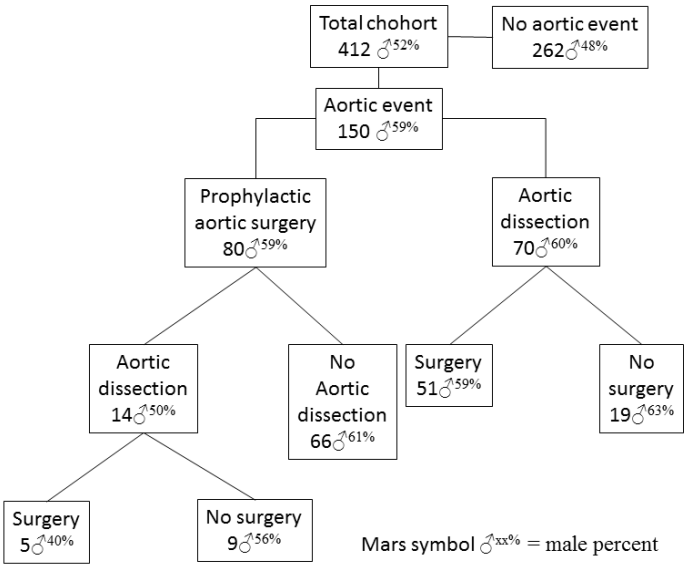

Figure 22: Aortic event data.

Males had a significantly increased risk of an aortic events (41.4%) compared to (31.0%) women. Furthermore, the aortic events appeared earlier in men with a median age of 36.5 years (range 4 to 74 years) compared to 39.2 years (range 4 to 69 years) in females. Based on these findings, we identified a significantly increased risk of aortic events in men, corresponding to a HR of 1.75 (CI 1.26 – 2.42, p=0.001) (Figure 24). Of the 48 (men=27) deceased patients, only ten patients (20.8%, men=4) did not experience an aortic event before they died.

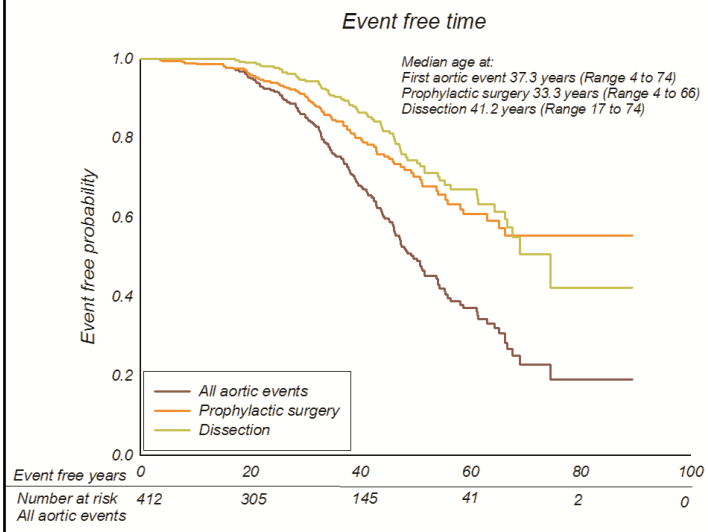

Figure 23: Curve of event free time to first aortic event divided on all aortic events, prophylactic aortic surgery and aortic dissections. The curves show the percentage of MFS patients free of aortic events at a given age. Patient years are calculated from birth date to date of first registered aortic event. Patients are censored at death or at follow up 31. December 2014. Numbers at risk only stated for all aortic events.

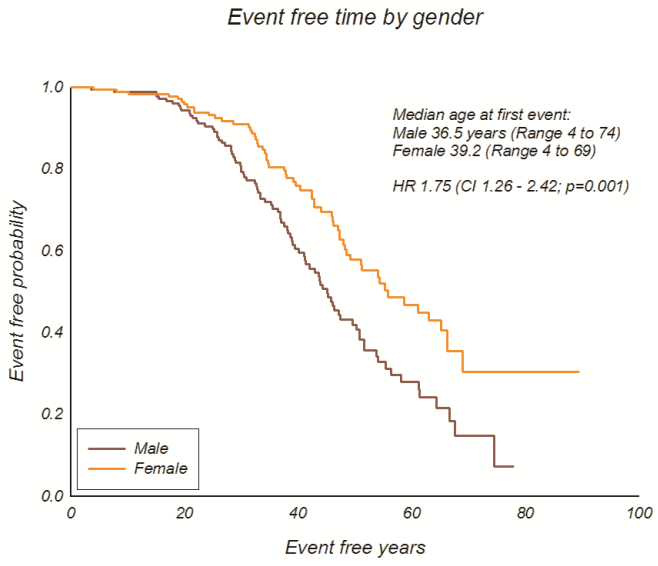

Figure 24: Curve of event free time to first aortic event divided on gender. The curves show the percentage of MFS patients free of aortic events at a given age. Patient years are calculated from birth date to date of first registered aortic event. Patients are censored at death or at follow up 31.

Table 6: Aortic event data.

		All	Men	Women	P-value
Number of patients		412	215 (52.2)	197 (47.8)	0.4
Deceased (%)		48	27 (56.3)	21 (43.8)	0.5
Deceased with aortic events (n)		38	23 (60.5)	15 (39.5)	0.3
First aortic event (%)*		150	89 (59.3)	61 (40.7)	0.03
Prophylactic aortic surgery (n)		80	47 (58.8)	33 (41.3)	0.1
All aortic dissection (%)	84	49 (58.3)	35 (41.7)	0.2	
Aortic dissection without prophylactic aortic surgery (n)	70	42 (60.0)	28 (40.0)	0.1	
With dissection and subsequent surgery (n)	56	32 (57.1)	24 (42.9)	0.4	
With dissection and no subsequent surgery (n)	28	17 (60.7)	11 (34.3)	0.3	
With dissection after prophylactic surgery (n)	14	7 (50.0)	7 (50.0)	1.00	

*First aortic event defined as either aortic surgery or aortic dissection.

Prophylactic aortic surgery

Prophylactic aortic surgery was registered in 80 patients. The age at surgery was ranging from 4 years to 66 years with a median of 33.3 years (Figure 25C). Around 25% of the operated patients had surgery before the age of 45 years (Figure 23). A 4-year old boy was the youngest to have prophylactic surgery.He was operated due to severe aortic regurgitation, aortic dilatation and a bicuspid valve.

Twelve patients with prophylactic surgery were dead at follow-up. For patients who died after prophylactic surgery the median survival time was 8.9 years (range 4 days to 25 years) and only one patient died within 30 days of surgery. Fewer females (n=33, 16.8%) compared to males (n=47, 21.9%) had prophylactic surgery.

Female patients also were older than male patients when they were operated with a median age of 34.7 years (range 4 to 66 years) for women and 32.9 years (range 4 to 58 years) for men. The hazard ratio was also significant (1.60 (CI 1.02 - 2.50, p=0.04)).

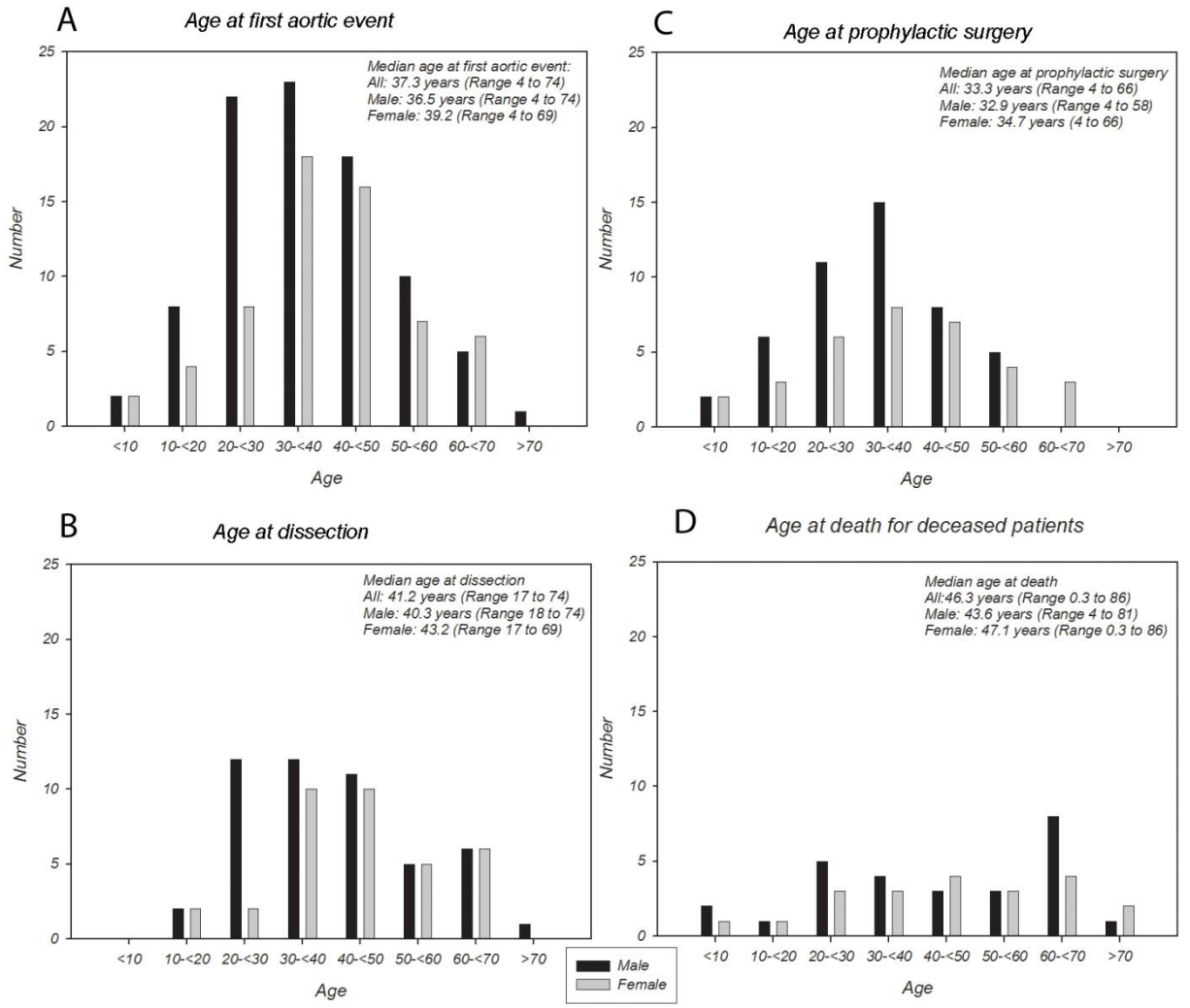

Figure 25 A-D: A: Age at first aortic event (aortic dissection or aortic surgery) divided in 10 year age intervals. B: Age at dissection divided in 10 year age intervals. C: Age at operation divided in 10 year age intervals. D: Age at death divided in 10 year intervals.

Prophylactic aortic surgery followed by aortic dissection

Fourteen (men n=7) patients who had prophylactic aortic surgery also experienced aortic dissection later on in life (Figure 22). This number represents 17.5% of all patients, who had prophylactic aortic surgery. The median time from prophylactic aortic surgery to aortic dissection was 11.4 years (range 7 days to 28.4 years) and three aortic dissections were registered within 30 of days of surgery.

Aortic dissection

In total, we registered 84 patients with aortic dissection. For 70 of the 84 patients the first aortic event was aortic dissection. When evaluating the subgroup of patients experiencing aortic dissection as their first event we found an age range from 17 to 74.5 years (Figure 25 B). Fewer female (n=28, 14.2%) than male patients (n=42, 19.5%) experienced aortic dissection as their first aortic event and women were older than men with a median age of 43.2 years (range 17 to 69 years) for female patients and 40.3 years (range 18 to 74 years) for male patients with a significant hazard ratio of 1.66 (CI 1.03 – 2.67, p=0.04).

Of patients with aortic dissection, 56 underwent subsequent aortic surgery. Forty-six had surgery within 30 days of dissection and ten patients had surgery at a later stage. In the group of patients receiving early surgery, 14 patients died. Of the dead patients in the subgroup with early surgery, the median survival time was 3.3 years (range: 1 day to 15.2 years) and only four died within 30 days of surgery. In the subgroup with late surgery (surgery after 30 days of dissection) six patients died. The median survival time after surgery in the late surgery group was 4.7 years (range: 3 days to 17.7 years) .Two died within 30 days and one 47 days after surgery.

In the group of patients with aortic dissection and no subsequent surgery 11 patients died before follow-up. The median survival time for diseased non-operated patients with aortic dissection was 4 days (range: 0 days to 13.6 years). Within the first five days of aortic dissection, six patients died and the remaining five patients survived at least 408 days.

Diagnosed with MFS after the first aortic event

We identified 53 patients (12.9%) that were diagnosed with MFS after their first aortic event. In this subgroup we found an overrepresentation of aortic dissections as the first aortic event, as 44 patients, representing 83% of the subgroup had aortic dissection as their first event. This subgroup represented 52.4% of all aortic dissections in the total cohort. For patients experiencing an aortic event before their MFS diagnosis the median age at the first aortic event was 39.2 years (range 18 to 74 years). We found no gender differences (males n=33, females n=20, p=0.12).

Discussion

Evaluating the MFS diagnosis

A vital part of this thesis concerns the evaluation of patients recorded as having MFS. The process of confirming or rejecting the MFS diagnosis was similar when evaluating genotype-phenotype associated to *FBN1* variants and when evaluating patient records in patients registered with MFS in the DNPR.

With the Ghent II criteria, the diagnostics of MFS has become simpler compared to Ghent I but establishing the MFS diagnosis is still highly complex. To determine the MFS diagnosis should be an interdisciplinary team effort where the team should consist of pediatricians, cardiologists, radiologists, ophthalmologists and geneticists. The complexity of the diagnostic features like aortic dilatation, ectopia lentis, dural ectasia, protusio acetabuli, wrist sign and thumb sign, scoliosis etc., demand that doctors within each specialist area are subspecialized with knowledge of the MFS disease and the special features of the condition.

Since the syndrome is a genetic disease, an attractive shortcut to the MFS diagnosis could be genetic testing of the *FBN1* gene and by this determining whether the patient has MFS or not. This strategy is not without problems since not all patients are genetically tested and furthermore the validity of the *FBN1* analysis is not much better than the clinical assessment and must be performed by a specialist with expert knowledge in the *FBN1* gene.

Evaluating *FBN1* variants for causality

While there is general consensus about “nonsense variants” and variants that segregate in a family are causal, there is no golden standard for when to determine a sporadic variant as causal. According to the Ghent I criteria a causal FBN1 variant has status as a major criterion in the MFS diagnosis. This means that a patient with an FBN1 variant, known to cause MFS in others, need one major criterion in one organ system and involvement of a second organ system to fulfill the Ghent I MFS diagnosis. In the Ghent II criteria, FBN1 variants need to be combined with the specific phenotypical features of aortic dilatation or ectopia lentis for variants associated with aortic dilatation. It is important to emphasize that the current thesis does not

attempt to compare the validity of the Ghent I and II criteria, neither clinically or conceptually. The Ghent II criteria for causal *FBN1* variants are only mentioned in a box and repeated in the general guideline text but without a single reference to the evidence behind these recommendations. It seems that the Ghent II criteria are inspired by the work of Faivre et al[25] published a couple of years before the guidelines. According to Omim.org, the *FBN1* gene is associated to no less than eight different conditions (Acromicric dysplasia, Familial thoracic aortic aneurysm, familial ectopia lentis, Geleophysic dysplasia 2, Marfan syndrome, MASS phenotype, Scheuermann kyphosis, Stiff skin syndrome, and Weill–Marchesani syndrome 2) of which some are in no way MFS-like. Even the presence of a causal variant in the *FBN1* gene does not necessarily cause MFS. It is our impression that laboratories do not follow the criteria mentioned the Ghent II and the changes in genetic demands in the diagnosis from Ghent I to Ghent II have not had a major impact on who obtain the diagnosis. In reality the process to determine causality of sporadic *FBN1* variants is reduced to a process of associating variants with MFS phenotype in either family members of the patient or in other patients. Evaluating families for segregation is extremely time-consuming and a final conclusion can take years. With a demand for a speedup in variant analysis caused by the high output from NGS, the laboratories will with increasing extend use variant databases to provide a source to associate variants with MFS. For this reason, we regard incorrect associations between variants and MFS in the databases as a serious problem. This thesis has only evaluated the *FBN1* gene and MFS but there is reason to assume that the problem is general and consist in other diseases and genes[54, 82, 83].

Inspired by the finding by Yang et al[54] we initially evaluated the reported 23 common variants and found that none of the variants were in fact clearly associated with MFS by the peer reviewed articles that the HGMD database was referring to. The selected 23 common variants were not representative for the entire HGMD database in the sense that the variants were common and therefore most likely not disease causing. To fully conclude the extent of the incorrect association of the database, it was needed to evaluate the entire database. Since there are several *FBN1* variant databases we also had to evaluate these to determine if the inaccuracy was only a problem in the HGMD database.

Our aim of the studies 1 and 2 was to evaluate the quality of the FBN1 databases and thereby the usability of the FBN1 databases, when used in determining causality of FBN1 variants in patients with possible MFS. As patients are diagnosed according to the Ghent II criteria we therefore evaluated variants according to the Ghent II guidelines so the resulting database could be used in an up to date clinical setting.

Establishing a curated *FBN1* database and the Marfan-score as a new tool in analyzing *FBN1* variants

A major accomplishment of this thesis is the formation of a new curated *FBN1* variant database and the design of the associated Marfan-score. The review of the data that form the basis of the curated database was also quite time-consuming since it was necessary to evaluate 4,904 records for MFS phenotype characteristics. However, the access to the published material in peer review journals is easy in Denmark with a well-functioning scientific library system. Almost all material was available and the scientific library at Aarhus University quickly obtained articles even from foreign scientific libraries. The variant evaluation is based on the Ghent II criteria even though many of the reported variants are genotyped in earlier days according to the older Berlin and Ghent I criteria. All phenotypical data provided was scored according to the Ghent II criteria and therefore in theory updated. Unspecific phenotypes classified as fulfilling the Berlin, Ghent I, incomplete MFS or polymorphism were scored according to how they resemble the Ghent II phenotype. As the scoring system in this thesis is used to determine is the quality of the FBN1 databases in causality validation, the scoring algorithm seems reasonable but could provide a problem if used as a standalone diagnostic tool and we therefore do not recommend it as a clinical tool. When evaluating material for genotype-phenotype association I also experienced difficulties in determining whether reported patients did in fact fulfill the Ghent II criteria since many reports were not very precise in relation to phenotype characteristics. For instance, imprecise statements such as “Classical Marfan”, “Incomplete Marfan”, “Marfan Habitus” etc. were often used[84, 85]. Changes in diagnostic criteria were overcome by either registering specific phenotypical traits or which criterion was used in the publication. Still, changes in how to define phenotypical traits like “arachnodactyly” vs. “thumb sign and wrist sign” was a problem that was difficult to truly overcome. In practical terms this was done by only registering clear-cut phenotypical

features according to the Ghent II criteria. If the article stated that a patient fulfilled a certain set of diagnostic criteria (e.g. Ghent I) we chose the highest scoring classification (clinical classification or nosology classification).

The Marfan-score is inspired by the Ghent II systemic point system. The idea of calculating a single numerical indicator of genotype-phenotype association was used in study 2 to evaluate the general quality of database information that associates *FBN1* variants and MFS. The Marfan-score was also intended to be used as a tool when analyzing new *FBN1* variants since the Marfan-score provides a genotype-phenotype association which is highly important in determining causality in variant analysis. The Marfan-score cannot be used as a standalone diagnostic tool and the scoring system has not been evaluated in a clinical setting. The cutoff limit of the Marfan-score is not determined and here we just introduced an arbitrary limit. The used cutoff limit at seven point is based on a qualified guess and is not clinically evaluated. The Marfan-score can be used as a supplement in variant evaluation and as a quick way of determining the quality of database material provided on specific FBN1 variants. With further evaluation and innovation of the ideas provided in this thesis, the Marfan-score could evolve to a helpful diagnostic tool in determining MFS causality in the FBN1 gene and the Marfan-score could be a tool to increase the speed of the analyses to cope with the demand from NGS. If the *FBN1* database is steadily updated with newly discovered *FBN1* variants and associated phenotypes it will quickly (within a few years) increase to a size that would provide a Marfan-score on the most possible disease causing *FBN1* variants. To increase the robustness of the Marfan-score on each variant (and thereby also the robustness of the database) already discovered variants also needs to be updated with more patient phenotypes. Just providing resources for this updating is of significance but the database would then also be a significant resource.

We expect the Marfan-score to be used as a genotype-phenotype association tool in future research in the *FBN1* gene.

Creating a Marfan cohort

A second very important part of this thesis is the formation of the MFS cohort. The cohort has already proven to be important in estimating prevalence, incidence, age at diagnosis and aortic events but in the future we also expect to evaluate the impact of MFS on general mortality, morbidity, and socioeconomic status.

Forming the MFS cohort has been challenging and we had to overcome several obstacles. Manually evaluating 1628 medical files takes time. A practical problem was the access to patient records spanning a period of 37 years back in time. Even in a highly organized health care system like the Danish this can be challenging. Surely, the E-journal system gave easy access to patient records but the system only covers recent years. Therefore, it was necessary to obtain access to several different computer systems at several hospitals as well as to gain access to older paper records in the hospital archives which in many cases resulted in travelling to hospitals throughout the country. Unfortunately, some paper records had been destroyed. Legal challenges also became an important aspect in getting access to medical records since it is no longer legal to access patient records without special permissions. Luckily the project obtained a permit to access patients records which probably would not be granted today as Danish legislation (and the interpretation of the law) becomes more and more rigid. Even with full access to patient medical records the effort of determining whether a patient fulfilled the Ghent II criteria was challenging because in many patient files clinicians had not been accurate when reporting the observed phenotype. Unprecise statements as “classical Marfan” were often used without any indication of what is “classical”. The statement of classical MFS is even more arbitrary since “classical” is somehow related to the diagnostic criteria which are changing over time and thereby not necessarily “classic” any more. The condition has a broad variability in clinical presentation[10], even within the same family and with the same causal FBN1 variant, underlining that typical or classical presentation do not exist.

Changing diagnostic criteria was also a considerable obstacle. As an example, the Berlin criteria accepted arachnodactyly as a characteristic clinical sign while the Ghent II equivalent was wrist and thumb sign.

Arachnodactyly is an unspecific statement about “spiderlike fingers”, while “wrist and thumb sign” are a more specific test for long and slender fingers. So it is difficult to conclude that a patient described with “arachnodactyly” have positive wrist and thumb sign. Even obsolete phenotypical characteristics from older diagnostic criteria like highly arched palate (Ghent I) or retinal detachment (Berlin) are still used by clinicians. Even self-invented phenotypical characteristics like “classical deposition of body fat on the abdomen” were reported by some clinicians. Even when clinicians report well established phenotypical characteristics like for example wrist sign, it is not evident that the patient in fact did fulfill this characteristic sign. If the clinician does not know that the wrist sign[12] only is positive if the thumb and the fifth finger overlaps with 1 to 2 cm (or the fingernail) the sign can be misinterpreted as positive and reported so in the patient record. Concluding a patient’s phenotype is therefore extremely difficult especially when two clinicians state opposite features. Many of the criteria in the Ghent II criteria have specific definitions; therefore the patient evaluation by non-expert clinicians should not be as trustworthy as by expert clinicians. We expected that genetic testing for *FBN1* variants would be a strong indicator to determine MFS status but even this parameter is not a very robust as it is based on variant evaluation that in many cases is just as challenged as the clinical assessment of the patient.

Prevalence of MFS

In a rare disease like MFS a simple and obvious question would be to ask “how rare?” We were surprised to discover that it was very difficult to identify the original origin of the most used prevalence figure in the literature. Furthermore the figure was based on an undefined cohort of the catchment of Johns Hopkins Hospital in Baltimore. It is not clear why this figure has established itself as a truth, since several publications have evaluated prevalence figures on a better basis than the one from Johns Hopkins. Many papers referring to the prevalence of 20/100,000 are actually not references to the original figure but to other articles that again refer to other articles and so on. We only found five studies evaluating MFS prevalence of which only one was written after the introduction of the Ghent II criteria[62]. This study was based on diagnosis registration but the diagnosis was not confirmed by patient record evaluation. As shown in study 3, a registry diagnosis of MFS does not necessarily prove that the patient in fact does have MFS; therefore the reported

prevalence of 10.2/100,000 could very well be an overestimation. On the other hand the prevalence of 6.5/100,000 reported in study 3 is probably a conservative estimate, since we cannot exclude the possibility that there are still several undiagnosed cases of MFS around – some may well have died before being diagnosed, while others are still at large without a MFS diagnosis.

The MFS cohort provided in study 3 and further evaluated in study 4 is based on Danish registries and the ICD codes provided by Danish doctors. Since we a priori did not know the validity of the reported ICD codes on MFS by Danish doctors, over- or underreporting could be a problem when forming our cohort.

Routinely collected health data are a valuable source that can be used for many different purposes from administrative health system planning to epidemiologic surveillance studies determining disease outcome over time [79]. Denmark is unique in having numerous continuously updated population-based health registries with a high level of completeness, where the individual-level data recording provides an invaluable opportunity to link registries and follow patients over time[79]. Further, it allows identification of specific cohorts rapidly. Nevertheless, using routine recorded health data, the risk of misclassification should always be considered, as codes used for administrative registration may not always be correct. Validity of registries is a general problem in epidemiological studies and therefore it is always important to evaluate validity in any patient population.

Therefore, we manually evaluated each reported patient, in this way handling possible over reporting of MFS. The DNPR also register ICD codes when doctors raise suspicion of MFS (observation diagnoses that may later be confirmed or disproved). We evaluated all patients with MFS diagnoses in an effort to identify putative MFS patients who did not fulfill diagnostic criteria. In this way we have made every effort to validate all diagnoses of MFS ever given by Danish doctors and can state that we have not overestimated the prevalence of MFS. The problem of possible underreporting is difficult to estimate which emphasizes that the prevalence figure is probably a conservative figure. To the extent that some patients exist, despite having been seen in the healthcare system with MFS-related complaints and **not** given the MFS-diagnosis, a certain amount of under-reporting may take place. We cannot quantify how small or large such under-reporting may be, but we speculate that it is not prominent. To the extent that we manually evaluated all patient files, we effectively avoided any false positives, at least clinically false positives, since they all fulfilled the Ghent 2 criteria. However, there could of course still be some patients that could be characterized as

false positives, in the sense that they fulfill the Ghent 2 criteria clinically (without being genetically tested), but if they were to be genetically tested in the future and not turn out to be positive for MFS, but for example turn out to suffer from Loeys-Dietz syndrome.

Incidence

We found an increasing incidence during the study period of 1977 to 2014. Our method of identifying MFS patients via patient records will contribute to the increased incidence since more patient records are available in the later part of the observation period revealing more patients for our cohort. We also experienced a larger focus on MFS. Especially family counseling and follow-up will contribute to identification of more MFS patients and some of them even with a mild MFS phenotype.

I expect both the incidence and prevalence to increase during the next years since there seems to be a buildup process of the DNPR. The increased focus on diagnosing and handling MFS will also contribute to the numbers and we have not seen the full effect of centralizing the management of the condition in two centers in Denmark.

The access to *FBN1* testing has also changed during the latest years and we expect that all patients in the future will be genetically tested before a final MFS diagnosis. Genetic testing of patients with MFS phenotypes could on one hand result in a decrease in MFS incidence since some patients would be genetically diagnosed with differential diagnosis as ex. Loeys-Dietz or some other hereditary thoracic aortic dilatation disorder. On the other hand we expect that more family members would be evaluated and patients with a discrete phenotype would eventually be diagnosed. Also a group of patients with no other phenotypical characteristics than aortic dilatation will be genetically tested with broad gene panels and some of these patients will eventually have *FBN1* variants and therefore be diagnosed MFS. A qualified guess would be that only a minor group of patients previously diagnosed with MFS would be re-diagnosed with another condition while a much larger group of patients would be diagnosed MFS due to genetic testing which would contribute to an increased prevalence.

Interestingly, the nosology of MFS changed three times (1986, 1996 and 2010) during the study period of paper 3, but we did not see any changes in incidence or prevalence related to changes in diagnostic criteria. The effect of changes in diagnostic criteria might be delayed and contribution to the increasing incidence.

Age at diagnosis

The diagnostic centers diagnosing MFS in Denmark are organized in the pediatrics departments and we expected that patients with MFS were diagnosed in childhood or at least in their teens. As shown in study 3 this is only the case for around half the patients, since the median age at diagnosis was 19.9 years and some patients live more or less a full life with MFS before diagnosed in their seventies. This is actually a major problem as undiagnosed patients have an increased risk of aortic dissection and a much worse outcome as shown in study 4.

Since MFS is a genetic disease you could argue that age at diagnosis larger than zero is actually a diagnostic delay because one should ideally be able to diagnose MFS at birth, but this is due to the fact that many patients do not show significant diagnostic signs of MFS before they grow up. Some clinicians actually describe that patients "grow into the diagnosis". The problem of not being able to diagnose the patients because they haven't developed their phenotypical characteristics does not exclude that the patient have the disease at the time, but only that we are not able to diagnose the disease at the time. While submitting study 3 we actually had this discussion with a reviewer and the paper was rejected on this basis, eventually it was published in another journal. The term "age at diagnosis" is a more uncontroversial term and was subsequently used in the paper even though we find age at diagnosis is an actual indicator of diagnostic delay (Figure 26).

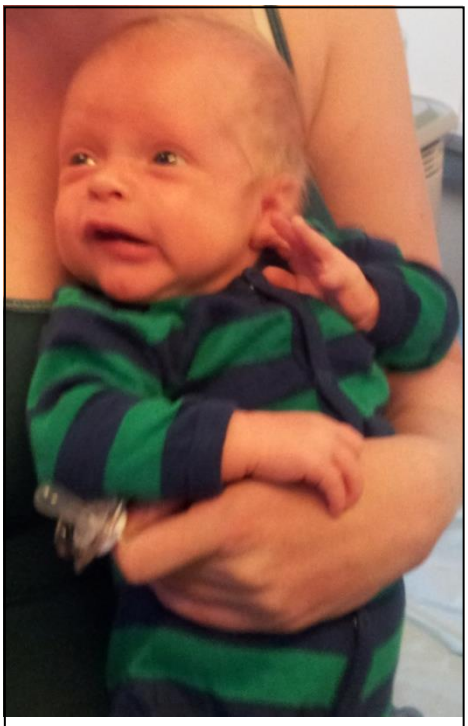

Figure 26: Baby with MFS. Courtesy: Niels Holmark Andersen.

With the increased access to genetic testing we expect that the age a diagnosis will change over the next couple of years. First of all, the family follow-up combined with genetic testing will probably identify some older MFS patients with a mild phenotype during the next couple of years. Secondly, the access to genetic testing would eventually result in an earlier diagnosis of MFS. In optimal conditions, all families with MFS should be fully evaluated and newborn patients could be diagnosed more or less at birth. Only sporadic MFS would then be a challenge to identify. The group with "sporadic MFS" is reported to be around 25% [59, 60] but all references are based on data from pedigrees and without *FBN1* testing. If this sporadic MFS percentage is true we should target to diagnose at least 75% during childhood.

Aortic events in MFS

A surprisingly large portion of MFS patients experience an aortic event of either aortic surgery or dissection in their life time (Figure 11B)[86]. It is obvious that aortic events appear with increasing age and that MFS

patients under 20 years of age only have a small risk of aortic dissection. Moreover, only a few patients have prophylactic aortic surgery in their childhood or teens. Our cohort is rather young so the data on aortic event in the elderly is not that robust but it seems that around 80% experience an aortic event during their life. One should still bear in mind how the cohort was made and since the data on the elderly is based on so few patients, just one or two patients wrongly diagnosed MFS would change these figures dramatically. It may be so that the 20% that did not experience an aortic event did actually get this old without an aortic event because they did in fact not have MFS. This is of course purely speculative.

Medical prophylactic therapy has for many years been beta blockers. Evidence is mainly based on one small study by Shores et al 1994[87] that showed a reduced dilatation rate of aorta and aortic complication. Most subsequent studies [88-95] showed a reduced dilatation rate when using beta blocker but not an effect on aortic event. Beta blockers do not provide an overwhelming effect and MFS patients still experience aortic dilatation as well as aortic events when treated with beta-blockers[87, 96]. In 2006 Habashi et al demonstrated that Lorsartan, an angiotensin II receptor blocker, could prevent aortic aneurisms to greater extent than beta blockers in a mouse model[97]. Several studies have since been publish with a tendency of smaller open-label studies showing effect of angiotensin II blockers[98-102] while the larger, placebo controlled and blinded studies showed no effect[96, 103]. For study 4 we did not have data on patient's medical therapy and therefore we unfortunately could not evaluate the effect of medical therapy on aortic events.

Gender difference in MFS

There was no gender difference in the number of MFS and the age at which the patients are diagnosed but we found a major gender difference in aortic events. Both prophylactic aortic surgery and dissection were more common in male than female patients. We have no explanation for this difference and we had actually expected that females had more aortic events than males due to guidelines recommending prophylactic aortic surgery earlier in some females planning pregnancy[104]. Our data show that a male MFS patient at any time in life will have a 75% increased chance of an aortic event compared to a female patient. Even though these data are based on MFS patients, the data might be of interest in a more general term as it seems that

the female gender has a protective feature when it comes to aortic disease. It is well known that in general male patients have an increased risk of abdominal aortic dilatation and dissection[105] and somehow it seem that this tendency also relates to the rest of the aorta. An obvious thought would be that estrogen could have a protective effect in aorta dilatation[106]. A study from 2005 found that female MFS patients dissected at an lower aortic diameter than male patients and therefore aortic intervention should be performed at a lower aortic diameter[107]. We did not have access to aorta dimension data and could therefore not correlate aorta size with events. Our study do not support a lower aortic intervention limit as we find an over representation of male patients with both prophylactic aortic surgery and aortic dissection. Older studies support our findings as they show a tendency of increased risk of aortic events in male patients[60, 62] while only one study from 1972 showed a significant difference in favor of the female gender[22]. None of the studies have shown such an overwhelming gender difference in aortic event as found in study 4.

MFS patient follow-up and a MFS database

During the process of evaluating MFS patients for the cohort, we identified patients that had fallen out of the control system. Several of these patients have re-entered a control program and many of these patient did have aortic dimensions that indicated sub-acute aortic surgery. These patients are not included in the aortic event study (study 4) as their aortic surgery was performed after follow-up. There is no centralized MFS database and no way to be sure that all patients in Denmark are in a follow up program. It is our impression that this is a significant problem especially for patients moving between the two MFS centers. A centralized MFS database with all diagnosed MFS patients would be a major benefit for the MFS patients in securing patient follow up and to prevent data loss in cases where patients move across the country. A MFS database could also be a major benefit for future research on MFS.

Data provided in study 3 could be the initial input in a Danish MFS database. A technical solution could be the already existing Raredis database with a few changes (ex. Updated to the Ghent II criteria). Raredis is a Nordic database developed in Denmark aimed at collecting clinical data on patients with a number of

different rare diagnoses (raredis.eu). To implement this setting the most significant barrier is that the clinicians need to find it relevant to provide data to the system.

Conclusions

The research performed in this thesis has shown that causality evaluation of variants based on public available *FBN1* databases must be done with caution. The quality of *FBN1* databases is low and they contain misleading information on causality of variants resulting in incorrect conclusions when evaluating *FBN1* variants. Misinterpretation of variants can lead to incorrect MFS diagnoses. The work of this thesis has provided a curated *FBN1* database as well as the introduction of a genotype-phenotype association score named "Marfan-score" – a score that can be compared across studies and across countries. The curated *FBN1* database and Marfan-score will in combination provide a fast and reliably tool to evaluate *FBN1* variants registered in the curated *FBN1* database.

The research forming this thesis also established a Danish MFS cohort based on evaluation of patient records of all patients registered with the MFS diagnosis in Denmark. From the cohort we established a prevalence figure of 6.5/100,000 among the Danish population and showed that prevalence as well as incidence is increasing and probably will continue to grow in the future years to come. The research also showed that MFS patients are diagnosed at a much higher age than expected with a median age at diagnosis at 19 years.

Based on the MFS cohort we also evaluated the number of aortic events of either aortic dissection or prophylactic aortic surgery. We showed that a large majority of MFS patients will experience an aortic event during their lifetime and highly interesting we showed that male patients at any age have a 75% increased risk of an aortic event compared to female patients.

Perspectives

This thesis consists of two major achievements within the area of MFS as mentioned above.

A future effort will be to continually update the *FBN1* database with more phenotypical entries on new as well as already known *FBN1* variants. The robustness of the database and the attached Marfan-score is determined by the number of registered *FBN1* variants and also the amount of phenotype data accessible to calculate the Marfan-score on each variant.

We expect to use the curated *FBN1* database and attached Marfan-score to continue research in the *FBN1* gene by evaluating different types of mutations in the gene and relate mutation types with the Marfan – score as a marker of severity of the MFS disease. This could potentially give new knowledge to mutation types in the *FBN1* gene and give a better understanding of correlation between specific types of mutations and severity of MFS.

The second major achievement of this thesis is the formation of a Danish MFS cohort that forms the basis of evaluating MFS in Denmark. The work in this thesis have already given new knowledge on prevalence and incidence of MFS in Denmark as well as evaluated the number of aortic events among Danish MFS patients. This information is vital in health care planning as well as a basis for MFS patient information on their condition. The Danish MFS cohort is a unique basis for further research in the condition. We have already established a control cohort via Statistical Denmark and expect to evaluate morbidity, mortality and socio-economic effects in future research projects. We also plan to update the Raredis database with the MFS cohort data and keep the MFS cohort updated with continues updates of clinical. In the future we therefore expect to perform research on updated material and even include new parameters such as aorta size based on the Raredis database.

Summaries

Background

Marfan syndrome (MFS) is a connective tissue disease caused by pathogenic variants in the *FBN1* gene. The MFS diagnosis is complex and the diagnosis should be evaluated by experts, familiar with the latest diagnostic criteria from 2010 (Ghent II). Among many syndromic traits, aortic disease is a major issue for MFS patients causing increased morbidity and mortality.

Aim

This thesis aims to uncover the quality of *FBN1* genetic variant databases. Additionally to investigate the frequency of MFS in Denmark and the age at diagnosis. Furthermore to determine the number of aortic event in Danish MFS patients.

Design

The thesis is based on four studies in two main areas:

1) Review of the quality of *FBN1* variant databases (Study 1 and Study 2)
2) Epidemiology, with focus on the frequency of MFS (prevalence and incidence study 3) and number of aorta events (Study 4).

Methods

Study 1: We reviewed all available background material on 23 common *FBN1* variants in four variant databases. Analysis was done according to recent diagnostic criteria and compared with the databases classification of the variants. Study 2: We reviewed all registered variants in four *FBN1* variant databases. Based on the most recent diagnostic criteria we developed a Marfan-score that is an aggregated variant specific single figure for the clinical observations. The Marfan-score was then compared with the databases conclusion. Study 3: From Statistical Denmark we received all patients registered with MFS. To verify the

diagnosis, all the patients records where examined according to the latest diagnostic criteria. Study 4: Based on patients identified in study 3 we extracted data on aorta events from Danish registries.

Findings

Study 1: None of the 23 variants were clearly associated with MFS though the databases indicated variants to be pathogenic. Study 2: The Marfan-score could confirm variant association with MFS in 35.8% of all registered variants, while 18.5% -33.3% (depending on database) of variants registered in the databases as associated with MFS, could not be confirmed by the Marfan score. Study 3: We identified 412 MFS patients, of whom 46 were deceased, which results in a prevalence of 6.5 / 100,000 at the end of 2014. We found a median age at diagnosis of 19 years. Study 4: In the cohort of 412 (from study 3) we found 150 (36.4%) with an aortic event. 80 (53.3%) had prophylactic aortic surgery and 70 (46.7%) had aortic dissection. The annual event rate was 0.02/year/patient. There was a massive over-representation of the aorta events in male compared with female patients resulting in a hazard ratio of 1.75.

Conclusion

The quality of *FBN1* databases is low and uncritical use of databases when evaluating a genetic diagnosis, can lead to misdiagnosing patients. MFS is a rare condition with a prevalence of approximately 6.5/100,000. Patients are diagnosed with MFS throughout life, and the majority is diagnosed in adulthood. More than one third of patients in our relatively young cohort had an aortic event. Male MFS patients have a massive risk for aortic events compared to female MFS patients.

Resumé

Marfan syndrome – diagnostik, epidemiologi og aortasygdom

Baggrund

MFS er en bindevævssygdom forårsaget af sygdomsfremkaldende varianter i *FBN1* genet. MFS diagnosen er kompleks og diagnosen bør stilles af eksperter med kendskab til de seneste diagnostiske kriterier fra 2010 (Ghent II). Blandt flere symptomer er aorta sygdom den væsentligste da den medfører betydelig mortalitet og morbiditet for MFS patienter.

Formål

Denne afhandling har til formål at afdække kvaliteten af *FBN1* variant databaser. Desuden at afklare hyppigheden af MFS i Danmark herunder patienternes alder når de bliver diagnosticeret med MFS. Desuden at opgører antallet, af aorta operationer og aorta dissektioner blandt danske MFS patienter.

Design

Afhandlingen er baseret på fire studier fordelt på to overordnede emner:

1) Gennemgang af kvaliteten af *FBN1* variantdatabaser (studie 1 og studie 2)

2) Epidemiologi med fokus på hyppighed af MFS (prævalens og incidens, studie 3) og antal aorta events (studie 4).

Metode

Studie 1: Vi gennemgik tilgængeligt baggrundsmateriale for 23 hyppige *FBN1* varianter i fire variantdatabaser. Gennemgang blev udført i henhold til de seneste diagnostiske kriterier og sammenlignede resultatet med databasernes klassificering af varianterne. Studie 2: Vi gennemgik fire *FBN1* variantdatabaser for samtlige registrerede varianter. Baseret på de seneste diagnostiske kriterier udviklede vi en Marfan-score der sammenregnede ét tal for de kliniske observationer der kunne fremskaffes for hver enkelt variant. Marfan-scoren blev herefter sammenholdt med databasernes diagnostiske konklusion. Studie 3: Vi

fremskaffede fra Danmarks Statistik alle patienter registreret med MFS. For at verificere diagnosen blev samtlige patienters journaler gennemgået efter de seneste diagnostiske kriterier. Studie 4: På baggrund af patienter identificeret i studie 3, blev der trukket data på patienternes aorta events.

Fund

Studie 1: Ingen af de 23 varianter var tydeligt associeret til MFS selvom databaserne angav varianterne til at være sygdomsfremkaldende. Studie 2: Med Marfan-scoren kunne vi bekræfte varianternes association med MFS i 35.8% af alle registrerede varianter, mens 18.5%-33.3% (alt efter database) af varianter registeret i databaserne som associeret med MFS ikke kunne bekræftes ved Marfan-scoren. Studie 3: Vi identificerede 412 MFS patienter, hvoraf 46 var afdøde, hvilket omregnet giver en prævalens på 6.5/100,000 pr. ultimo 2014. Vi fandt en median alder for diagnosen på 19 år. Studie 4: I kohorten på 412 (fra studie 3) fandt vi 150 (36.4%) med en aorta events. 80 (53.3%) var forbyggende aorta opereret og 70 (46.7%) havde dissektion. Den årlige event rate var 0.02/år/patient. Der var en massiv overrepræsentation af aorta events for mænd sammenlignet med kvinder med en hazard ratio på 1.75.

Konklusion

Kvaliteten i *FBN1* database er lav og ukritisk anvendelse af databaserne ved genetisk diagnostik kan fører til fejldiagnoser af patienter. MFS er en sjælden tilstand med en prævalens på ca. 6.5/100,000. Patienter diagnosticeres med MFS igennem hele livet og flertallet diagnosticeres i voksenalderen. Mere end hver tredje patient i vores relativt unge kohorte havde en aorta event. Mænd har en massiv overrisiko for aorta event i forhold til kvinder.

List of references

(1) Beighton P, de PA, Danks D et al. International Nosology of Heritable Disorders of Connective Tissue, Berlin, 1986. *Am J Med Genet* 1988 March;29(3):581-94.

(2) De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. *Am J Med Genet* 1996 April 24;62(4):417-26.

(3) Marfan AB. Un cas de deformation des quatre membre plus prononcee aux extremitre characterisee par l'allongment des os avec un certain degre d'amincissement. Bull Mem Soc Med Hop Paris 13, 220-221. 1896.

(4) Dietz HC, Cutting GR, Pyeritz RE et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. *Nature* 1991 July 25;352(6333):337-9.

(5) McKusick VA. Heritable disorders of connective tissue. III. The Marfan syndrome. *J Chronic Dis* 1955 December;2(6):609-44.

(6) von Kodolitsch Y, De BJ, Schuler H et al. Perspectives on the revised Ghent criteria for the diagnosis of Marfan syndrome. *Appl Clin Genet* 2015 June 16;8:137-55. doi: 10.2147/TACG.S60472. eCollection@2015.:137-55.

(7) Rybczynski M, Bernhardt AM, Rehder U et al. The spectrum of syndromes and manifestations in individuals screened for suspected Marfan syndrome. *Am J Med Genet A* 2008 December 15;146A(24):3157-66.

(8) Loeys BL, Dietz HC, Braverman AC et al. The revised Ghent nosology for the Marfan syndrome. *J Med Genet* 2010 July;47(7):476-85.

(9) Faivre L, Collod-Beroud G, Ades L et al. The new Ghent criteria for Marfan syndrome: what do they change? *Clin Genet* 2012 May;81(5):433-42.

(10) Rand-Hendriksen S, Lundby R, Tjeldhorn L et al. Prevalence data on all Ghent features in a cross-sectional study of 87 adults with proven Marfan syndrome. *Eur J Hum Genet* 2009 October;17(10):1222-30.

(11) Steinberg I. A simple screening test for the Marfan syndrome. *Am J Roentgenol Radium Ther Nucl Med* 1966 May;97(1):118-24.

(12) Walker BA, Murdoch JL. The wrist sign. A useful physical finding in the Marfan syndrome. *Arch Intern Med* 1970 August;126(2):276-7.

(13) Brown OR, DeMots H, Kloster FE, Roberts A, Menashe VD, Beals RK. Aortic root dilatation and mitral valve prolapse in Marfan's syndrome: an ECHOCARDIOgraphic study. *Circulation* 1975 October;52(4):651-7.

(14) Pyeritz RE, Fishman EK, Bernhardt BA, Siegelman SS. Dural ectasia is a common feature of the Marfan syndrome. *Am J Hum Genet* 1988 November;43(5):726-32.

(15) Canadas V, Vilacosta I, Bruna I, Fuster V. Marfan syndrome. Part 1: pathophysiology and diagnosis. *Nat Rev Cardiol* 2010 May;7(5):256-65.

(16) De CP, De PG, Martens L, de PA. Craniofacial structure in Marfan syndrome: a cephalometric study. *Am J Med Genet A* 2004 December 15;131(3):240-8.

(17) Lundby R, Rand-Hendriksen S, Hald JK, Pripp AH, Smith HJ. The pulmonary artery in patients with Marfan syndrome: a cross-sectional study. *Genet Med* 2012 November;14(11):922-7.

(18) Velvin G, Bathen T, Rand-Hendriksen S, Geirdal AO. Systematic review of the psychosocial aspects of living with Marfan syndrome. *Clin Genet* 2015 February;87(2):109-16.

(19) Velvin G, Bathen T, Rand-Hendriksen S, Geirdal AO. Satisfaction with life in adults with Marfan syndrome (MFS): associations with health-related consequences of MFS, pain, fatigue, and demographic factors. *Qual Life Res* 2016 January 4.

(20) Vis JC, Timmermans J, Post MC et al. Increased prevalence of migraine in Marfan syndrome. *Int J Cardiol* 2009 August 21;136(3):330-4.

(21) Chow K, Pyeritz RE, Litt HI. Abdominal visceral findings in patients with Marfan syndrome. *Genet Med* 2007 April;9(4):208-12.

(22) Murdoch JL, Walker BA, Halpern BL, Kuzma JW, McKusick VA. Life expectancy and causes of death in the Marfan syndrome. *N Engl J Med* 1972 April 13;286(15):804-8.

(23) Schaeffer BN, Rybczynski M, Sheikhzadeh S et al. Heart rate turbulence and deceleration capacity for risk prediction of serious arrhythmic events in Marfan syndrome. *Clin Res Cardiol* 2015 December;104(12):1054-63.

(24) Loeys B. The search for genotype/phenotype correlation in Marfan syndrome: to be or not to be? *Eur Heart J* 2016 April 20.

(25) Faivre L, Collod-Beroud G, Loeys BL et al. Effect of mutation type and location on clinical outcome in 1,013 probands with Marfan syndrome or related phenotypes and FBN1 mutations: an international study. *Am J Hum Genet* 2007 September;81(3):454-66.

(26) De BJ, Loeys B, Leroy B, Coucke P, Dietz H, de PA. Utility of molecular analyses in the exploration of extreme intrafamilial variability in the Marfan syndrome. *Clin Genet* 2007 September;72(3):188-98.

(27) Loeys BL, Schwarze U, Holm T et al. Aneurysm syndromes caused by mutations in the TGF-beta receptor. *N Engl J Med* 2006 August 24;355(8):788-98.

(28) Faivre L, Masurel-Paulet A, Collod-Beroud G et al. Clinical and molecular study of 320 children with Marfan syndrome and related type I fibrillinopathies in a series of 1009 probands with pathogenic FBN1 mutations. *Pediatrics* 2009 January;123(1):391-8.

(29) Pomianowski P, Elefteriades JA. The genetics and genomics of thoracic aortic disease. *Ann Cardiothorac Surg* 2013 May;2(3):271-9.

(30) Milewicz DM, Guo DC, Tran-Fadulu V et al. Genetic basis of thoracic aortic aneurysms and dissections: focus on smooth muscle cell contractile dysfunction. *Annu Rev Genomics Hum Genet* 2008;9:283-302. doi: 10.1146/annurev.genom.8.080706.092303.:283-302.

(31) de SG, Roman MJ, De MM et al. Hemodynamic Correlates of Abnormal Aortic Root Dimension in an Adult Population: The Strong Heart Study. *J Am Heart Assoc* 2015 September 28;4(10):e002309.

(32) Biddinger A, Rocklin M, Coselli J, Milewicz DM. Familial thoracic aortic dilatations and dissections: a case control study. *J Vasc Surg* 1997 March;25(3):506-11.

(33) Coady MA, Davies RR, Roberts M et al. Familial patterns of thoracic aortic aneurysms. *Arch Surg* 1999 April;134(4):361-7.

(34) Pyeritz RE. Heritable thoracic aortic disorders. *Curr Opin Cardiol* 2014 January;29(1):97-102.

(35) Sheikhzadeh S, Kade C, Keyser B et al. Analysis of phenotype and genotype information for the diagnosis of Marfan syndrome. *Clin Genet* 2012 September;82(3):240-7.

(36) Loeys B, De BJ, Van AP et al. Comprehensive molecular screening of the FBN1 gene favors locus homogeneity of classical Marfan syndrome. *Hum Mutat* 2004 August;24(2):140-6.

(37) Magenis RE, Maslen CL, Smith L, Allen L, Sakai LY. Localization of the fibrillin (FBN) gene to chromosome 15, band q21.1. *Genomics* 1991 October;11(2):346-51.

(38) Pereira L, D'Alessio M, Ramirez F et al. Genomic organization of the sequence coding for fibrillin, the defective gene product in Marfan syndrome. *Hum Mol Genet* 1993 July;2(7):961-8.

(39) Zeyer KA, Reinhardt DP. Engineered mutations in fibrillin-1 leading to Marfan syndrome act at the protein, cellular and organismal levels. *Mutat Res Rev Mutat Res* 2015 July;765:7-18. doi: 10.1016/j.mrrev.2015.04.002. Epub@2015 May 5.:7-18.

(40) Wagenseil JE, Mecham RP. New insights into elastic fiber assembly. *Birth Defects Res C Embryo Today* 2007 December;81(4):229-40.

(41) Neptune ER, Frischmeyer PA, Arking DE et al. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. *Nat Genet* 2003 March;33(3):407-11.

(42) Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A* 1977 December;74(12):5463-7.

(43) Collins FS, Morgan M, Patrinos A. The Human Genome Project: lessons from large-scale biology. *Science* 2003 April 11;300(5617):286-90.

(44) Richards S, Aziz N, Bale S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015 May;17(5):405-23.

(45) Plon SE, Eccles DM, Easton D et al. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum Mutat* 2008 November;29(11):1282-91.

(46) Deans Z, Fairley JA, den Dunnen JT, Clark C. HGVS Nomenclature in Practice: An Example from the United Kingdom National External Quality Assessment Scheme. *Hum Mutat* 2016 February 26;10.

(47) von Kodolitsch Y, Kutsche K. Interpretation of sequence variants of the FBN1 gene: analog or digital? A commentary on decreased frequency of FBN1 missense variants in Ghent criteria-positive Marfan syndrome and characterization of novel FBN1 variants. *J Hum Genet* 2015 September;60(9):465-6.

(48) Tiecke F, Katzke S, Booms P et al. Classic, atypically severe and neonatal Marfan syndrome: twelve mutations and genotype-phenotype correlations in FBN1 exons 24-40. *Eur J Hum Genet* 2001 January;9(1):13-21.

(49) Faivre L, Collod-Beroud G, Callewaert B et al. Clinical and mutation-type analysis from an international series of 198 probands with a pathogenic FBN1 exons 24-32 mutation. *Eur J Hum Genet* 2009 April;17(4):491-501.

(50) Stenson PD, Ball EV, Mort M et al. Human Gene Mutation Database (HGMD): 2003 update. *Hum Mutat* 2003 June;21(6):577-81.

(51) Collod-Beroud G, Le BS, Ades L et al. Update of the UMD-FBN1 mutation database and creation of an FBN1 polymorphism database. *Hum Mutat* 2003 September;22(3):199-208.

(52) Landrum MJ, Lee JM, Riley GR et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res* 2014 January;42(Database issue):D980-D985.

(53) UniProt Consortium. Activities at the Universal Protein Resource (UniProt). *Nucleic Acids Res* 2014 January;42(Database issue):D191-D198.

(54) Yang RQ, Jabbari J, Cheng XS et al. New population-based exome data question the pathogenicity of some genetic variants previously associated with Marfan syndrome. *BMC Genet* 2014 June 18;15:74. doi: 10.1186/1471-2156-15-74.:74-15.

(55) Pepe G, Lapini I, Evangelisti L et al. Is ectopia lentis in some cases a mild phenotypic expression of Marfan syndrome? Need for a long-term follow-up. *Mol Vis* 2007 November 29;13:2242-7.:2242-7.

(56) Gillis E, Kempers M, Salemink S et al. An FBN1 deep intronic mutation in a familial case of Marfan syndrome: an explanation for genetically unsolved cases? *Hum Mutat* 2014 May;35(5):571-4.

(57) Pyeritz RE. Marfan Syndrome and Related Disorders. Sixth ed. 2013. p. 1-51.

(58) LYNAS MA. Marfan's syndrome in Northern Ireland; an account of thirteen families. *Ann Hum Genet* 1958 July;22(4):289-309.

(59) Sun QB, Zhang KZ, Cheng TO et al. Marfan syndrome in China: a collective review of 564 cases among 98 families. *Am Heart J* 1990 October;120(4):934-48.

(60) Gray JR, Bridges AB, Faed MJ et al. Ascertainment and severity of Marfan syndrome in a Scottish population. *J Med Genet* 1994 January;31(1):51-4.

(61) Fuchs J. Marfan syndrome and other systemic disorders with congenital ectopia lentis. A Danish national survey. *Acta Paediatr* 1997 September;86(9):947-52.

(62) Chiu HH, Wu MH, Chen HC, Kao FY, Huang SK. Epidemiological profile of Marfan syndrome in a general population: a national database study. *Mayo Clin Proc* 2014 January;89(1):34-42.

(63) Silverman DI, Burton KJ, Gray J et al. Life expectancy in the Marfan syndrome. *Am J Cardiol* 1995 January 15;75(2):157-60.

(64) Gray JR, Bridges AB, West RR et al. Life expectancy in British Marfan syndrome populations. *Clin Genet* 1998 August;54(2):124-8.

(65) Chan YC, Ting CW, Ho P, Poon JT, Cheung GC, Cheng SW. Ten-year epidemiological review of in-hospital patients with Marfan syndrome. *Ann Vasc Surg* 2008 September;22(5):608-12.

(66) Yuan SM, Jing H. Cystic medial necrosis: pathological findings and clinical implications. *Rev Bras Cir Cardiovasc* 2011 January;26(1):107-15.

(67) Hiratzka LF, Bakris GL, Beckman JA et al. 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with thoracic aortic disease: executive summary. A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. *Catheter Cardiovasc Interv* 2010 August 1;76(2):E43-E86.

(68) Benedetto U, Melina G, Takkenberg JJ, Roscitano A, Angeloni E, Sinatra R. Surgical management of aortic root disease in Marfan syndrome: a systematic review and meta-analysis. *Heart* 2011 June;97(12):955-8.

(69) Erbel R, Aboyans V, Boileau C et al. 2014 ESC Guidelines on the diagnosis and treatment of aortic diseases: Document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. The Task Force for the Diagnosis and Treatment of Aortic Diseases of the European Society of Cardiology (ESC). *Eur Heart J* 2014 November 1;35(41):2873-926.

(70) Treasure T, Takkenberg JJ, Pepper J. Surgical management of aortic root disease in Marfan syndrome and other congenital disorders associated with aortic root aneurysms. *Heart* 2014 October;100(20):1571-6.

(71) David TE, David CM, Manlhiot C, Colman J, Crean AM, Bradley T. Outcomes of Aortic Valve-Sparing Operations in Marfan Syndrome. *J Am Coll Cardiol* 2015 September 29;66(13):1445-53.

(72) David TE, Feindel CM. An aortic valve-sparing operation for patients with aortic incompetence and aneurysm of the ascending aorta. *J Thorac Cardiovasc Surg* 1992 April;103(4):617-21.

(73) Price J, Magruder JT, Young A et al. Long-term outcomes of aortic root operations for Marfan syndrome: A comparison of Bentall versus aortic valve-sparing procedures. *J Thorac Cardiovasc Surg* 2015 October 27;(15):10.

(74) Jondeau G, Detaint D, Tubach F et al. Aortic event rate in the Marfan population: a cohort study. *Circulation* 2012 January 17;125(2):226-32.

(75) Rylski B, Bavaria JE, Beyersdorf F et al. Type A aortic dissection in Marfan syndrome: extent of initial surgery determines long-term outcome. *Circulation* 2014 April 1;129(13):1381-6.

(76) Arbustini E, Grasso M, Ansaldi S et al. Identification of sixty-two novel and twelve known FBN1 mutations in eighty-one unrelated probands with Marfan syndrome and other fibrillinopathies. *Hum Mutat* 2005 November;26(5):494.

(77) Judge DP, Dietz HC. Marfan's syndrome. *Lancet* 2005 December 3;366(9501):1965-76.

(78) Stenson PD, Mort M, Ball EV, Shaw K, Phillips AD, Cooper DN. The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Hum Genet* 2013 September 28.

(79) Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sorensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. *Clin Epidemiol* 2015 November 17;7:449-90. doi: 10.2147/CLEP.S91125. eCollection@2015.:449-90.

(80) Andersen TF, Madsen M, Jorgensen J, Mellemkjoer L, Olsen JH. The Danish National Hospital Register. A valuable source of data for modern health sciences. *Dan Med Bull* 1999 June;46(3):263-8.

(81) Helweg-Larsen K. The Danish Register of Causes of Death. *Scand J Public Health* 2011 July;39(7 Suppl):26-9.

(82) Risgaard B, Jabbari R, Refsgaard L et al. High prevalence of genetic variants previously associated with Brugada syndrome in new exome data. *Clin Genet* 2013 November;84(5):489-95.

(83) Jabbari J, Jabbari R, Nielsen MW et al. New exome data question the pathogenicity of genetic variants previously associated with catecholaminergic polymorphic ventricular tachycardia. *Circ Cardiovasc Genet* 2013 October;6(5):481-9.

(84) Perez AB, Pereira LV, Brunoni D, Zatz M, Passos-Bueno MR. Identification of 8 new mutations in Brazilian families with Marfan syndrome. Mutations in brief no. 211. Online. *Hum Mutat* 1999;13(1):84.

(85) Oh MR, Kim JS, Beck NS et al. Six novel mutations of the fibrillin-1 gene in Korean patients with Marfan syndrome. *Pediatr Int* 2000 October;42(5):488-91.

(86) Rasmussen M, Pedersen SF, Sunde L, Andersen NH, Ostergaard JR, Lildballe DL. A novel FBN1 variant in a large Marfan family with high penetrance of aortic dissection or rupture. *Dan Med J* 2014 November;61(11):A4949.

(87) Shores J, Berger KR, Murphy EA, Pyeritz RE. Progression of aortic dilatation and the benefit of long-term beta-adrenergic blockade in Marfan's syndrome. *N Engl J Med* 1994 May 12;330(19):1335-41.

(88) Barbier M, Gross MS, Aubart M et al. MFAP5 loss-of-function mutations underscore the involvement of matrix alteration in the pathogenesis of familial thoracic aortic aneurysms and dissections. *Am J Hum Genet* 2014 December 4;95(6):736-43.

(89) Selamet Tierney ES, Feingold B, Printz BF et al. Beta-blocker therapy does not alter the rate of aortic root dilation in pediatric patients with Marfan syndrome. *J Pediatr* 2007 January;150(1):77-82.

(90) Salim MA, Alpert BS, Ward JC, Pyeritz RE. Effect of beta-adrenergic blockade on aortic root rate of dilation in the Marfan syndrome. *Am J Cardiol* 1994 September 15;74(6):629-33.

(91) Rossi-Foulkes R, Roman MJ, Rosen SE et al. Phenotypic features and impact of beta blocker or calcium antagonist therapy on aortic lumen size in the Marfan syndrome. *Am J Cardiol* 1999 May 1;83(9):1364-8.

(92) Ladouceur M, Fermanian C, Lupoglazoff JM et al. Effect of beta-blockade on ascending aortic dilatation in children with the Marfan syndrome. *Am J Cardiol* 2007 February 1;99(3):406-9.

(93) Gersony DR, McClaughlin MA, Jin Z, Gersony WM. The effect of beta-blocker therapy on clinical outcome in patients with Marfan's syndrome: a meta-analysis. *Int J Cardiol* 2007 January 18;114(3):303-8.

(94) Gao L, Mao Q, Wen D, Zhang L, Zhou X, Hui R. The effect of beta-blocker therapy on progressive aortic dilatation in children and adolescents with Marfan's syndrome: a meta-analysis. *Acta Paediatr* 2011 September;100(9):e101-e105.

(95) Phomakay V, Huett WG, Gossett JM, Tang X, Bornemeier RA, Collins RT. beta-Blockers and angiotensin converting enzyme inhibitors: comparison of effects on aortic growth in pediatric patients with Marfan syndrome. *J Pediatr* 2014 November;165(5):951-5.

(96) Lacro RV, Dietz HC, Sleeper LA et al. Atenolol versus losartan in children and young adults with Marfan's syndrome. *N Engl J Med* 2014 November 27;371(22):2061-71.

(97) Habashi JP, Judge DP, Holm TM et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science* 2006 April 7;312(5770):117-21.

(98) Brooke BS, Habashi JP, Judge DP, Patel N, Loeys B, Dietz HC, III. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. *N Engl J Med* 2008 June 26;358(26):2787-95.

(99) Pees C, Laccone F, Hagl M, Debrauwer V, Moser E, Michel-Behnke I. Usefulness of losartan on the size of the ascending aorta in an unselected cohort of children, adolescents, and young adults with Marfan syndrome. *Am J Cardiol* 2013 November 1;112(9):1477-83.

(100) Mueller GC, Stierle L, Stark V et al. Retrospective analysis of the effect of angiotensin II receptor blocker versus beta-blocker on aortic root growth in paediatric patients with Marfan syndrome. *Heart* 2014 February;100(3):214-8.

(101) Chiu HH, Wu MH, Wang JK et al. Losartan Added to beta-Blockade Therapy for Aortic Root Dilation in Marfan Syndrome: A Randomized, Open-Label Pilot Study. *Mayo Clin Proc* 2013 January 12;(12):10.

(102) Groenink M, den Hartog AW, Franken R et al. Losartan reduces aortic dilatation rate in adults with Marfan syndrome: a randomized controlled trial. *Eur Heart J* 2013 December;34(45):3491-500.

(103) Milleron O, Arnoult F, Ropers J et al. Marfan Sartan: a randomized, double-blind, placebo-controlled trial. *Eur Heart J* 2015 August 21;36(32):2160-6.

(104) Groth KA, Greisen JR, Nielsen BB, Andersen NH. [High-risk pregnancy in a woman with Marfan syndrome, a bicuspid aortic valve, and a dilated aortic sinus.]. *Ugeskr Laeger* 2015 January 26;177(2A):V0313020.

(105) Lo RC, Schermerhorn ML. Abdominal aortic aneurysms in women. *J Vasc Surg* 2016 March;63(3):839-44.

(106) Wu XF, Zhang J, Paskauskas S, Xin SJ, Duan ZQ. The role of estrogen in the formation of experimental abdominal aortic aneurysm. *Am J Surg* 2009 January;197(1):49-54.

(107) Meijboom LJ, Timmermans J, Zwinderman AH, Engelfriet PM, Mulder BJ. Aortic root growth in men and women with the Marfan's syndrome. *Am J Cardiol* 2005 November 15;96(10):1441-4.

(108) Tjeldhorn L, Rand-Hendriksen S, Gervin K et al. Rapid and efficient FBN1 mutation detection using automated sample preparation and direct sequencing as the primary strategy. *Genet Test* 2006;10(4):258-64.

(109) Rommel K, Karck M, Haverich A, Schmidtke J, Arslan-Kirchner M. Mutation screening of the fibrillin-1 (FBN1) gene in 76 unrelated patients with Marfan syndrome or Marfanoid features leads to the identification of 11 novel and three previously reported mutations. *Hum Mutat* 2002 November;20(5):406-7.

(110) de PA, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. *Am J Med Genet* 1996 April 24;62(4):417-26.

(111) Hung CC, Lin SY, Lee CN et al. Mutation spectrum of the fibrillin-1 (FBN1) gene in Taiwanese patients with Marfan syndrome. *Ann Hum Genet* 2009 November;73(Pt 6):559-67.

(112) Comeglio P, Johnson P, Arno G et al. The importance of mutation detection in Marfan syndrome and Marfan-related disorders: report of 193 FBN1 mutations. *Hum Mutat* 2007 September;28(9):928.

(113) Robinson DO, Lin F, Lyon M et al. Systematic screening of FBN1 gene unclassified missense variants for splice abnormalities. *Clin Genet* 2012 September;82(3):223-31.

(114) Stheneur C, Collod-Beroud G, Faivre L et al. Identification of the minimal combination of clinical features in probands for efficient mutation detection in the FBN1 gene. *Eur J Hum Genet* 2009 September;17(9):1121-8.

(115) Attanasio M, Lapini I, Evangelisti L et al. FBN1 mutation screening of patients with Marfan syndrome and related disorders: detection of 46 novel FBN1 mutations. *Clin Genet* 2008 July;74(1):39-46.

(116) Yuan B, Thomas JP, von KY, Pyeritz RE. Comparison of heteroduplex analysis, direct sequencing, and enzyme mismatch cleavage for detecting mutations in a large gene, FBN1. *Hum Mutat* 1999;14(5):440-6.

(117) Hayward C, Porteous ME, Brock DJ. A novel mutation in the fibrillin gene (FBN1) in familial arachnodactyly. *Mol Cell Probes* 1994 August;8(4):325-7.

(118) Hayward C, Porteous ME, Brock DJ. Mutation screening of all 65 exons of the fibrillin-1 gene in 60 patients with Marfan syndrome: report of 12 novel mutations. *Hum Mutat* 1997;10(4):280-9.

(119) Dorschner MO, Amendola LM, Turner EH et al. Actionable, pathogenic incidental findings in 1,000 participants' exomes. *Am J Hum Genet* 2013 October 3;93(4):631-40.

(120) Howarth R, Yearwood C, Harvey JF. Application of dHPLC for mutation detection of the fibrillin-1 gene for the diagnosis of Marfan syndrome in a National Health Service Laboratory. *Genet Test* 2007;11(2):146-52.

(121) Waldmuller S, Muller M, Warnecke H et al. Genetic testing in patients with aortic aneurysms/dissections: a novel genotype/phenotype correlation? *Eur J Cardiothorac Surg* 2007 June;31(6):970-5.

(122) Montgomery RA, Geraghty MT, Bull E et al. Multiple molecular mechanisms underlying subdiagnostic variants of Marfan syndrome. *Am J Hum Genet* 1998 December;63(6):1703-11.

(123) Matyas G, de PA, Halliday D, Boileau C, Pals G, Steinmann B. Evaluation and application of denaturing HPLC for mutation detection in Marfan syndrome: Identification of 20 novel mutations and two novel polymorphisms in the FBN1 gene. *Hum Mutat* 2002 April;19(4):443-56.

(124) Liu WO, Oefner PJ, Qian C, Odom RS, Francke U. Denaturing HPLC-identified novel FBN1 mutations, polymorphisms, and sequence variants in Marfan syndrome and related connective tissue disorders. *Genet Test* 1997;1(4):237-42.

(125) Ng PC, Henikoff S. Accounting for human polymorphisms predicted to affect protein function. *Genome Res* 2002 March;12(3):436-46.

(126) Collod-Beroud G, Beroud C, Ades L et al. Marfan Database (third edition): new mutations and new routines for the software. *Nucleic Acids Res* 1998 January 1;26(1):229-3.

(127) Biggin A, Holman K, Brett M, Bennetts B, Ades L. Detection of thirty novel FBN1 mutations in patients with Marfan syndrome or a related fibrillinopathy. *Hum Mutat* 2004 January;23(1):99.

(128) Comeglio P, Evans AL, Brice GW, Child AH. Erratum: Detection of six novel FBN1 mutations in British patients affected by Marfan syndrome. *Hum Mutat* 2001 December;18(6):546-7.

(129) Callier P, Aral B, Hanna N et al. Systematic molecular and cytogenetic screening of 100 patients with marfanoid syndromes and intellectual disability. *Clin Genet* 2013 December;84(6):507-21.

(130) Rand-Hendriksen S, Tjeldhorn L, Lundby R et al. Search for correlations between FBN1 genotype and complete Ghent phenotype in 44 unrelated Norwegian patients with Marfan syndrome. *Am J Med Genet A* 2007 September 1;143A(17):1968-77.

(131) Sakai H, Visser R, Ikegawa S et al. Comprehensive genetic analysis of relevant four genes in 49 patients with Marfan syndrome or Marfan-related phenotypes. *Am J Med Genet A* 2006 August 15;140(16):1719-25.

(132) Loeys B, Nuytinck L, Delvaux I, De BS, de PA. 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 November 12;161(20):2447-54.

(133) Turner CL, Emery H, Collins AL et al. Detection of 53 FBN1 mutations (41 novel and 12 recurrent) and genotype-phenotype correlations in 113 unrelated probands referred with Marfan syndrome, or a related fibrillinopathy. *Am J Med Genet A* 2009 February;149A(2):161-70.

(134) Milewicz DM, Grossfield J, Cao SN, Kielty C, Covitz W, Jewett T. A mutation in FBN1 disrupts profibrillin processing and results in isolated skeletal features of the Marfan syndrome. *J Clin Invest* 1995 May;95(5):2373-8.

(135) Buoni S, Zannolli R, Macucci F et al. The FBN1 (R2726W) mutation is not fully penetrant. *Ann Hum Genet* 2004 November;68(Pt 6):633-8.

(136) Van Dijk FS, Hamel BC, Hilhorst-Hofstee Y et al. Compound-heterozygous Marfan syndrome. *Eur J Med Genet* 2009 January;52(1):1-5.

(137) Pepe G, Nistri S, Giusti B et al. Identification of fibrillin 1 gene mutations in patients with bicuspid aortic valve (BAV) without Marfan syndrome. *BMC Med Genet* 2014 February 24;15:23. doi: 10.1186/1471-2350-15-23.:23-15.

Supplemental material

Table 1S

Diagnosis codes and codes of operation indicating aortic events.

ICD 10 codes of diagnosis	
I710	Dissection of aorta [any part]
I710A	Dissection of aorta, type A
I710B	Dissection of aorta, type B
I711	Thoracic aortic aneurysm, ruptured
I713	Abdominal aortic aneurysm, ruptured
I715	Thoracoabdominal aortic aneurysm, ruptured
I718	Aortic aneurysm of unspecified site, ruptured
ICD-8 codes of diagnosis	
44109	Aneurysma dissecans aortae (ALL locations)
44111	Ruptura aneurysmatis aortae thoracalis(non syphiliticum)
44121	Ruptura aneurysmatis aortae abdominalis
NCSP codes of operation	
FCA00	Suture of ascending aorta
FCA10	Repair of ascending aorta by division and suture
FCA20	Reinforcement of ascending aorta with wrapping
FCA30	Partial resection and suture of ascending aorta
FCA40	Repair of ascending aorta using patch
FCA45	Percutaneous insertion of stent into ascending aorta
FCA50	Resection and reconstruction of ascending aorta using tube graft
FCA60	Resection of aortic root and ascending aorta with reimplantation of coronary arteries and use of composite graft with mechanical valve prosthesis

FCA70	Resection of aortic root and ascending aorta with reimplantation of coronary arteries and use of biological valve prosthesis and tube graft
FCA90	Other repair of ascending aorta
FCB00	Suture of aortic arch
FCB10	Repair of aortic arch by division and suture
FCB20	Partial resection and suture of aortic arch
FCB30	Repair of aortic arch using patch
FCB35	Percutaneous insertion of stent into aortic arch
FCB40	Resection and reconstruction of aortic arch using tube graft
FCB50	Resection of aortic arch and reimplantation of branches
FCB96	Other repair of aortic arch
FCC00	Suture of descending aorta
FCC10	Repair of descending aorta by division and suture
FCC30	Partial resection and suture of descending aorta
FCC40	Repair of descending aorta using patch
FCC45	Percutaneous insertion of stent into descending aorta
FCC50	Resection and reconstruction of descending aorta using tube graft
FCC60	Resection of descending aorta and reimplantation of branches
FCC70	Bypass to descending aorta using tube graft
FCC76	Bypass to thoracoabdominal aorta using tube graft
FCC96	Other repair of descending aorta
FCD00	Suture of thoracoabdominal aorta
FCD10	Reinforcement of thoracoabdominal aorta using suture

FCD30	Repair of thoracoabdominal aorta using patch
FCD35	Percutaneous insertion of stent into thoracoabdominal aorta
FCD40	Partial resection and suture of thoracoabdominal aorta
FCD50	Resection and reconstruction of thoracoabdominal aorta using tube graft
FCD60	Resection of thoracoabdominal aorta and reimplantation of branches
FCD70	Bypass of thoracoabdominal aorta using tube graft
FCD96	Other repair of thoracoabdominal aorta
ICD-8 codes of operation	
31249	Operatio plastica valvulae aortae
31250	Resectio aortae
31268	Excisio valvulae aortae c. Insert. Prostheseos biologica
31269	Excisio valvulae aortae c. Insert. Prostheseos mechanica
31280	Excis. Valv. Aortae et resect. Aneurysma aort.c.insert.prosth
31300	Sutura aortae thoracalis
31301	Sutura laesionis aortae thoracalis
31319	Reconstructio aortae thoracalis

31400	Resectio aneurysmatis aortae ascendentis
31401	Resectio aneurysmatis aortae ascend. C. Insert. Prostheseos
31419	Resectio aneurysmatis aortae cum insertione prostheseos
31420	Resectio aneurysmatis aortae descendentis
31439	Resect. Aneurysmatis aortae descend. C. Insert. Prosthes.
31440	Resect. Aneurysmatis arcus aortae c. Implant. Art. Colli
31459	Resect. Aneurysmatis arcus aortae cum insert. Prostheseos
31700	Resectio aneurysmatis aortae ascendentis
31760	Operatio pro ruptura aortae c.implant.prostheseos vascularis
31780	Resectio arcus aortae cum implant. Prostheseos vascular.
31820	Resectio aneurysmatis aortae ascend.cum implant.art.coron.

AARHUS UNIVERSITY

Table S1

References for each variant.

Nr	Variant	HGMD references	UMD-*FBN1* references	ClinVar references	UniProt references	Comments	Able to conclude association with MFS
1	c.59A>G	[76]	[76]	Not registered	[76]	The study is based on screening of 81 patients referred for MFS or MFS like phenotype. They found 74 *FBN1* mutations in 81 patients. They found one patient, a 14 year old boy, with the *FBN1* variant c.59A>G. The patient is registered with a "positive family history" and the phenotype is described with skeletal (but not a major criteria) involvement and pneumothorax but he had no cardiovascular or ocular manifestations. With the given phenotype the patient does not fulfill the Ghent criteria for MFS.	No
2	c.1027G>A	[108]	[108]	Not registered	Not registered	The study is based on *FBN1* screening of 105 patients suspected of Marfan syndrome. Of the 105 included patients 86 fulfilled the criteria for Marfan syndrome according to the 1996 nosology. In 105 patients they found 70 mutations in 69 patients (one had two). In all patients in which a mutation was detected the patient fulfilled the 1996 Ghent criteria. There is no other phenotype data on the patients. The phenotype-genotype correlation is not mentioned and it is therefore unclear whether the specific patient fulfilled the 2010 Ghent II criteria.	No
3	c.1345G>A	[109]	[109]	Not registered	Not registered	The study I based on *FBN1* analyses of 76 unrelated patients referred with a tentative diagnosis of MFS. Only 18 fullfilled the 1996 Ghent criteria[110]. One patient presented a c.1345G>A variant c.1345. The patient phenotype is registered as a "15-year old boy with tall stature but no typical marfanoid appearance and no	Maybe

						ocular symptoms. The cardiovascular manifestation of Marfan syndrome was severe insufficiency of aortic valve due to dilatation of aortic root." The authors declare the variant a "provisional" disease-causing variant that requires further family and/or functional studies. The patients father had mitral valve insufficiency but none of the parents was genetically testet. The patient did have aorta dilatation and a *FBN1* mutation which could be associated in which case the patient would fulfill the Ghent II criteria. Further variant and family evaluation is needed.	
4	c.2056G>A	[111]	[111]	Not registered	Not registered	The study is based on screening of 294 patients in 157 families for the presence of *FBN1* mutations. The cohort of 294 patient consisted of patients suspicious of MFS, their families and 50 controls. They found 56 mutations in 62 of the 157 families. 47 out of 157 the families fit the Ghent criteria from 1996[110]. One patients was found with a c.2056G>A variant. The registered phenotype of this patient is without cardiovascular, skeletal or ocular manifestations. Only "other manifestations" is mentioned. The patient did not fulfill the 1996 Ghent criteria.	No
5	c.2927G>A	[112]	[112]	[112]	Not registered	The study is based on *FBN1* screening of 508 patients. They found 193 variants . One 22 year old patient had a c.2927G>A variant. The patient was classified as classic MFS but the authors either not have access to phenotypical data or the data should be accessible on UMD.be UMD-*FBN1* in which case it is not public accessible.	No
			[113]			The study was made to evaluate RNA analysis on *FBN1* with focus on splicesite effects. They analysed 40 cases with 36 different *FBN1* mutations and identified 2 mutations that caused abnormality of splicing. One of the analyzed variants was *FBN1* c.2927G>A but	No

						this variant did not cause a splicing effect on the RNA.	
6	c.3058A>G	[48]	[48]	[48]	Not registered	The study is based on *FBN1* screening of 127 patients with MFS or related disorders. They found 12 mutations. One 3 year old girl did have c.3058A>G. Her phenotype was described as being tall and with hyperextensible joints and borderline wrist and thumb sign. There was at the time of examination (3 years old) no aorta dilatation or any other cardiovascular manifestation.	NO
			[114]			The study is based on 586 blood samples from probands referred for molecular diagnosis of MFS. One patient was found with the c.3058A>G variant but the variant is not found in a MFS patient but in a patient with Lujan Fryns syndrome. The syndrome is with MFS habitus but is X-linked.	No
			[115]			The study is based in *FBN1* screening of 99 patients with MFS or MFS related disorders. One patient was found with c.3058A>G. The patient was diagnosed “incomplete Marfan” and the phenotype of the patient only registered scoliosis. The patient did not have phenotype of cardiovascular, ocular, skeletal or any other MFS phenotype. The patient was not checked for phenotype from the central nervous system. The phenotype is highly unlikely fulfilling the Ghent criteria for MFS.	No
7	c.3422C>T	[116]	[116]	[116]	Not registered	The study screened 39 patients with MFS, 7 with MASS, 4 with familial aortic aneurysm plus 23 from tumor tissue with primary hepatocellular carcinoma. They screened for mutations in *FBN1* and p53 and found 19 mutations in *FBN1* and 6 in p53. The study was designed to compare different sequencing methods and the results were not associated with patients and their phenotype. On sample had a c.3422C>T variant but no phenotype associated with the variant is recorded. In reality the sample could be from tumor tissue. The data	No

						is not valid for concluding that the variant is associated with MFS.	
			Boileau C. (personnal communicatio n 2013)			Female with isolated skeletal features.	No
8	c.3509G>A	[117]	[117]		[117]	The study is a case report describing a "novel mutation in two related individuals who have a marfanoid phenotype which does not conform to the clinical criteria defining MFS". The mutation is c.3509G>A. The criteria used is the Berlin criteria from 1988 [1]. Both patients (mother and daughter) did not have ocular or cardiovascular manifestations. Only phenotype was "dolichostenomalia and arachnodactyly". The mother was 47 years and the daughter 20 years. Data is not sufficient to conclude an association between the variant and MFS.	No
					[118]	The study screened 20 MFS families where at least two affected members where available for analysis plus 30 families with one member available and 10 sporadic cases. All in all 60 patients was screened. The patient with c.3509G>A was described with skeletal findings only.	No
		[119]				The study was formed to evaluate how many incidentals findings that are identified with exome sequencing. They evaluated 1000 exomes from National Heart, Lung, and Blood Institute Exome Sequencing Project (ESP). 500 European- and 500 African-decent. They screen the 1000 exomes for variants in 114 genes associated with medically actionable genetic conditions. One of the 114 genes was *FBN1*. They found 585 instances of 239 unique variants indentified as disease causing in the Human Gene Mutations Database (HGMD). They found 7 *FBN1* variants that they all classify variant of uncertain significance (VUS).	No

						One patient carried c.3509G>A which is classified as VUS. The data is not associated with phenotype and data is not sufficient for associating the variant with MFS.	
			[112]			For general detail se case 5 (c.2927G>A) One 35 year old patient presented a c.3509G>A variant. The patient was classified as incomplete MFS. Phenotype is classified with minor involvement of the skeletal system. No other system involvement.	No
			[120]			The article intended to evaluate the dHPLC technique in detecting *FBN1* mutation. They screened 262 unrelated patients and additional 173 clinically affected and non-affected family members. In the 262 patients they found 103 mutations, of these 93 were unique. The authors mention that they were "able to prove segregation of the mutation with disease phenotype in 66 of the 103 mutation-positive patients (64.1%)." c.3509G>A was found in two unrelated patients. There is no specific data related to any specific mutation. Data from the article does not provide phenotype-genotype documentation for the variants association of MFS.	No
			[121]			The study analyzed 36 patients *FBN1* and TGFBR2. It is not clear which indications were used to include the patients. Of the included patients 17 (61%) were suspected of having MFS, 8 patients had a history of thoracic aortic aneurism/dissection. One patient presented a c.3509G>A variant and noted in a table but there is no connection with a patient phenotype.	NO
			[113]			For general details se case 5 (c.2927G>A) One of the analyzed variants was *FBN1* c.3509G>A but this variant did not cause any splicing effect on the RNA.	No
		[122]		[122]		The study aims to examine the relationship between *FBN1* genotype and phenotype in families with	No

						extremely mild phenotypes and in those that show striking clinical variation. They analysed two families. The proband in the c.3509G>A family is described as a patient with “non-specific connective-tissue disorder”. Phenotype with dolichostenomelia, joint hypermobility, kyphoscoliosis, pes planus, posistive wrist and thumb signs, stria, myopia and myxomatous mitral leaflets with mitral prolapse. No lens dislocation and no aorta dilatation. Family members (four children, a brother, her father and paternal uncle) had connective-tissue disorder finding but none MFS like. The affected family members did all have the same variant. All in all 8 patients was found with c.3509G>A.	
			Black C, Boxer M (Personnal communicatio n 2000).			Patient from the UK with classical MFS but no clinical data.	No
			Boileau C. (personnal communicatio n 2013)			Female with incomplete MFS.	No
			Boileau C. (personnal communicatio n 2013)			Male from France with no clinical data.	No
9	c.3797A>T	[123]	Not registered	Not registered	Not registered	The study is based on screening 64 DNA samples containing 60 unique known *FBN1* sequence variants. The study is designed to test DHPLC technique in detecting *FBN1* mutations. As a supplement they also screened 3 MFS patients which did not carry the mentioned variant. In the study there is no geno-phenotype correlation.	No
10	c.3845A>G	[124]		Not registered	Not registered	The study is designed to test DHPLC technique in detecting *FBN1* mutations. There is no genotype-phenotype correlation. The article mentions: “Of the 95 coded DNA samples screened for *FBN1* mutations, 62	No

						samples were from individuals with definitive MFS and 33 were from patients with isolated MFS-related phenotypes such as ascending aortic aneurysm (n = 16), characteristic skeletal features (n = 8), mitral valve prolapse and skeletal features (n = 2), ectopia lentis (n = 2), familial retinal detachment (n = 1), orthostatic headaches due to chronic cerebrospinal fluid (CSF) leakage (n = 2), or various nondiagnostic connective tissue manifestations (n = 2)." One patient carried c.3845A>G. It is not clear which group the patient carrying the variant is from.	
		[125]				The study is based on an evaluation of the SIFT program to predict whether SNP's are damaging or not. They also looked at c.3845A>G and found it "tolerated" and made a reference to [124]. The study has no patients and phenotype involved and cannot be used as documentation of variant connection to Marfan syndrome.	No
			Boileau C. (personnal communicatio n 2013)			Female from France with MFS no other clinical data. It is unclear which diagnostic criteria were used for the diagnosis.	No
11	c.4270C>G	[126]		[126]		Is an early version of the UMB-*FBN1* database. The entry in some cases has phenotype data. In this specific variant the entry mentions the presence of skeletal and ocular manifestations but no cardiovascular, pulmonary, skin or central nervous manifestations.	No
			[127]		[127]	The study is based on screening of 57 unrelated patients with MFS or a MFS like phenotype. They found 49 *FBN1* mutations. c.4270C>G was found in one patient phenotyped with skeletal manifestation of positive hand and wrist sign, joint hypermobility, characteristic facial appearance and increased axial length of globe in the eye. No reported cardiovascular, pulmonary, skin or dural involvement.	No

			[128]	[128]		The study is a screening of 10 MFS patients for *FBN1* mutations. c.4270C>G was found in one patient with a phenotype described with skeletal involvement, aorta dilatation, aortic regurgitation, mitral valve prolapse with regurgitation. The patient had a family history of MFS.	Yes
			[112]			For general detail se case 5 (c.2927G>A) Three patients presented a c.4270C>G variant. One 47 year old patient was classified as MFS and two (one 47 year old and one 30 year old) were classified as incomplete MFS. The MFS patient had a major criteria involvement of the cardiovascular system. The 47 year old incomplete MFS patient had major criteria involvement of the cardiovascular system and minor involvement of skeletal, pulmonary and skin. With major involvement of the cardiovascular system and a *FBN1* mutation associated with aortadilatation (as for the reference [128]) the patient could fulfill the Ghent II criteria for MFS. The 30 year old had minor involvement of the skeletal system.	Yes/Yes/No
			[114]			For general detail se case 6 (c.3058A>G) The c.4270C>G variant was not found in a MFS patient but in a Shprintzen Goldberg syndrome patient. This syndrome is associated with SKI mutations.	No
			[120]			See case 8 (c.3509G>A) c.4270C>G was found in one patient.	No
			[129]			The article describes screening of 100 patients with MFS habitus and intellectual disability. The authors define MFS habitus as “patients with skeletal signs of MFS but who do not meet the international criteria”.c.4270C>G was found in two patients. One with phenotype: Skeletal features: 188 cm, asymmetric pectus excavatum, dolichostenomelia, arachnodactyly, joint laxity, severe thoraco-lumbar cyphosis, protrusio acetabulae.	No

						The other with phenotype: Skeletal features: long and thin habitus, pectus excavatum, dolichostenomelia, arachnodactyly. Other MFS features: severe mitral valve prolapse with massive mitral insufficiency requiring surgery at age 36 years, myopia. Other features: ASD, vascular embolization of two intracranial sylvian aneurysms, dolichocephaly, plagiocephaly, down slanting palpebral fissures, small and round ears	
			Boileau C. (personnal communication 2013)			Male from France with Shprintzen-Goldberg syndrome. No clinical data.	No
			Boileau C. (personnal communication 2013)			Female from France with MFS. No clinical data. Female from France with MFS no other clinical data. It is unclear which diagnostic criteria were used for the diagnosis.	No
			Boileau C. (personnal communication 2013)			Male from France with unknown condition and no clinical data.	No
12	c.6055G>A	[35]	[35]	Not registered	Not registered	The article is based on evaluation of 300 patients referred for confirmation or exclusion of MFS. They were evaluated first by genetic evaluation with *FBN1* and TGFBR1/2 screening second by the Ghent I criteria[110], thirdly by the Ghent II criteria[8]. They found 140 MFS genotypes, 139 Ghent I phenotypes, 124 Ghent II phenotypes. MFS syndrome was consistently by all classifications confirmed in 94 and excluded in 129 and was discordant in 77 patients. Genotype with Ghent I gave 126 MFS. Genotype with Ghent II gave 125. Both among the 140 with genotype of MFS (aka positive *FBN1*). Exclusion of MFS was achieved with Ghent I in 139 and Ghent II in 141 among the 160 without MFS genotype. c.6055G>A is mentioned for two patients screened for *FBN1* but do not fulfill Ghent 1[110] or Ghent II[8]. The	No

						article clearly indicates that the patients do not fulfill the criteria for MFS and therefore do not have MFS.	
13	c.6700G>A	[108]	[108]	[108]	Not registered	Se comments on case 2 (c.1027G>A)	No
		[119]				For general detail se case 8 (c.3509G>A) One patient carried c.6700G>A which is classified as VUS. The data is not associated with phenotype and data is not sufficient for associating the variant with MFS.	No
			[35]			For general details se case 12 (c.6055G>A) c.6700G>A is found in one patient screened for *FBN1* but do not fulfill Ghent 1[110] or Ghent II[8]. The article clearly indicates that the patient do not fulfill the criteria for MFS and therefore do not have MFS.	No
			[112]	[112]		For general details se case 5 (c.2927G>A) One patient presented a c.6700G>A variant. The patient was classified as incomplete MFS. Phenotype is classified with major involvement of the skeletal system and minor involvement of the cardiovascular system. No other system involvement.	No
				[130]		The study screened 105 patients from the Norwegian Marfan Syndrome Study for *FBN1 mutations*. The 105 patients representing 66 families, 73 individuals from 44 families had mutations in *FBN1*. Each family was represented by an index case. The aim of the study was to test for correlations between FBN1 genotype and phenotype in adults where all individuals were equally and completely examined for all manifestations in the Ghent I nosology [110]. The carrier of c.6700G>A had two variants in which c.6700G>A was only one of them. The patient phenotype was registered as dural ectasia, ectopia lentis, flat cornea, increased globe length of the eye, mitral valve proplapse, dilatation of the decending aorta, pectus carinatum, displacement of the medial malleolus causing pes planus, facial appearace and skin	No

						striae athrophicae and apical pulmonary blebs. As the patient had two variants it is not possible to distinguish which variant is accountable for the phenotype.	
14	c.7241G>A	[131]	[131]	Not registered	Not registered	The study screened 49 patients with MFS or suspected MFS for *FBN1*/2 and TGFBR1/2. They found 27 *FBN1* mutations in 27 patients. c.7241G>A is mentioned in a table for two patients and clearly indicated that the patients do not fulfill the Ghent criteria[110]. On the other hand both the patients are indicated to have cardiovascular manifestations. One patient 6 years and one patient 32. If both patients have aorta dilatation this would indicate MFS according to Ghent II[8]. The phenotype is not clearly stated and the article cannot be used as documentation of an association between the variant and Marfan syndrome.	No
15	c.7379A>G	[120]	[120]	Not registered	Not registered	Se case 8 (c.3509G>A)	No
		[119]				For general details se case 8 (c.3509G>A) One 47 year old patient carried c.7379A>G which is classified as VUS. The data is not associated with phenotype and data is not sufficient for associating the variant with MFS.	No
			[113]			For general detail se case 5 (c.2927G>A) One of the analyzed variants was *FBN1* c.7379A>G but this variant did not cause any splicing effect on the RNA.	No
16	c.7660C>T	[112]	[112]	Not registered	Not registered	For general detail se case 5 (c.2927G>A) One 70 year patient presented a c.7660C>T variant. The patient was classified as incomplete MFS. Phenotype I classified with major involvement of the cardiovascular system. No other system involvement. If the *FBN1* variant is associated with MFS and aorta dilatation the patient would have MFS according to the newest Ghent criteria. The age and the non-existing data connecting the variant with MFS indicates that c.7660C>T is not disease causing but data is inconclusive.	Yes/No
17	c.7661G>A	[35]	[35]	Not registered	Not registered	For general details se case 12 (c.6055G>A)	No

						c.7661G>A is found in one patient screened for *FBN1* but do not fulfill Ghent 1[110] or Ghent II[8]. The article clearly indicates that the patient does not fulfill the criteria for MFS and therefore does not have MFS.	
			Hyland JC (Personal communicatio n 2003).			Male from the USA with classical MFS. Phenotype described with aorta dilatation, mitralvalve prolapse, myopia, arachnodactyly, high arched palate, increased body length and joint hypemobility.	Yes
18	c.7702G>A	[120]	[120]	Not registered	Not registered	Se case 8 (c.3509G>A)	No
		[119]				For general details se case 8 (c.3509G>A) One patient carried c.7702G>A which is classified as VUS. The data is not associated with phenotype and data is not sufficient for associating the variant with MFS.	No
19	c.7846A>G	[120]	[120]	Not registered	Not registered	Se case 8 (c.3509G>A)	No
		[119]				For general details se case 8 (c.3509G>A) One patient carried c.7846A>G which is classified as VUS. The data is not associated with phenotype and data is not sufficient for associating the variant with MFS.	No
20	c.7852G>A	[123]		[123]		Se case 9 (c.3797A>T)	No
			[132]	[132]	[132]	The study was based on a review of 171 patients genotype and phenotype. The aim was to investigate if patients fulfilling the Ghent I criteria[110] had more *FBN1* mutations than patients who did not fulfill the criteria. 94 patient fulfilled the criteria and of these 62 (66%) had a *FBN1* mutation. 77 patients did not fulfill the criteria of which 9 (12%) did have a *FBN1* mutation. The patient with c.7852G>A had major skeletal involvement and a family history of MFS. The patient did not have cardiovascular, ocular, pulmonary or skin involvement. The patient was 15 years old.	No
			[112]	[112]		For general details se case 5 (c.2927G>A) One 40 year old patient presented a c.7852G>A variant. The patient was classified as incomplete MFS.	No

						Phenotype is classified with major involvement of the skeletal system and minor involvement of the cardiovascular system and skin. No other system involvement.	
			[120]			Se case 8 (c.3509G>A)	No
				[133]		The study screened 113 patients with MFS or MFS like features. They found 57 mutations in 52 patients. c.7852G>A was found in a male who did not fulfill the Ghent criteria and only with sparse MFS phenotype of minor skeletal involvement. He had no cardiovascular involvement and no family history of MFS.	No
21	c.8081G>A	[111]	[111]	Not registered	Not registered	For general details se case 4 (c.2056G>A) They found one patient with c.8081C>A but no phenotype data was recorded.	No
22	c.8176C>T	[134]	[134]	[134]	[134]	The article is a case report on a 13-year old tall boy with very few MFS phenotypical characteristics. The boy and six other family members' with the specific mutation did not have cardiovascular or ocular manifestations of MFS. The case patient only had skeletal manifestations with tall stature, long arms (equal to height which is not enough to score point in the Ghent criteria where the ratio should be 1.05), scoliosis, pectus carinatum and arachnodactyly. The patients do not in any way fulfill the Ghent criteria (both Ghent I from 1996[110] and Ghent II from 2010[8])	No
		[135]	[135]	[135]		The study reports a family of which two out of four individuals had the specific mutation one of these had MFS like phenotype and the other did not.	No
		[136]	[136]	[136]		The study reports two families in which the probands have compound-heterozygous MFS. One of the families carried the specific mutation. One proband had the specific mutation and another *FBN1* mutation and fulfilled the Ghent I[110] criteria. His brother did also carry the specific mutation but not the other one and this brother did not fulfill the criteria. Of the parents the	No

						mother was diagnosed MFS and carried the “other” mutation but not the specific mutation. The father carried the specific mutation but not the “other” and did not have MFS. The case clearly shows that the specific mutation is not disease causing.	
		[119]				For general details se case 8 (c.3509G>A) One patient carried c.8176C>T which is classified as VUS. The data is not associated with phenotype and data is not sufficient for associating the variant with MFS.	No
		[137]				The study aims at evaluating patients with bicuspid aortic valve and aortic dilatation without MFS. They screened 8 patients for *FBN1* mutations and found three mutations of which one patient had two mutations. The patient with two mutations carried c.8176C>T as one of the mutations. According to the newest Ghent guidelines a patient with a *FBN1* mutation associated with aortic dilation and aorta dilatation fulfills the diagnostic criteria but in this case the patient with c.8176C>T carried two mutations and it is not possible to conclude which variant is disease causing.	No
			[76]			For general details se case 8 (c.59A>G) c.8176C>T is mentioned in a patient with phenotype with tricuspidal valve prolapse, increased axial length of the globe in the eye, skeletal involvement but not a major criteria, no dura involvement examined by MR, no skin and lung involvement.	No
			[114]			For general details se case 6 (c.3058A>G) The c.8176C>T in three patients who all had double mutation in *FBN1* of which one was the c.8176C>T. As the three patients had a different combination of double mutations it is unlikely that the patients were related. The fact that the same mutation was found three times in an unrelated cohort (and always as a	No

						double mutation) indicates that it is either very common or a technical error in the laboratory.	
			[112]			For general detail se case 5 (c.2927G>A) One 17 year old patient presented a c.3509G>A variant. The patient was classified as incomplete MFS. Phenotype is classified with minor involvement of the skeletal system and skin. No other system involvement.	No
			[115]			For general details see case 6 (c.3058A>G) c.8176C>T was found in a 28 year old female patient with incomplete MFS. She had mitral valve prolapse, skin striae and myopia. She did not have skeletal or pulmonary involvement and no family history.	No
			[129]			For general details see case 11 (c.4270C>G) c.8176C>T was found in a 21 year old male. The patient had skeletal involvement of dolichostenomelia, scoliosis, join laxity, arachnodactyly, hyperlaxity with recurrent sprains, crowded teeth with irregular shape. This supplemented with myopia does not fulfill the criteria for MFS.	No
			Barber R, Boxer M (Personal communicatio n 2001).			Patient from UK med classical MFS. No clinical data.	No
			Boileau C. (personnal communicatio n 2013)			Female from France with MFS. No clinical data.	No
			Boileau C. (personnal communicatio n 2013)			Male from France with AAA. No other clinical data.	No
			Boileau C. (personnal communicatio n 2013)			Male from France with incomplete MFS. No clinical data.	No

23	c.8494A>G	[111]	[111]	Not registered	Not registered	For general details se case 4 (c.2056G>A) They found one patient with c.8494A>G but no phenotype data was recorded.	No
		[119]				For general details se case 8 (c.3509G>A) One patient carried c.8494A>G which is classified as VUS. The data is not associated with phenotype and data is not sufficient for associating the variant with MFS.	No

AARHUS
UNIVERSITY

Appendix

Study 1

Genetics inMedicine | BRIEF REPORT

Difficulties in diagnosing Marfan syndrome using current *FBN1* databases

Kristian A. Groth, MD[1,2], Mette Gaustadnes, MSc, PhD[2], Kasper Thorsen, PhD, MSc[2], John R. Østergaard, MD[3], Uffe Birk Jensen, MD[4], Claus H. Gravholt, PhD, DMSc[2,5] and Niels H. Andersen, PhD, DMSc[1]

Purpose: The diagnostic criteria of Marfan syndrome (MFS) highlight the importance of a *FBN1* mutation test in diagnosing MFS. As genetic sequencing becomes better, cheaper, and more accessible, the expected increase in the number of genetic tests will become evident, resulting in numerous genetic variants that need to be evaluated for disease-causing effects based on database information. The aim of this study was to evaluate genetic variants in four databases and review the relevant literature.

Methods: We assessed background data on 23 common variants registered in ESP6500 and classified as causing MFS in the Human Gene Mutation Database (HGMD). We evaluated data in four variant databases (HGMD, UMD-FBN1, ClinVar, and UniProt) according to the diagnostic criteria for MFS and compared the results with the classification of each variant in the four databases.

Results: None of the 23 variants was clearly associated with MFS, even though all classifications in the databases stated otherwise.

Conclusion: A genetic diagnosis of MFS cannot reliably be based on current variant databases because they contain incorrectly interpreted conclusions on variants. Variants must be evaluated by time-consuming review of the background material in the databases and by combining these data with expert knowledge on MFS. This is a major problem because we expect even more genetic test results in the near future as a result of the reduced cost and process time for next-generation sequencing.

Genet Med advance online publication 26 March 2015

Key Words: ClinVar; Exome Sequencing Project; Human Gene Mutation Database; next-generation sequencing; variant databases

Patients with classic Marfan syndrome (MFS) tend to be tall, with long limbs and fingers, as described by Marfan[1] in 1896. The syndrome's description has since evolved considerably, however, and a variety of clinical manifestations are now well established. The genetic background, that is, mutations in the fibrillin-1 gene (*FBN1*),2 was discovered much later but is now a cornerstone in the diagnosis of MFS.

The definition of MFS is based on several sets of criteria, the latest being the Ghent II nosology.[3] It is considered to be an autosomal-dominant disorder associated with a mutation in *FBN1* and phenotypical manifestations of MFS. The Ghent II criteria highlight the importance of a *FBN1* mutation test; for this reason, the focus on genetic testing has increased considerably when either diagnosing or excluding MFS.[4]

With the introduction of next-generation sequencing, the sequencing price and the process time have been reduced considerably, and the amount of genetic sequencing data has been multiplied, resulting in an enormous amount of data that need to be evaluated. Moreover, access to genetic testing in the clinical setting is becoming more common and widespread, and the number of patients who are genetically tested is rapidly increasing.

The vast majority of genetic variants found are benign and represents a part of our genetic variation, but some—maybe just one in a given patient—may be pathogenic and the cause of a given disease. So even though sequencing has become easier and more accessible, the evaluation of these sequencing data has not evolved with the same speed as the technical evolution of next-generation sequencing. This is a considerable problem given that a patient must receive the correct genetic diagnosis.

The majority of variants are single-nucleotide variants. In reality, the genotype–phenotype correlation is essential to determining the pathogenicity of *FBN1* in MFS.

A range of tools for evaluating variants has been developed, but a majority of these tools are not exact and can be used only for guidance when evaluating genetic variants. In addition, a number of databases exist with data collected for published and nonpublished variants. The databases are often incorrect and have many incorrect interpretations of published data.[5,6]

Yang et al.[6] recently presented an evaluation of common variants in the National Heart, Lung, and Blood Institute GO Exome Sequencing Project (ESP) classified as "disease causing" in the widely used Human Gene Mutation Database (HGMD).[7] Yang et al. expected to find a maximum of two patients with disease-causing *FBN1* variants in the ESP database but found 100 individuals with 23 different variants, indicating a misinterpretation of variants in the HGMD database. The aim of this

[1]Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark; [3]Centre for Rare Diseases, Department of Paediatrics, Aarhus University Hospital, Aarhus, Denmark; [4]Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark; [5]Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark. Correspondence: Kristian A. Groth (kristian.groth@ki.au.dk)

Submitted 20 November 2014; accepted 9 February 2015; advance online publication 26 March 2015. doi:10.1038/gim.2015.32

study was to evaluate the quality of variant databases as they relate to these 23 likely benign variants.

MATERIALS AND METODS

Yang et al.[6] identified 23 *FBN1* variants (**Supplementary Table S1** online) in ESP that were classified as disease-causing in HGMD. We searched the HGMD professional database (**Supplementary Table S1** online), the UMD-*FBN1* database[8] (**Supplementary Table S2** online), the ClinVar database[9] (**Supplementary Table S3** online), and the UniProt database[10] (**Supplementary Table S4** online) for the 23 variants. In each database we identified reference material in as much detail as possible (**Supplementary Tables S1–S4** online). Published peer-reviewed articles were all identified via PubMed searches, and all material was accessible. The UMD-*FBN1* database also contained data classified as "personal communication," which is not published in the literature and therefore accessible only via the UMD-*FBN1* database homepage (www.umd.be/FBN1/).

Each variant was evaluated according to published information (**Supplementary Table S5** online). The accessible data were evaluated according to the Ghent II nosology, and each variant was evaluated as "not MFS", "maybe MFS" and "inconclusive" (**Table 1**). "Not MFS" indicates that the variant probably does not cause MFS based on a majority of reported phenotypes that do not fulfill the Ghent II nosology. "Maybe MF" indicates that the variant could cause MFS, but there is not full documentation for a genotype–phenotype association. "Inconclusive" indicates that the data were insufficient to evaluate the variant's effect on the phenotype. The term "MFS" also was intended to be used to describe variants that cause an evident MFS phenotype, but none of the variants had a clear genotype–phenotype association with an MFS phenotype fulfilling the Ghent II nosology. The manual evaluation of the variants then was compared with the database conclusions (**Table 2**).

RESULTS

Only the HGMD database contained all variants. The UMD-*FBN1* database contained all but one variant, ClinVar contained eight variants, and UniProt contained five variants (**Table 2**). The majority of references were overlapping in all databases. None of the databases did cover all references, and all four databases had unique references that were not recorded in the three other databases.

As expected, the HGMD database classified all 23 variants as "disease-causing mutations" and associated all the variants with MFS. The UMD-*FBN1* database had records for 22 variants, all classified as "mutation," but one variant was in a single subrecord classified as a "polymorphism." This specific variant was

Table 1 Summary of articles reviewed and the diagnostic conclusion as assessment for the documented phenotype

Number	Variant	Patients with MFS (*n*)	Patient with unknown MFS status (*n*)	Patients without MFS (*n*)	Diagnostic conclusion[a]
1	c.59A>G	0	0	1	Inconclusive
2	c.1027G>A	0	1	0	Inconclusive
3	c.1345G>A	(1)	0	0	Maybe MFS
4	c.2056G>A	0	0	1	Inconclusive
5	c.2927G>A	(1)	1	0	Maybe MFS
6	c.3058A>G	0	0	3	Not MFS
7	c.3422C>T	0	2	0	Inconclusive
8	c.3509G>A	0	8	12	Not MFS
9	c.3797A>T	0	1	0	Inconclusive
10	c.3845A>G	0	3	0	Inconclusive
11	c.4270C>G	3	4	5	Maybe MFS
12	c.6055G>A	0	0	2	Not MFS
13	c.6700G>A	0	3	2	Not MFS
14	c.7241G>A	0	2	0	Inconclusive
15	c.7379A>G	0	3	0	Inconclusive
16	c.7660C>T	0	1	0	Inconclusive
17	c.7661G>A	1	0	1	Inconclusive
18	c.7702G>A	0	2	0	Inconclusive
19	c.7846A>G	0	2	0	Inconclusive
20	c.7852G>A	0	2	3	Not MFS
21	c.8081G>A	0	1	0	Inconclusive
22	c.8176C>T	0	11	15	Not MFS
23	c.8494A>G	0	2	0	Inconclusive

[a]The conclusions are based on published data and according to the Ghent II criteria. "Not MFS" variants probably do not cause MFS based on the fact that the majority of reported phenotypes with this variant do not fulfill the Ghent II criteria. "Maybe MFS" indicates that the variant could result in the MFS phenotype but the full documentation on genotype–phenotype association is not provided. "Inconclusive" indicates that assessable data on the variant is inconclusive to assess the variant effect on phenotype.

MFS, Marfan syndrome.

Table 2 Summary of conclusions in databases and conclusions of the manual evaluation of background material in this study

Number	Variant	ESP count	Database				This study's conclusion
			HGMD	UMD-*FBN1*	ClinVar	UniProt	
1	c.59A>G; p.Y20C	3	DCM: MFS	Mutation: incomplete MFS	NR	MFS	Inconclusive
2	c.1027G>A; p.G343R	2	DCM: MFS	Mutation	NR	NR	Inconclusive
3	c.1345G>A; p.V449I	2	DCM: MFS	Mutation: incomplete MFS	NR	NR	Maybe MFS
4	c.2056G>A; p.A686T	1	DCM: MFS	Mutation	NR	NR	Inconclusive
5	c.2927G>A; p.R976H	2	DCM: MFS	Mutation: classic MFS	Uncertain significance: all highly penetrant	NR	Maybe MFS
6	c.3058A>G; p.T1020A	3	DCM: MFS	(2)Mutation/(1)polymorphism: classic MFS/Lujan-Fryns syndrome/isolated skeletal features	Pathogenic/likely pathogenic: MFS	NR	Not MFS
7	c.3422C>T; p.P1141L	14	DCM: MFS	Mutation: classic MFS/isolated skeletal features	Uncertain significance: all highly penetrant	NR	Inconclusive
8	c.3509G>A; p.R1170H	25	DCM: MFS	Mutation: classic MFS/isolated skeletal features/incomplete MFS	Pathogenic/likely pathogenic: MFS, subdiagnostic variant of	MFS	Not MFS
9	c.3797A>T; p.Y1266F	4	DCM: MFS	Mutation	NR	NR	Inconclusive
10	c.3845A>G; p.N1282S	3	DCM: MFS	Mutation: MFS	NR	NR	Inconclusive
11	c.4270C>G; p.P1424A	4	DCM: MFS	Mutation: incompletes MFS/classic MFS/Shprintzen-Goldberg syndrome/ unknown marfanoid syndrome	Pathogenic/likely pathogenic: MFS	MFS	Maybe MFS
12	c.6055G>A; p.E2019K	1	DCM: MFS	Mutation: probable MFS	NR	NR	Not MFS
13	c.6700G>A; p.V2234M	8	DCM: MFS	Mutation: incomplete MFS	Conflicting data from submitters: MFS, all highly penetrant	NR	Not MFS
14	c.7241G>A; p.R2414Q	1	DCM: MFS	Mutation	NR	NR	Inconclusive
15	c.7379A>G; p.K2460R	2	DCM: MFS	Mutation: MFS	NR	NR	Inconclusive
16	c.7660C>T; p.R2554W	1	DCM: MFS	Mutation: incomplete MFS	NR	NR	Inconclusive
17	c.7661G>A; p.R2554Q	1	DCM: MFS	Mutation: classic MFS	NR	NR	Inconclusive
18	c.7702G>A; p.V2568M	1	DCM: MFS	Mutation	NR	NR	Inconclusive
19	c.7846A>G; p.I2616V	4	DCM: MFS	Mutation	NR	NR	Inconclusive
20	c.7852G>A; p.G2618R	2	DCM: MFS	Mutation: incomplete MFS	Pathogenic/likely pathogenic: MFS	MFS	Not MFS
21	c.8081G>A; p.R2694Q	1	DCM: MFS	Mutation	NR	NR	Inconclusive
22	c.8176C>T; p.R2726W	14	DCM: MFS	Mutation: isolated skeletal features/ classic MFS/incomplete MFS/MASS/ marfanoid syndrome/Lujan-Fryns syndrome/AAA	Conflicting data from submitters: MFS	MFS	Not MFS
23	c.8494A>G; p.S2832G	1	DCM: MFS	mutation	NR	NR	Inconclusive

ESP individuals is the number of alleles registered in ESP6500 with the specific variant. HGMD, UMD-*FBN1*, and ClinVar are stated as classification conclusion and disease association.

DCM, disease-causing mutation; ESP, Exome Sequencing Project; HGMD, Human Gene Mutation Database; MFS, Marfan syndrome; NR, not registered.

still classified as a mutation in the overall database, and only by analyzing the specific input record of the patient with X-linked Lujan-Fryns syndrome was it clear that the record had been classified as a polymorphism. A polymorphism is historically defined as a variant more common than 1% in the background population, but the term is seldom used in the modern literature. In some records in the UMD-*FBN1* database, the variant was associated with a variety of syndromes and characteristic phenotypes. In many cases the same variant was associated with more than one syndrome/phenotype. Even syndromes not associated with *FBN1*-like Lujan-Fryns syndrome were found in the UMD-*FBN1* database.

ClinVar had records of eight variants, of which four were classified under the term "clinical significance" as "pathogenic/likely pathogenic"; two were classified as being of "uncertain significance" and two were classified as "conflicting data from submitters." The six variants classified as either "pathogenic/likely pathogenic" or "conflicting data from submitters" were connected with MFS, whereas the "uncertain significance" variants were labeled as "all highly penetrant."

The UniProt database had records of five variants, which all were associated with MFS.

Manual evaluation of the database references did not find evidence of any of the 23 variants being associated with MFS. Of the 23 variants, only 3 variants were classified as "maybe MFS," indicating that the variant could result in the MFS phenotype but none of the identified references provided full documentation of a genotype–phenotype association. We classified 14 variants as "inconclusive," indicating that the accessible literature on the variant precluded any definite conclusions concerning genotype–phenotype relations. Six variants were classified as "not MFS" because they most likely do not cause MFS based on the fact that the majority of the reported patients with this variant do not fulfill the Ghent II criteria.

DISCUSSION

The evaluation of 23 variants shows that the databases do contain misleading information on variants and their genotype–phenotype associations. Thus, clearly, the databases cannot be used as a direct source for diagnostics, but only as a tool for seeking additional information about the specific variant. An MFS diagnosis is based on a rather complex set of diagnostic criteria, which has changed over time. Interpretation of *FBN1* variants for defining MFS is rather difficult, and the analyst must have expert knowledge about MFS phenotypes and the diagnostic criteria. Descriptions such as "classic MFS" or "fulfilling the MFS criteria" are not precisely defined and not useful for classifying a given phenotype. MFS can be considered as a diagnosis about which knowledge is constantly evolving, and the diagnostic criteria therefore have to change over time.[3,11,12] Describing specific phenotypical characteristics in the databases, such as aortic dilatation, ectopia lentis, or scoliosis, is necessary because they do not change with new diagnostic criteria, and reevaluating the MFS diagnosis if the nosology changes would be possible.

MFS is a rare disease, but the exact incidence is not fully known. Different estimates have been reported: from 17.2 per 100,000 (ref. 13) to 4.6 per 100,000 (ref. 14), but 10 in 100,000 is widely quoted.[15] Precisely calculating the expected incidents of disease-causing *FBN1* mutations in the ESP cohort is difficult. ESP contains 6,503 samples from a cohort with heart, lung, and blood disorders. One small subcohort of 29 subjects, notated as "thoracic aortic aneurysms leading to acute aortic dissections," is noteworthy because aortic dissection is highly associated with MFS. The ESP phenotype data are not publicly available, and verifying in which cohort each variant is detected is not possible. In the general ESP cohort one would expect 0.3 to 1.1 patients with MFS, but this could be supplemented with up to 29 extra patients because of the aortic dissection cohort. An expected maximum of around 30 patients compared with the actual 100 patients indicates an overrepresentation of benign variants among the 23 variants found in ESP and HGMD.

The introduction of next-generation sequencing into clinical diagnostics obviously will provide an increased amount of genotype versus phenotype information, but much of this information will never be reported in the databases because the main source of data in the databases is published peer-reviewed articles. Currently, publication of new or reconfirmed variants is not prioritized by most scientific journals. For this reason, publication bias is inevitable. Because the diagnostic strength of each variant is correlated with the amount of data collected about genotype versus phenotype, the future of MFS diagnostics is dependent on data collection from genetic testing and input directly from laboratories, not via published, peer-reviewed articles. Variant databases need to accept data on an individual level, but providing phenotype information as well is crucial.

The UMD-*FBN1* database does have the possibility for "personal communication" on variants, but such information relies on personal communication and in many cases does not contain specific information about the phenotype. The ClinVar database does provide data from a few laboratories, but these also do not provide phenotype data.

In a broad perspective, the *FBN1* databases do not seem to be ready to incorporate the benefit of the high output of data from next-generation sequencing. The many daily analyses are not incorporated in the databases, and the databases are not ready to "learn" from new data input. Only the ClinVar database provides information on "conflicting data from submitters." The UMD-*FBN1* database registers all variants as mutations even though the variants may be associated with a variety of (up to seven different) phenotypes. It is necessary for variant databases to be able both to handle many data inputs with different and even conflicting data and to present these data in a way that provides the user with a clear understanding of the currently accessible information on the specific variant.

The evaluation of these 23 highly selected and likely benign variants in the *FBN1* gene shows that not all data in the databases are correctly classified. That references with no other

connection to MFS other than mentioning a variant in the *FBN1* gene can be found in the UMD-*FBN1* (ref. 16), HGMD,[17] and ClinVar[18] databases is worrying. Even a reference based on a publication about incidental findings of *FBN1* variants is, for some reason, suddenly associated with MFS in the HGMD database.[17] UniProt contains only a minority of references compared with HGMD and UMD-*FBN1*, and this might be the reason why this database does not have references to any of these articles. Among the five UniProt records labeled "MFS," three do not have MFS, one is inconclusive, and one might have MFS.

Yang et al.[6] concluded that the genotype prevalence of MFS was 1:65 but question the causality of some of these variants and suggest "that these variants may not be the monogenic cause of MFS." We think that their study shows that some researchers tend to use the databases rather uncritically.

Conclusion

The genetic diagnosis of MFS cannot be made reliably using only variant databases; it must be made through time-consuming evaluation of the background material in the databases and by combining these data with expert knowledge on MFS. Because the databases do not provide a reliable interpretation of variants, there is a substantial possibility of misdiagnosing MFS.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

ACKNOWLEDGMENTS

The authors thank the National Heart, Lung, and Blood Institute GO Exome Sequencing Project and its ongoing studies, which produced and provided exome variant calls for comparison: the Lung GO Sequencing Project (HL-102923), the WHI Sequencing Project (HL-102924), the Broad GO Sequencing Project (HL-102925), the Seattle GO Sequencing Project (HL-102926), and the Heart GO Sequencing Project (HL-103010).

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Marfan AB. Un cas de deformation des quatre membre plus prononcee aux extremitre characterisee par l'allongment des os avec un certain degre d'amincissement. *Bull Mem Soc Med Hop Paris* 1896;13:220–221.
2. Dietz HC, Cutting GR, Pyeritz RE, et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. *Nature* 1991;352:337–339.
3. Loeys BL, Dietz HC, Braverman AC, et al. The revised Ghent nosology for the Marfan syndrome. *J Med Genet* 2010;47:476–485.
4. Sheikhzadeh S, Kade C, Keyser B, et al. Analysis of phenotype and genotype information for the diagnosis of Marfan syndrome. *Clin Genet* 2012;82:240–247.
5. Bell CJ, Dinwiddie DL, Miller NA, et al. Carrier testing for severe childhood recessive diseases by next-generation sequencing. *Sci Transl Med* 2011;3:65ra4.
6. Yang RQ, Jabbari J, Cheng XS, et al. New population-based exome data question the pathogenicity of some genetic variants previously associated with Marfan syndrome. *BMC Genet* 2014;15:74.
7. Stenson PD, Ball EV, Mort M, et al. Human Gene Mutation Database (HGMD): 2003 update. *Hum Mutat* 2003;21:577–581.
8. Collod-Béroud G, Le Bourdelles S, Ades L, et al. Update of the UMD-FBN1 mutation database and creation of an FBN1 polymorphism database. *Hum Mutat* 2003;22:199–208.
9. Landrum MJ, Lee JM, Riley GR, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res* 2014;42(Database issue):D980–D985.
10. UniProt Consortium. Activities at the universal protein resource (UniProt). *Nucleic Acids Res* 2014;42:D191–D198.
11. De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. *Am J Med Genet* 1996;62:417–426.
12. Beighton P, de Paepe A, Danks D, et al. International Nosology of Heritable Disorders of Connective Tissue, Berlin, 1986. *Am J Med Genet* 1988;29:581–594.
13. Sun QB, Zhang KZ, Cheng TO, et al. Marfan syndrome in China: a collective review of 564 cases among 98 families. *Am Heart J* 1990;120:934–948.
14. Fuchs J. Marfan syndrome and other systemic disorders with congenital ectopia lentis. A Danish national survey. *Acta Paediatr* 1997;86:947–952.
15. Gray JR, Bridges AB, Faed MJ, et al. Ascertainment and severity of Marfan syndrome in a Scottish population. *J Med Genet* 1994;31:51–54.
16. Robinson DO, Lin F, Lyon M, et al. Systematic screening of FBN1 gene unclassified missense variants for splice abnormalities. *Clin Genet* 2012;82:223–231.
17. Dorschner MO, Amendola LM, Turner EH, et al.; National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project. Actionable, pathogenic incidental findings in 1,000 participants' exomes. *Am J Hum Genet* 2013;93:631–640.
18. Yuan B, Thomas JP, von Kodolitsch Y, Pyeritz RE. Comparison of heteroduplex analysis, direct sequencing, and enzyme mismatch cleavage for detecting mutations in a large gene, FBN1. *Hum Mutat* 1999;14:440–446.

Study 2

ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

Evaluating the quality of Marfan genotype–phenotype correlations in existing *FBN1* databases

Kristian A. Groth[1,2], Yskert Von Kodolitsch[3], Kerstin Kutsche[4], Mette Gaustadnes[2], Kasper Thorsen[2], Niels H. Andersen[1] and Claus H. Gravholt[2,5]

Background: Genetic *FBN1* testing is pivotal for confirming the clinical diagnosis of Marfan syndrome. In an effort to evaluate variant causality, *FBN1* databases are often used. We evaluated the current databases regarding *FBN1* variants and validated associated phenotype records with a new Marfan syndrome geno-phenotyping tool called the Marfan score.

Methods and results: We evaluated four databases (UMD-FBN1, ClinVar, the Human Gene Mutation Database (HGMD), and Uniprot) containing 2,250 *FBN1* variants supported by 4,904 records presented in 307 references. The Marfan score calculated for phenotype data from the records quantified variant associations with Marfan syndrome phenotype. We calculated a Marfan score for 1,283 variants, of which we confirmed the database diagnosis of Marfan syndrome in 77.1%. This represented only 35.8% of the total registered variants; 18.5–33.3% (UMD-FBN1 versus HGMD) of variants associated with Marfan syndrome in the databases could not be confirmed by the recorded phenotype.

Conclusion: *FBN1* databases can be imprecise and incomplete. Data should be used with caution when evaluating *FBN1* variants. At present, the UMD-FBN1 database seems to be the biggest and best curated; therefore, it is the most comprehensive database. However, the need for better genotype–phenotype curated databases is evident, and we hereby present such a database.

Genet Med advance online publication 1 December 2016

Key Words: database-management systems; early diagnosis; heart disease; musculoskeletal diseases

INTRODUCTION

The first systematic definition of Marfan syndrome (MFS) was the so-called Berlin criteria[1] of 1986. After the discovery of *FBN1* as the disease-causing gene for MFS in 1991,[2] the criteria were revised in Ghent in 1996 (Ghent I).[3] In 2010, the criteria were again revised (Ghent II), highlighting *FBN1* mutations, aortic dilatation, and ectopia lentis as cornerstones in the MFS diagnosis.[4]

According to the Ghent II criteria, it is possible to diagnose MFS by evaluating clinical manifestations, but genetic testing for diagnosing MFS has proven to be increasingly important.[5] According to the American College of Medical Genetics and Genomics (ACMG),[6] a variant should be classified with one of the following modifiers: (i) pathogenic, (ii) likely pathogenic, (iii) uncertain significance, (iv) likely benign, or (v) benign. ACMG suggests that the term "likely" be used when a variant is at least 90% likely to be either benign or pathogenic and, of course, with even stronger evidence for the terms "benign" or "pathogenic." Variants with insufficient evidence are termed variants of uncertain significance (VUS). In clinical practice, a variant evaluation that results in a VUS statement is of minor diagnostic utility.[7] When categorizing a variant, a VUS classification calls for gathering sufficient additional evidence in the direction of either "benign" or "pathogenic.".

The Ghent II criteria for causality of variants[4] in the *FBN1* gene are listed in a box in the original guideline paper and repeated in the general guideline text. Some of the them, including "nonsense mutations," "in-frame and out-of-frame deletion/insertion," and "mutations previously shown to segregate in the Marfan family" are generally accepted in the genetics community.[6,8] Others are specific for the *FBN1* gene, such as "missense affecting/creating cysteine residues"[4] and "missense affecting conserved residues of the EGF consensus sequence ((D/N)X(D/N)(E/Q)Xm(D/N)Xn(Y/F), with *m* and *n* representing variable numbers of residues: D, aspartic acid; N, asparagine; E, glutamic acid; Q, glutamine; Y, tyrosine; F, phenylalanine)."[4] No references to substantiate these recommendations are cited, but some of the recommendations (for example, regarding the effect of cysteine mutations) were inspired by the work by Faivre et al.,[9] which was published a few years before the guidelines. According to the OMIM database (http://omim.org), the *FBN1* gene is associated with no fewer than nine conditions (acromicric dysplasia, familial thoracic aortic aneurysm, familial ectopia lentis, geleophysic dysplasia 2, MFS, MASS phenotype, Scheuermann kyphosis, stiff skin syndrome, and Weill–Marchesani syndrome 2), some of which are in no way like MFS. Thus, the presence of a causal variant in the *FBN1* gene is not necessarily associated with or causes

[1]Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark; [3]German Aorta Centre of Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [4]Institute of Human Genetics, University Medical Center Hamburg–Eppendorf, Hamburg, Germany; [5]Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark. Correspondence: Kristian A. Groth (kristian.groth@ki.au.dk)

Submitted 22 April 2016; accepted 3 October 2016; advance online publication 1 December 2016. doi:10.1038/gim.2016.181

MFS. In reality, laboratories seldom report results referring to the Ghent II variant criteria, and it seems that each laboratory has its own criteria for evaluating variant causality, and even the nomenclature can be troublesome for some laboratories.[10]

The variant evaluation process is usually performed according to local and generalized guidelines used to evaluate any kind of variant. Variant databases such as the Human Gene Mutation Database (HGMD) are used as a resource for linkage to peer-reviewed articles on disease-associated variants,[11] but these databases have shown inaccuracies when used to diagnose MFS.[12] Sequencing techniques such as next-generation sequencing and the use of large gene panels as well as whole-exome sequencing increase the demand for complex computer algorithms to handle data from various sources, including genotype–phenotype databases.[11] Therefore, a well-curated variant database focused on genotype–phenotype correlation is essential when evaluating previously described variants.

We hereby provide a comprehensive quality evaluation of the present databases with information on *FBN1* variants as well as variant-associated phenotypes and introduce a new MFS genotype–phenotype tool called the Marfan score.

MATERIALS AND METHODS

We created a database with all publicly available *FBN1* variants and all known associated case-based MFS phenotype data. All data were manually evaluated on a case-based level, and relevant phenotype data according to the Ghent II nosology[4] were extracted and entered into our new database. Each record was linked to a reference representing the source of the record. We searched the Universal Mutation Database for *FBN1* (UMD-FBN1; http://www.umd.be/FBN1/),[13] the HGMD professional database (http://www.hgmd.cf.ac.uk/ac/index.php), the ClinVar database (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/clinvar/),[14] and the Universal Protein Resource (UniProt; http://www.uniprot.org/) database[15] for all known *FBN1* variants. In each database, we identified reference articles and other material in as much detail as possible. Published peer-reviewed articles were all identified by PubMed searches. All material written in English was evaluated; nine papers in Chinese were not evaluated. It was not possible to gain access to 12 papers indexed in PubMed, representing 15 of 4,904 entries in the database.

The UMD-FBN1 mutations database also contains data classified as "personal communication," which is not published in the literature and is therefore accessible only at the database's homepage (http://www.umd.be/FBN1/).

Evaluating papers for variants referred in the databases, we found additional variants (n = 168) that, for unknown reasons, were not registered in any of the databases. These variants were as well registered in our database and evaluated in the current setup.

Each record for a variant was regarded as a specific individual representing a specific phenotype. We classified records only when it was possible to identify a specified individual representing a specific phenotype. In cases of multiple reports for the same individual, only one record was evaluated. For all individuals reported more than once in the literature, the report with the most detailed phenotype was used and no other record representing the same individual was evaluated.

Each record was classified into one of seven groups:

1. "Nonclassified," representing records in which no phenotypic data were available or the recorded individual was already registered in the database
2. "Polymorphism," representing records stating that the variant was found in individuals not having MFS or stated it as a polymorphism
3. "MFS Berlin," representing records without detailed phenotypical data but describing the individual as fulfilling the Berlin criteria of MFS[1]
4. "MFS Ghent I," representing records without detailed phenotypical data but describing the individual as fulfilling the first revised Ghent criteria of MFS[3]
5. "MFS Ghent II," representing records without detailed phenotypical data but describing the individual as fulfilling the second revised Ghent criteria of MFS[4]
6. "Incomplete MFS," representing records without detailed phenotypical data but describing the individual as having incomplete MFS, with MFS habitus, MFS-like phenotype, or something else
7. "Clinical classification," representing records with phenotypic data

During evaluation of *FBN1* databases, we registered whether the database associated the variant with MFS. If the variant was associated with MFS at least once, then we defined the variant as a database MFS diagnosis (database-MFS).

Marfan score

To provide phenotypical data with a numeric value when handling multiple references, the Marfan score was established. A numeric score was chosen because the databases often have numerous records for the same variant when the phenotype information points toward differing effects in different patients and because phenotype information for individual patients is often incomplete. A numeric Marfan score enables the management of such scenarios because it can handle variant associations with MFS and specific and relevant phenotypes, and it differentiates references with good phenotype descriptions from those with unspecific and insufficient descriptions. The present Marfan score is not intended to be a tool used in daily clinical practice. At this time, the Marfan score should be used only to evaluate the feasibility and quality of current databases that include information on MFS.

For all cases classified as "clinical classification," the Marfan score (**Table 1**) was based on the "systemic criteria" in the Ghent II nosology,[4] which is based on the provided clinical data. Because aortic dilatation/dissection is not among the systemic criteria in the Ghent II nosology but is still a very important clinical feature, we chose to score aorta dilation/dissection

Table 1 Marfan score

		Points
Polymorphism		−10
Nonclassified		0
MFS Berlin		5
MFS Ghent I		8
MFS Ghent II		10
Incomplete MFS		2
Clinical classification	Wrist sign + thumb sign	3
	Only wrist sign	1
	Only thumb sign	1
	Spontaneous pneumothorax	2
	Pectus carinatum	2
	Pectus excavatum	1
	Hindfoot deformity	2
	Plain flat foot	1
	Dural ectasia	2
	Protucio acetabulae	2
	Upper/lower segment and arm/height ratio	1
	Scoliosis or thoracolumbar kyphosis	1
	Reduced elbow extension	1
	Three of five facial features	1
	Skin striae	1
	Severe myopia	1
	Mitral valve prolapse	1
	Aorta dilatation/dissection	10
	Fulfilling Ghent II criteria	≥20 systemic points

Assignment of points in the Marfan score according to reference material. The clinical classification is based on phenotypical data in the reference material. If the phenotype fulfilled the Ghent II nosology for the MFS diagnosis, the Marfan score was given an additional 20 points.

MFS, Marfan syndrome.

as 10 points. Moreover, a causal *FBN1* variant combined with aortic dilatation are sufficient for diagnosing MFS according to Ghent II nosology.[4]

We also considered the clinical presentation of a patient with a clear MFS phenotype as the best marker for the genotype–phenotype association of a variant and MFS. To highlight the effect of high-quality phenotype records and to ensure that these records were reflected in the mean of a variant's Marfan score, cases fulfilling the Ghent II criteria—based solely on phenotypical data (e.g., lens luxation and aorta dilatation)—were scored an additional 20 points.

We considered a Marfan score ≥7 points as indicating an association with MFS (score-MFS), but it is obvious that a higher Marfan score had greater association significance than a lower score. In theory, it is possible to have a Marfan score >7 points without fulfilling the Ghent II criteria, but a score of 7 points would be found only in patients with at least some phenotypical characteristics typical for MFS. Therefore, a cutoff of 7 points is a conservative estimate when evaluating database diagnoses.

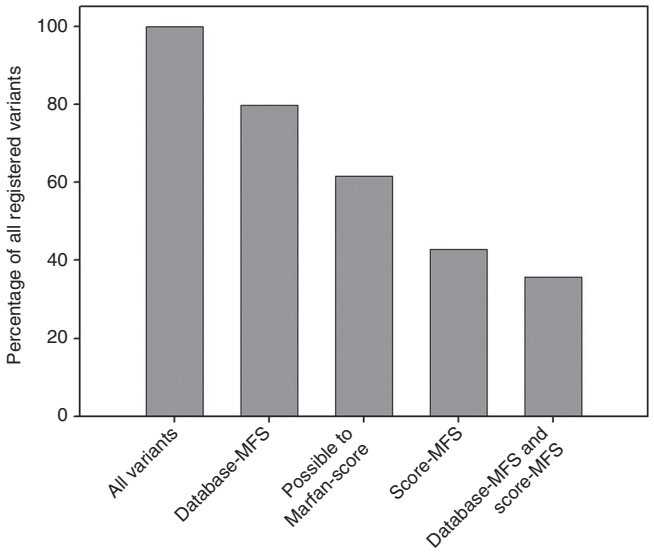

Figure 1 Percentages of all variants in different subgroups. "Database-Marfan syndrome" (MFS) represents variants associated with MFS in at least one *FBN1* database. "Possible to Marfan score" represents all variants classifiable with a Marfan score. "Score-MFS" represents variants with a Marfan score ≥7. "Database-MFS" and "score-MFS" represent all variants associated with MFS in at least one *FBN1* database and with a Marfan score ≥7.

For all cases defined as a "polymorphism," "MFS Berlin," "MFS Ghent I," "MFS Ghent II," or "incomplete MFS," the Marfan score was defined as shown in **Table 1**. No nonclassified records were scored.

RESULTS

Database

We registered 2,250 *FBN1* variants in our data set based on 4,904 single records presented in 307 references. It was possible to identify a specific individual in 2,303 records. For the remaining 2,601 records, either we were not able to determine whether the records represented a specific individual or we identified the records as representing an already published individual. We found 168 variants in searched references that were not registered in the databases, resulting in 2,082 database variants.

Database-MFS and score-MFS

Of the total 2,082 variants (**Figure 1** and **Table 2**, row 1), we were able to calculate a Marfan score for 1,283 variants, of which 69.7% (n = 893) had a Marfan score ≥7 and were therefore regarded as score-MFS. These were distributed as 830 (74.6% of all variants possible for the Marfan score in the database) in UMD-FBN1, 542 (73.3%) in HGMD, 73 (57.9%) in ClinVar, and 171 (69.7%) in Uniprot.

In all four databases, 1,661 variants (**Figure 1** and **Table 2**, row 3) were registered at least once as being associated with MFS; therefore, they were regarded as database-MFS. In general, the Marfan scores were higher for database-MFS, with mean Marfan scores ranging from 10.51 in ClinVar to 13.64 in

Table 2 Overview of database characteristics

	UMD-FBN1	Human Gene Mutation Database	ClinVar	Uniprot	Total
Total number of variants (%)[a]	1,840 (88.0%)	994 (47.5%)	329 (15.7%)	252 (12.0%)	2,082
Unique variants registered only in the specific database (%)[b]	857 (46.6%)	50 (5.0%)	198 (60.2%)	2 (0.8%)	1,107 (53.2%)
Database-MFS (%)[b]	1,254 (68.2%)	894 (89.9%)	240 (72.9%)	226 (89.7%)	1,661 (79.8%)
Possible to Marfan score (%)[b]	1,113 (60.5%)	739 (74.3%)	126 (38.3%)	230 (91.3%)	1,283 (61.6%)
Score-MFS (%)[c]	830 (74.6%)	542 (73.3%)	73 (57.9%)	171 (74.1%)	893 (69.7%)
Database-MFS and possible to Marfan score (%)[c]	751 (67.5%)	673 (91.1%)	104 (82.5%)	220 (95.7%)	967 (75.4%)
Mean Marfan score for database-MFS (range)	13.64 (–10 to 33)	12.22 (0 to 34)	10.51 (–10 to 30)	12.99 (0 to 29)	
Score-MFS and database-MFS (%)[d]	612 (81.5%)	516 (76.7%)	71 (68.3%)	169 (76.8%)	746 (77.1%)
Non-score-MFS and database-MFS (%)[d]	139 (18.5%)	157 (33.3%)	33 (31.7%)	51 (23.2%)	221 (22.9%)
Non-database-MFS and possible to Marfan score (%)[c]	180 (16.2%)	43 (5.8%)	17 (13.5%)	4 (1.7%)	195 (20.2%)
Mean Marfan score for non-database-MFS (range)	8.67 (–10 to 32.5)	4.4 (–10 to 28)	–7.16 (–10 to 0)	0.25 (0 to 1)	
Score-MFS and non-database-MFS (%)[e]	106 (58.9%)	13 (30.2%)	0 (0%)	0 (0%)	106 (54.4%)
Non-score-MFS and non-database-MFS (%)[e]	74 (41.1%)	30 (69.8%)	17 (100%)	4 (100%)	89 (46.6%)

Score-MFS represents variants with a Marfan score ≥7. Non-score-MFS represents variants with a Marfan score <7. Database-MFS represents variants associated with MFS in the *FBN1* database. Non-database-MFS represents variants *not* associated with MFS by an *FBN1* database.

MFS, Marfan syndrome.

[a]Percentage of all variants registered in all four databases. [b]Percentage of total registered variants in the specific database. [c]Percentage of variants possible to Marfan score in the database. [d]Percentage of possible to Marfan score and database-MFS. [e]Percentage of possible to Marfan score and non-database-MFS.

UMD-FBN1, compared with non-database MFS variants with mean Marfan scores ranging from −7.16 in ClinVar to 8.67 in UMB-FBN1. The negative ClinVar figure indicates that the database contains a high percentage of variants correctly not linked to MFS.

When evaluating database-MFS versus score-MFS correlations, we could only confirm score-MFS in 746 database-MFS variants (35.8% of all variants (**Figure 1**), representing 77.1% of all variants for which assignment of a Marfan score was possible. In the specific databases, there were 612 (81.5% of all classifiable database-MFS variants in the database) in UMD-FBN1, 516 (76.7 %) in HGMD, 71 (68.3%) in ClinVar, and 169 (76.8%) in Uniprot (**Table 2** (row 8)). Most of the variants appear in several of the databases.

If we accepted a Marfan score cutoff of ≤7 as a marker for no phenotypical association of the variant with MFS (non-score-MFS), then the UMD-FBN1 database associated variants with MFS without clinical evidence for 18.5% of variants, HGMD did so for 33.3%, ClinVar for 31.7%, and UniProt for 23.2% (**Table 2**, row 9). When evaluating score-MFS with non-database-MFS, we found score-MFS for 54.4% of all scoreable non-database-MFS. This indicates that databases contain incorrect conclusions for variants.

Supplementary data

Individual variants and the data used for calculating the Marfan score are provided in **Supplementary Data S1** online. We searched the ExAC (Exome Aggregation Consortium) data set[16] for allele frequency data for each recorded variant. **Supplementary Data S2** presents these data. We manually searched all recorded variants for the in silico scores of SIFT,[17] Mutation Taster,[18] and PhyloP using the Alamut v2.3 software package (Interactive Biosoftware, Rouen, France). We also manually searched all non-synonymous variants for PolyPhen 2 HumDiv via the PolyPhen 2 homepage. **Supplementary Data S3** online contains the in silico score data. When available in the databases, we also recorded expected variant effects on amino acids. **Supplementary Data S4** online shows the collected amino acid data.

DISCUSSION

At present, the four databases collectively provide the diagnostic tools for evaluating genetic test results when diagnosing MFS. Based on these four databases, we compiled a large, comprehensive, and well-curated *FBN1* database with detailed descriptions of genotype–phenotype relations. We also defined a new Marfan score to test currently used databases to gauge the quality of the information in these curated databases. MFS is a well-defined monogenic disorder with a widely accepted systematic phenotypical scoring criteria described in the Ghent II nosology.[4] The Marfan score is an effort to operationalize the MFS phenotype in one figure in accordance with the diagnostic criteria of Ghent II. To minimize loss of valuable data in the Marfan score, we also used imprecise information such as "incomplete Marfan" or "classical Marfan." We regarded the Ghent II criteria as the gold standard for diagnosing MFS, but older criteria or imprecise definitions also affect the Marfan score.

Our evaluation of the four *FBN1* variant databases showed that they have different characteristics. We considered the size of the database as a key parameter because the likelihood that a variant was in a database must be correlated with the size of the database. However, the number of unique variants could also be

important because the variant undergoing evaluation could be a unique variant represented in only one database.

We observed large differences between databases regarding the absolute number as well as the percentage of unique variants. The size of the database was not an indicator of the proportion of unique variants because HGMD had only 5.0% of the total unique variants even though the database contained 47.5% of the total variants. Strikingly, 60.2% of all variants in the rather small ClinVar database were unique variants. Still, many unique variants do not necessarily indicate that the database is better than other databases because variants without supporting data are of very little value. Our data indicate that the ClinVar database did indeed have many unique variants, but a majority (61.7%) of variants could not be classified with a Marfan score based on the available data.

Only 47 variants were present in all four databases. It is therefore very important to use more databases when looking for variant data. We cannot recommend using any of the databases as a sole indicator of variant pathogenicity. However, the current collated database could prove to be an important tool for diagnosing MFS in the future.

We found 168 variants in searched references that were not recorded in any of the databases. None of the variants was clearly associated with MFS, and we could score only 63 variants, of which 61 scored as polymorphisms, 1 variant scored 1 point due to mitral prolapse, and 1 variant scored 2 points due a registration of "incomplete MFS." This shows that relevant published data overall are recorded in the databases.

The databases provided very little information regarding the variants registered in the database. Only 61.6% of the registered variants were classifiable with a Marfan score, indicating that the databases have many variants registered without accessible documentation for any disease-causing effect. There was a large variation in classifiable variants between the databases, with Uniprot having 91.3% of the registered variants classifiable with a Marfan score compared with only 38.3% in ClinVar. Both databases have a low number of registered variants compared with UMD-FBN1 and HGMD, which could explain the magnitude of variance. The fact that the smallest database (Uniprot) also had the highest degree of phenotypes that we were able to score could be explained by the fact that large databases may be less critical with the data they record.

We have found that laboratories, when provided with genetic material to analyze, do not receive comprehensive phenotype data from the referring clinicians. For this reason, they cannot provide detailed variant-associated phenotype data to the databases. This is a major reason why databases contain large quantities of variant data but not of appropriate and detailed phenotype data.

In evaluating database-MFS, it is alarming that up to one-third of variants (in the HGMD) do not score enough points with the conservative cutoff limit of ≥7 points in score-MFS. The database-MFS and score-MFS correlation proportion represent only 35.8% of all registered variants (**Figure 1**), indicating that an even larger proportion of the information in the databases is based on undocumented diagnosis statements than we can evaluate using the Marfan score.

As more genetic tests are being performed owing to reduced costs and easier access to sequencing facilities, we expect that new variants will be discovered more frequently. We also predict a more frequent single-patient setting where segregation data are unavailable. We therefore expect an increasing demand for fast and reliable classification of variants in diagnostic laboratories. It is our impression that variant databases, at least to some extent, are already used by laboratories when analyzing variants. To our knowledge, there are no data regarding how precise these databases are when used as a diagnostic tool, but the present data indicate that one should be cautious when using these databases.

Evaluation of reference literature regarding MFS and associated variants is an expert effort because the genotype–-phenotype association for specific variants must be conducted by an evaluator with specific knowledge of the MFS phenotype as well as the history of the diagnostic criteria. Clinical manifestations may also vary considerably, both interfamiliarly and intrafamiliarly,[19] making it even more difficult for non-MFS experts to determine whether a genotype–phenotype exists.

ACMG accepts the use of databases as supporting evidence but warns about "how frequently the database is updated, whether data curation is supported, and what methods were used for curation."[6] Previously, we showed that all current databases include data of equivocal quality.[12] The arbitrary goal of 90% certainty that a variant is either likely benign or likely pathogenic is often difficult or impossible to achieve for missense variants when following the ACMG 2015 guidelines. Still, the ACMG guidelines recommend that laboratories use and report to variant databases, including the ClinVar database.[6] In the present study, we did not find any data that could specifically support use of the ClinVar database for assessing *FBN1* variants. Instead, we recommend well-curated databases based on phenotype data associated with each variant. We also suggest using some sort of phenotype scoring system and propose our Marfan score as a method for scoring *FBN1* variants associated to MFS. The database data used in this study can be found in the Supplementary Information and are free to use as a transient update and curation of the *FBN1* databases. We also provide three separate supplementary variant data sets: one for ExAC allele frequency;[16] one for expected amino acid effects; and one for in silico scores from SIFT,[17] Mutation Taster,[18] PhyloP, and PolyPhen 2 HumDiv.[20,21]

Conclusion

The current *FBN1* databases should be used with caution when evaluating *FBN1* variant pathogenicity. Therefore, it is of great importance to use more than one database when searching for variant data. At present, the UMD-FBN1 database seems to be the biggest and best curated and therefore the most comprehensive database. However, the need is evident for better-curated databases containing clear phenotype–genotype associations. A systematic phenotype scoring system could aid in clinical decision making.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

ACKNOWLEDGMENTS

This study was supported by the Department of Clinical Medicine, Aarhus University Hospital (http://clin.au.dk/en/) and Aarhus University (http://au.dk/en), by a PhD scholarship to K.A.G. and by the Novo Nordisk Foundation (http://www.novonordiskfonden.dk/en). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Beighton P, de Paepe A, Danks D, et al. International Nosology of Heritable Disorders of Connective Tissue, Berlin, 1986. *Am J Med Genet* 1988;29:581–594.
2. Dietz HC, Cutting GR, Pyeritz RE, et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. *Nature* 1991;352:337–339.
3. De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. *Am J Med Genet* 1996;62:417–426.
4. Loeys BL, Dietz HC, Braverman AC, et al. The revised Ghent nosology for the Marfan syndrome. *J Med Genet* 2010;47:476–485.
5. Sheikhzadeh S, Kade C, Keyser B, et al. Analysis of phenotype and genotype information for the diagnosis of Marfan syndrome. *Clin Genet* 2012;82:240–247.
6. Richards S, Aziz N, Bale S, et al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405–424.
7. von Kodolitsch Y, Kutsche K. Interpretation of sequence variants of the FBN1 gene: analog or digital? A commentary on decreased frequency of FBN1 missense variants in Ghent criteria-positive Marfan syndrome and characterization of novel FBN1 variants. *J Hum Genet* 2015;60:465–466.
8. Plon SE, Eccles DM, Easton D, et al.; IARC Unclassified Genetic Variants Working Group. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum Mutat* 2008;29:1282–1291.
9. Faivre L, Collod-Beroud G, Loeys BL, et al. Effect of mutation type and location on clinical outcome in 1,013 probands with Marfan syndrome or related phenotypes and FBN1 mutations: an international study. *Am J Hum Genet* 2007;81:454–466.
10. Deans ZC, Fairley JA, den Dunnen JT, Clark C. HGVS Nomenclature in practice: an example from the United Kingdom National External Quality Assessment Scheme. *Hum Mutat* 2016;37:576–578.
11. Brookes AJ, Robinson PN. Human genotype-phenotype databases: aims, challenges and opportunities. *Nat Rev Genet* 2015;16:702–715.
12. Groth KA, Gaustadnes M, Thorsen K, et al. Difficulties in diagnosing Marfan syndrome using current FBN1 databases. *Genet Med* 2016;18:98–102.
13. Collod-Béroud G, Le Bourdelles S, Ades L, et al. Update of the UMD-FBN1 mutation database and creation of an FBN1 polymorphism database. *Hum Mutat* 2003;22:199–208.
14. Landrum MJ, Lee JM, Riley GR, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res* 2014;42(Database issue):D980–D985.
15. UniProt Consortium. Activities at the Universal Protein Resource (UniProt). *Nucleic Acids Res* 2014;42:D191–D198.
16. Lek M, Karczewski KJ, Minikel EV, et al.; Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285–291.
17. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res* 2003;31:3812–3814.
18. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods* 2010;7:575–576.
19. Dietz HC, Pyeritz RE. Mutations in the human gene for fibrillin-1 (FBN1) in the Marfan syndrome and related disorders. *Hum Mol Genet* 1995;4 Spec No:1799–1809.
20. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. *Curr Protoc Hum Genet* 2013;Chapter 7:Unit7.20.
21. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7:248–249.

Study 3

Groth et al. Orphanet Journal of Rare Diseases (2015) 10:153
DOI 10.1186/s13023-015-0369-8

Orphanet Journal of Rare Diseases

RESEARCH Open Access

Prevalence, incidence, and age at diagnosis in Marfan Syndrome

Kristian A. Groth[1,2*], Hanne Hove[3,4], Kasper Kyhl[5], Lars Folkestad[6,7], Mette Gaustadnes[2], Niels Vejlstrup[5], Kirstine Stochholm[8,9], John R. Østergaard[8], Niels H. Andersen[1] and Claus H. Gravholt[2,9]

Abstract

Background: Marfan syndrome is a genetic disorder with considerable morbidity and mortality. Presently, clinicians use the 2010 revised Ghent nosology, which includes optional genetic sequencing of the *FBN1* gene, to diagnose patients. So far, only a few studies based on older diagnostic criteria have reported a wide range of prevalence and incidence. Our aim was to study prevalence, incidence, and age at diagnosis in patients with Marfan syndrome.

Method: Using unique Danish patient-registries, we identified all possible Marfan syndrome patients recorded by the Danish healthcare system (1977–2014). Following, we confirmed or rejected the diagnosis according to the 2010 revised Ghent nosology.

Results: We identified a total of 1628 persons with possible Marfan syndrome. We confirmed the diagnosis in 412, whereof 46 were deceased, yielding a maximum prevalence of 6.5/100,000 at the end of 2014. The annual median incidence was 0.19/100,000 (range: 0.0–0.7) which increased significantly with an incidence rate ratio of 1.03 (95 % CI: 1.02–1.04, $p < 0.001$). We found a median age at diagnose of 19.0 years (range: 0.0–74). The age at diagnosis increased during the study period, uninfluenced by the changes in diagnostic criteria. We found no gender differences.

Conclusion: The increasing prevalence of Marfan syndrome during the study period is possibly due to build-up of a registry. Since early diagnosis is essential in preventing aortic events, diagnosing Marfan syndrome remains a task for both pediatricians and physicians caring for adults.

Keywords: Epidemiology, Rare diseases, Aortic aneurism, Lens subluxation, Aortic dissection

Background

Since the first description of Marfan syndrome (MFS), decades of research in the syndrome [1] have contributed to the knowledge about the phenotypical presentation and the genetic background. In 1986, the definition of MFS described by the Berlin criteria [2], was purely based on the clinical phenotype. Later on, Dietz et al. found a connection between MFS and *FBN1*, the gene coding for the fibrillin protein [3]. The first Ghent criteria from 1996 (Ghent-I) [4], which were a revision of the Berlin criteria, used the newly discovered *FBN1* mutations as a component in the diagnostic criteria. In 2010, the revised Ghent criteria (Ghent-II) [5] highlighted *FBN1* mutation, aortic dilatation and ectopia lentis as cornerstones in the MFS diagnosis [5].

The most frequently quoted prevalence of MFS is 20/100,000 [6, 7]. The source is an early version of the textbook of Emery and Rimoins: Principles and practice of Medical Genetics [8], but the latest version only refers to a crude calculation of 4–6/100,000 based on MFS patients found in the catchment area of Johns Hopkins Hospital in Baltimore. During the last 70 years, only five studies report MFS prevalence, all but one based on the Berlin criteria. In 1958, Lynas et al. reported a prevalence of 1.5/100,000 in a population from Northern Ireland [9]. Sun et al. reported a prevalence of 17.2/100,000 in China in 1990 [10]. Gray et al. [11] reported a prevalence of 6.8/100,000 in the north-east Scottish population. A Danish study from 1997 by Fuchs et al. showed a prevalence of 4.6/100,000 [12]. Here the diagnosis was based on data from medical records and all

* Correspondence: kristian.groth@ki.au.dk
[1]Department of Cardiology, Aarhus University Hospital, DK-8200 Aarhus N, Denmark
[2]Department of Molecular Medicine, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus N, Denmark
Full list of author information is available at the end of the article

cases were diagnosed before 1993. Chiu et al. in 2014 reported a much higher prevalence of 10.2/100,000, but the figures were solely based on data collected from 2000–2012 and without any regard to diagnostic criteria or clinical presentation [13]. Thus, there are no publications on the prevalence of clinically verified MFS based on the Ghent-I or the Ghent-II criteria and no prevalence studies report data including *FBN1* mutations. As the clinical manifestations of MFS may vary even within families with the same genetic background, it is not only difficult to diagnose MFS but also to assess the true prevalence of MFS based on clinical phenotyping of patients [14]. However, use of *FBN1* genotyping may represent a new dimension in diagnosing MFS and thereby provide a more accurate identification and classification of MFS [15].

Therefore, we set out to determine the prevalence and incidence of Marfan syndrome in Denmark using the current diagnostic approach as well as to describe the age diagnostic as a marker for the diagnostic delay in MFS defined as the time from birth until diagnosis.

Methods

Since 1968, all Danish citizens have a unique personal identification number (CPR-number) in the Danish Central Person Register (www.cpr.dk) which is used in a number of Danish registers, thus providing a unique opportunity for record linkage, including The National Patient Register (NPR) [16] and The Danish Register of Cause of Death (DRCD) [17]. From 1977 and onwards, the NPR registered all in-patient contacts with the Danish healthcare system and from 1995, also registered all outpatient contacts. All contacts were given an International Classification of Diseases (ICD) code (ICD-8 until 1993 and ICD-10 from 1994 and onwards). DRCD record all death certificates since 1973 according to the ICD system, and used ICD-8 in 1973–1983, and ICD-10 from 1984 and onwards. DRCD was updated through 2013.

We retrieved CPR-numbers from all persons recorded in at least one of the two registries with the ICD-10 diagnosis Q87.4 "Marfan Syndrome" or ICD-8 759.80 "Arachnodactylia (syndroma Marfan)".

As several persons were noted with an ICD-8 or ICD-10 diagnosis of MFS only based on the suspicion of suffering from MFS in the NPR register, all medical records were manually evaluated, to confirm or reject the diagnosis. As the MFS diagnosis has evolved significantly during the years with the changing criteria, Berlin [2], Ghent-I [4] and II [5], we decided to perform the medical record evaluation according to the Ghent-II criteria [5]. Medical records were accessed via a central electronic patient journal system (E-journal) provided by the Danish Healthcare System. If E-journal material was insufficient to determine whether the person had MFS or not, the original paper medical file was retrieved.

If we, during the evaluation, found other persons such as family members that could also have MFS, we evaluated their MFS status as well (Fig. 1).

There are seven ways a person can meet the Ghent-II criteria (Table 1). All who fulfilled at least one of the seven principal diagnostic features were classified as "MFS", whereas all who did not fulfill any of the seven possible diagnostic criteria were classified as "not MFS".

If medical records were insufficient (or non-existing) in both electronic and non-electronic versions, or if for some reason (ex. deceased or emigrated) it was not possible to fully determine the persons MFS status, a committee of three MFS specialist physicians evaluated the available persons data and determined the MFS status by consensus. All persons with no clinical data were classified as "not MFS".

The study was approved by the Scientific Ethical Committee and the Danish Data Protection Agency.

Statistical analysis

Age at diagnosis was studied by median age at diagnosis with range interval and time trends were studied with quantile regression including 95 % confidence intervals (CI). Time trends in incidence including 95 % confidence intervals (CI) were analyzed using Poisson regression. To graphically illustrate time trends in incidence we used linear regression lines. Gender difference and difference between the cohort with MFS and without MFS were studied using Mann-Whitney's nonparametric test. $P < 0.05$ was considered significant. Stata 12.1 for Windows (StataCorp LP, College Station, TX, USA) was used for all calculations.

Results

From NPR and DRCD, we extracted all persons registered with a relevant ICD-8 or ICD-10 diagnosis, which resulted in 1559 unique CPR-numbers (Fig. 1). During the evaluation of their medical records, we found 69 additional potential MFS persons resulting in a total cohort of 1628. During the evaluation process, we found 22.5 % ($n = 366$) patients fulfilling one of the seven ways to obtain the MFS diagnosis and rejected 1078 cases (66.2 %). In 184 (11.3 %), it was not possible to accurately determine whether the persons fulfilled the diagnostic criteria. Thus, sufficient data was present in 1444 (88.7 %) of the total cohort. 73 (4.5 %) had no clinical data and were either deceased ($n = 69$) or emigrated ($n = 4$). They were classified as "not MFS". A committee of three physicians specialized in MFS (KAG, NHA and CHG), evaluated every remaining case ($n = 111$) and reached consensus on their MFS status. Forty-six were determined to have MFS and the remaining 65 were registered as "not

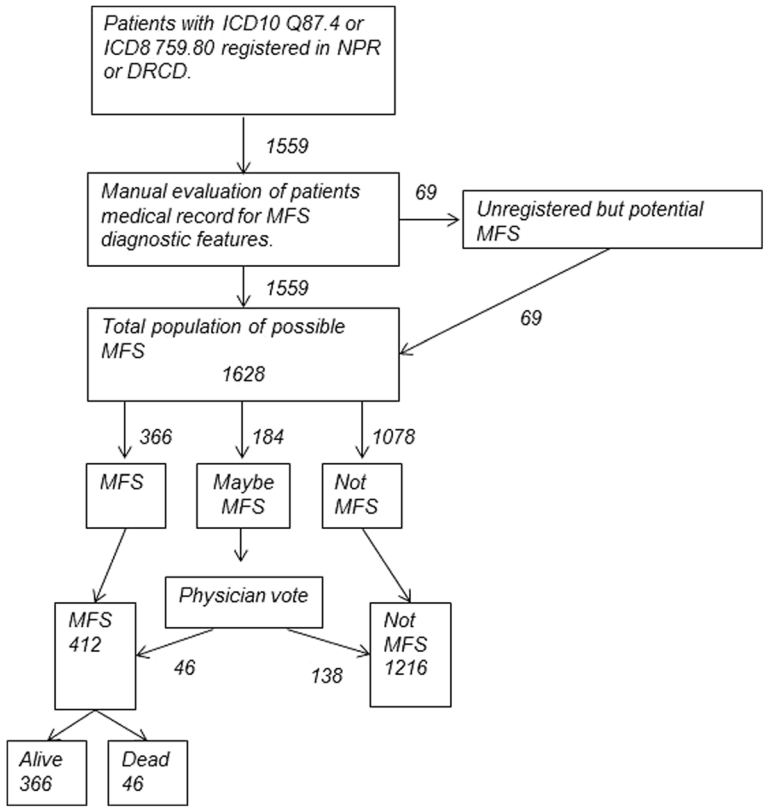

Fig. 1 The total cohort and evaluation process defining patients with MFS

MFS". Thus, 1216 (74.7 %) had "not MFS" and 412 (male $n = 215$) classified as "MFS". Among the 412 classified as MFS, 366 (male $n = 189$) were still alive at the end of 2014 (Fig. 2a).

There was no difference in gender ($p = 0.3$) and birth year between persons classified with or without MFS ($p = 0.2$).

Prevalence and incidence

As of January 1st 2015, the population of Denmark was 5,659,715 inhabitants (www.dst.dk) yielding a point prevalence of MFS of 6.5/100,000. We also calculated an average prevalence increase of 0.17/100,000 per year during the study period. The average number of MFS diagnosed patients annually was 11.1 with a significantly increasing incidence during the study period (Fig. 2a, b).

The median annual incidence was 0.19/100,000 (0.0–0.7) (Table 2). During the study period, the absolute number of patients diagnosed with MFS annually increased significantly with an incidence rate ratio (IRR) of 1.03 (95 % CI: 1.02–1.04, $p < 0.001$) (Fig. 3). Since this increase could be due to lack of access to patient records early in the study period, we calculated the IRR for the last 10 years of the study period (2004–2014) resulting in an increasing IRR of 1.11 (95 % CI 1.01–1.21 $p = 0.018$). We identified no difference in IRR between the two genders during the study period ($p = 0.47$).

Based on the current prevalence of MFS in our data and exploring different scenarios with different relative risk of mortality of 1.1, 1.25, 1.5, 2.0 or 3.0 in comparison with the general population and using forecasts for the development of the Danish population, we generated future trajectories of the prevalence of MFS (Fig. 4).

Age at diagnosis

The median age at diagnosis for the entire MFS group was 19.0 (0.0–74.5) years. There was no difference in age at diagnosis between males and females (median age at diagnosis: males 18.3 years (0.0–74.5) and females 19.9 years (0.0–72.1) ($p = 0.3$)). By the age of 1.5 years 10 %, 6.5 years 25 % and 38.8 years 75 % of the entire cohort were diagnosed, respectively, but age at diagnosis extended into the seventies (Fig. 5a). There was a tendency towards an increasing age at diagnosis of 0.29 (95 % CI -0.03–0.60, $p = 0.075$) years per year of diagnosis during the study period (Fig. 5b).

FBN1 evaluation

Of the total cohort of 412 MFS patient 196 had been tested for *FBN1* mutations, with 193 having a *FBN1* mutation

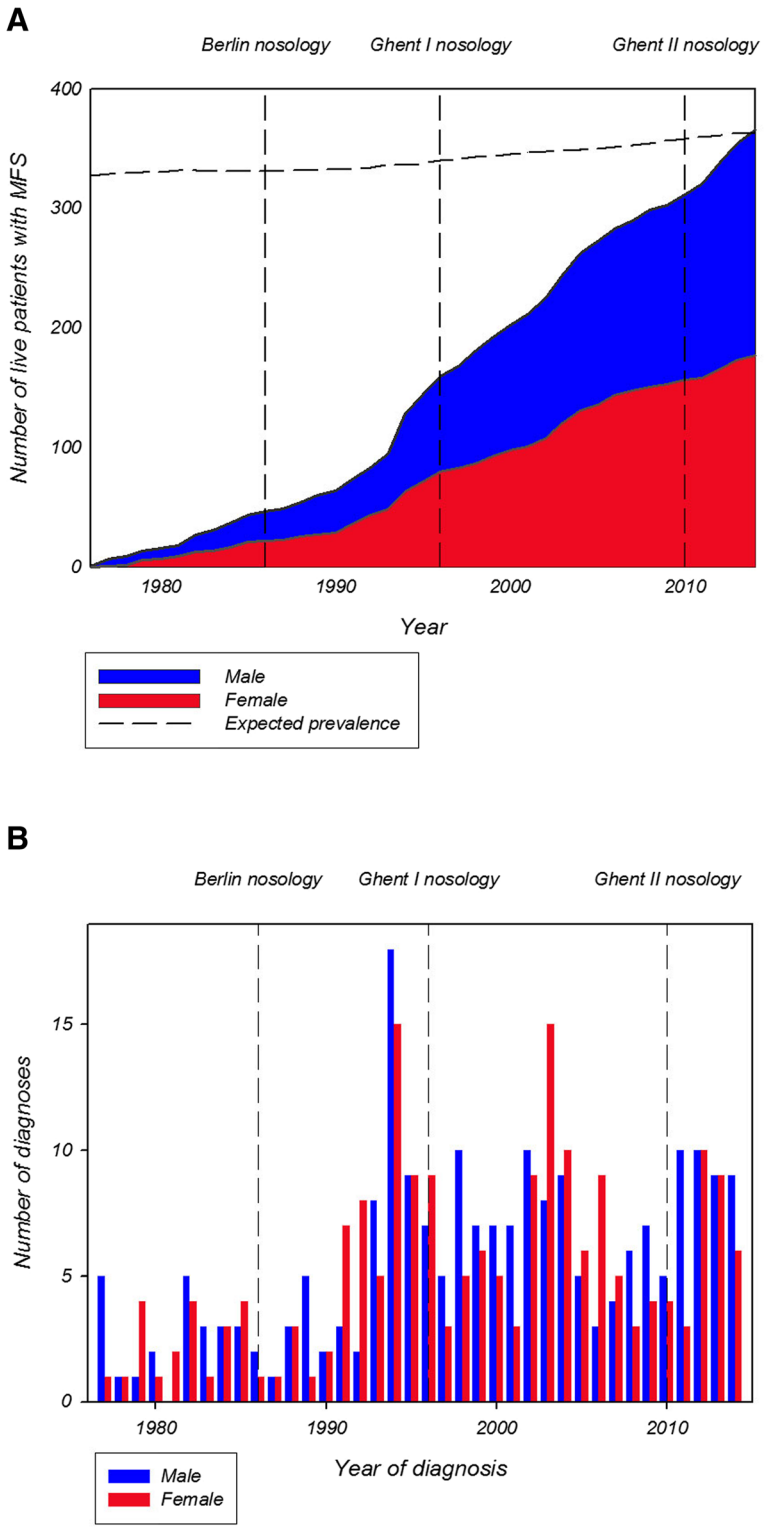

Fig. 2 a Observed cumulated absolute number of Marfan syndrome patients alive per year during the study period from 1977 to 2014. The dashed line (expected prevalence) indicates the expected number of Marfan syndrome patients assuming a prevalence of 6.5 per 100,000 Danish inhabitants. The year of change of nosology is indicated by a horizontal line and marked with the nosology name. **b** Number of Marfan syndrome patients diagnosed per year during the study period from 1977 to 2014. Bars divided by sex. The year when the MFS nosology was changed, is indicated by a horizontal line and marked with the nosology name

Table 1 The seven principal ways a person can meet the Ghent II criteria in the Marfan syndrome diagnosis

1) Ascending aorta dilatation[a] & ectopia lentis
2) Ascending aorta dilatation[a] & a *FBN1* mutation
3) Ascending aorta dilatation[a] & minimum seven systematic points
4) Ectopia lentis with a *FBN1* mutation known to cause ascending aorta dilatation[a]
5) Family history of MFS & ectopia lentis
6) Family history of MFS & minimum seven systematic points
7) Family history of MFS & ascending aorta dilatation[a]

[a]Or dissection of the ascending aorta

known to cause MFS. In three cases no known mutation was found, however they fulfilled the Ghent-II nosology by other criteria (aorta ascendens dilatation and minimum seven systemic points ($n = 2$) or by a family history of MFS and aorta ascendens dilatation ($n = 1$)). One patient was only evaluated for FBN1 mutations and could have a MFS related disorder. One patient was evaluated with a wide genetic panel spanning all Marfan related disorders. One patient was evaluated for FBN1 mutations and collagen anomaly. However, since the three patients fulfilled the criteria for MFS, we included them in the study cohort.

Preimplantation and prenatal diagnostics

Since 2000, only extremely few patients have chosen preimplantation diagnostics due to limited service and long waiting times. A total of 24 MFS patients chose prenatal diagnostics and of these, ten fetuses carried an FBN1 mutation. In only three cases did the parents choose an abortion before the 12th gestation week, indicating that currently such low numbers of legal abortions are unlikely to affect the prevalence and incidence of MFS (unpublished data from the Danish Cytogenetic Central Register).

Discussion

As the first study of MFS according to the Ghent-II nosology, this report shows a prevalence of MFS to be 6.5/100,000 in the uniform health care system in Denmark. We also find that the diagnosis of MFS is made throughout the entire lifetime, with only half of all diagnoses confirmed before the age of 19 years. Importantly, it seems as if the diagnostic vigilance is increasing during the study period, illustrated by the significant increase in incidence.

We identified the Danish MFS prevalence to be 41 % higher than the previously reported Danish prevalence of 4.6/100,000 published almost 20 years ago [12]. In the 1990'ies, patients were diagnosed according to the Berlin nosology and the study primarily focused on ectopia lentis [12], whereas the present study subjected every single patient file to close scrutiny including every aspect of MFS. Interestingly, the nosology of MFS changed three times (1986, 1996 or 2010) during the study period, but we did not see any changes in incidence or prevalence related to different diagnostic criteria (Fig. 2a, b).

It is difficult to estimate the true prevalence of MFS and we are well aware that some patients with MFS in Denmark still need to be diagnosed and identified. Based on the present data, we expect that the prevalence of MFS will increase by approximately 0.17 patients/100.000 the next many years. The reasons behind the imprecision are multifactorial – i.e. multiple factors exert an effect and some may tend to decrease and others may tend to increase the observed prevalence. Thus mortality, and to a lesser extent diagnostic practice, will influence the absolute number of MFS in the Danish population. Since the exact relative risk of death is not known in MFS, we have illustrated this with a set of different scenarios, where it can be appreciated that if the relative risk of death is below 2.0, we will continue to see an expanding population of MFS (Fig. 4). Newer literature seems to suggest that mortality is decreasing for contemporarily treated MFS [18, 19], which would obviously increase the prevalence, as illustrated in our future projections (Fig. 4). Another important component in the increasing prevalence is the build-up of a registry, where more patients are often diagnosed than censured (deceased or emigrated) in the beginning of the history of a registry. This phenomenon is seen in many other studies of rare syndromes [20, 21]. Moreover, our data also illustrate a significantly increasing incidence rate ratio, which was evident even during the last 10 years of the study period. This increase in incidence could be caused by an increased focus on the disease and better knowledge about the syndrome by healthcare professionals, resulting in more patients being diagnosed even at a high age. Better diagnostics and the increased use of genotyping could also explain the increasing incidence, as could more intense investigations of affected families, currently recommended in guidelines [5]. Factors expected to decrease prevalence, such as preimplantation diagnostics followed by induced abortions currently only seem to play a very minor role. On the other hand more surviving well-treated individuals with a disease causing MFS mutation could also lead to increased transmission of MFS mutations.

Since 1996 there have been two centers in Denmark handling rare diseases including MFS. We believe that the centralization of rare diseases has resulted in an increased focus on examining pedigrees of MFS families, and thereby diagnosing adult family members with MFS.

Given that MFS is a potentially life-threatening disorder due to aortic disease [22–24], an early diagnosis is important and will provide better overall health for the MFS patient [22, 25]. It is our impression that some

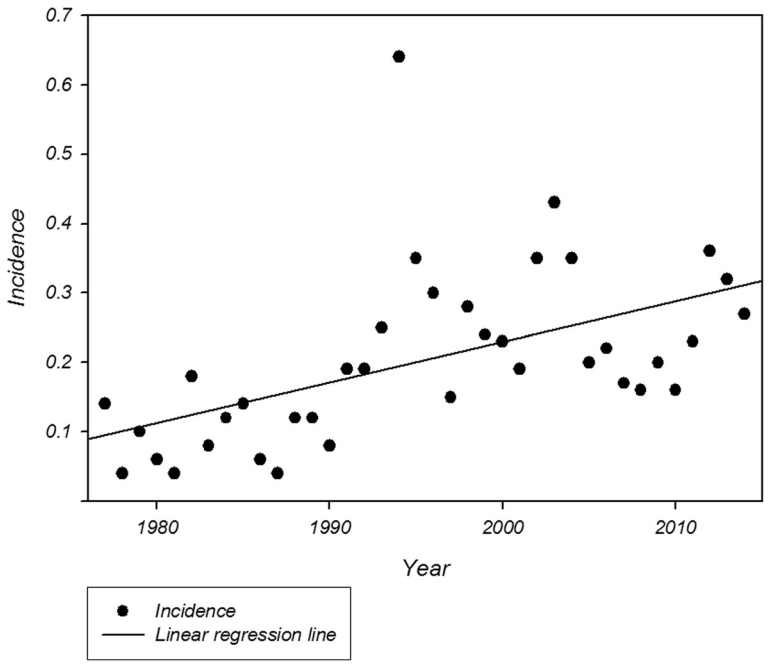

Fig. 3 Yearly incidence of Marfan syndrome in Denmark during the study period 1977 to 2014. For clarity, the significant increase in incidence during the study period is visualized by linear regression

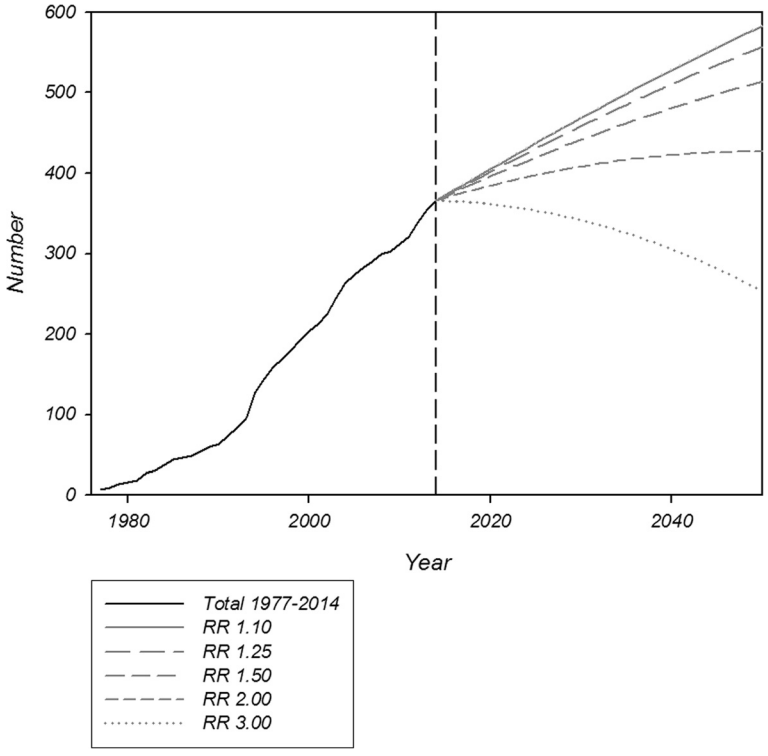

Fig. 4 Absolute numbers of Marfan syndrome in Denmark during the study period 1977 to 2014 and the absolute theoretical numbers extrapolated onwards to 2050. Extrapolation is based on the expected Danish population according to Statistical Denmark (www.dst.dk). Incidence is set to 0.19 per 100,000 as found in this study. Since there has been no studies reporting mortality ratios in comparison with general population, we have for illustration plotted five different relative risks (RR) of mortality compared to the general Danish population

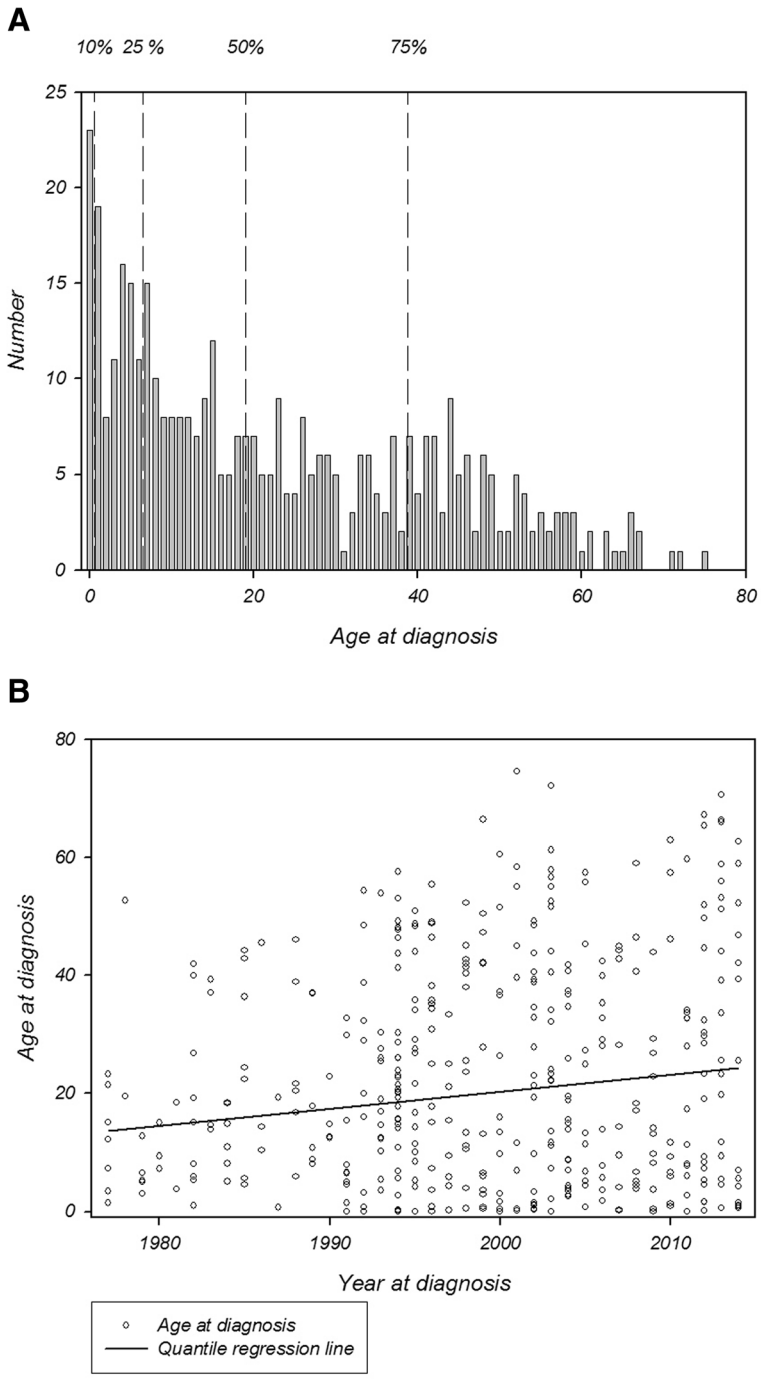

Fig. 5 a Number of Marfan syndrome patients by age at diagnosis. Patients diagnosed during the study period 1977 to 2014. Dashed lines indicating the age when 10, 25, 50 and 75 % of MFS patients are diagnosed. **b** Age at diagnosis versus year of diagnosis during the study period 1977 to 2014. The non-significant increase in age at diagnosis is visualized by quantile regression

physicians expect diagnosing MFS to be mainly a task for pediatricians. However, our data clearly indicate that many MFS patients are not diagnosed until late in life which means that all medical specialties should focus on even subtle clinical signs [26] and not hesitate to refer potential undiagnosed MFS patients, even from an elderly population. Early diagnosis should be the goal since this could reduce health expenditures and possibly avoid cases of dissection and sudden death [27]. The significant increase in age at diagnosis in the current cohort and

Table 2 Yearly incidence per 100,000 of Marfan syndrome in Denmark

Year of diagnosis	Male			Female			Total		
	Diagnosed	Population	Incidence	Diagnosed	Population	Incidence	Diagnosed	Population	Incidence
1977	6	2,513,000	0.24	1	2,567,000	0.04	7	5,079,879	0.12
1978	1	2,520,000	0.04	1	2,577,000	0.04	2	5,096,959	0.04
1979	1	2,526,000	0.04	4	2,586,000	0.15	5	5,111,537	0.10
1980	2	2,529,053	0.08	1	2,593,012	0.04	3	5,122,065	0.06
1981	0	2,528,225	0.00	2	2,595,764	0.08	2	5,123,989	0.04
1982	5	2,523,825	0.20	4	2,595,330	0.15	9	5,119,155	0.18
1983	3	2,521,220	0.12	1	2,595,244	0.04	4	5,116,464	0.08
1984	3	2,517,942	0.12	3	2,594,188	0.12	6	5,112,130	0.12
1985	3	2,517,072	0.12	4	2,594,036	0.15	7	5,111,108	0.14
1986	2	2,520,563	0.08	1	2,595,710	0.04	3	5,116,273	0.06
1987	1	2,526,020	0.04	1	2,598,774	0.04	2	5,124,794	0.04
1988	3	2,527,996	0.12	3	2,601,258	0.12	6	5,129,254	0.12
1989	5	2,528,165	0.20	1	2,601,613	0.04	6	5,129,778	0.12
1990	2	2,530,597	0.08	2	2,604,812	0.08	4	5,135,409	0.08
1991	3	2,536,391	0.12	7	2,610,078	0.27	10	5,146,469	0.19
1992	2	2,544,454	0.08	8	2,617,672	0.31	10	5,162,126	0.19
1993	8	2,554,594	0.31	5	2,626,020	0.19	13	5,180,614	0.25
1994	18	2,563,442	0.70	15	2,633,200	0.57	33	5,196,642	0.64
1995	9	2,573,324	0.35	9	2,642,394	0.34	18	5,215,718	0.35
1996	7	2,592,222	0.27	9	2,658,805	0.34	16	5,251,027	0.30
1997	5	2,604,937	0.19	3	2,670,184	0.11	8	5,275,121	0.15
1998	10	2,615,669	0.38	5	2,679,191	0.19	15	5,294,860	0.28
1999	7	2,625,421	0.27	6	2,688,156	0.22	13	5,313,577	0.24
2000	7	2,634,122	0.27	5	2,695,898	0.19	12	5,330,020	0.23
2001	7	2,644,319	0.26	3	2,704,893	0.11	10	5,349,212	0.19
2002	10	2,654,146	0.38	9	2,714,208	0.33	19	5,368,354	0.35
2003	8	2,662,423	0.30	15	2,721,084	0.55	23	5,383,507	0.43
2004	9	2,670,135	0.34	10	2,727,505	0.37	19	5,397,640	0.35
2005	5	2,677,292	0.19	6	2,734,113	0.22	11	5,411,405	0.20
2006	3	2,685,846	0.11	9	2,741,613	0.33	12	5,427,459	0.22
2007	4	2,696,662	0.15	5	2,750,422	0.18	9	5,447,084	0.17
2008	6	2,712,666	0.22	3	2,763,125	0.11	9	5,475,791	0.16
2009	7	2,732,020	0.26	4	2,779,431	0.14	11	5,511,451	0.20
2010	5	2,743,286	0.18	4	2,791,452	0.14	9	5,534,738	0.16
2011	10	2,756,582	0.36	3	2,804,046	0.11	13	5,560,628	0.23
2012	10	2,766,776	0.36	10	2,813,740	0.36	20	5,580,516	0.36
2013	9	2,778,852	0.32	9	2,823,776	0.32	18	5,602,628	0.32
2014	9	2,792,279	0.32	6	2,834,956	0.21	15	5,627,235	0.27
1977–2014	215	2,609,146	0.22	197	2,671,729	0.19	412	5,281,280	0.20

Data on gender only, with an accuracy of 1000 individuals for the years 1977–1979. For the line 1977–2014 the presented data are the summed number of patients diagnosed with Marfan syndrome, mean values for the Danish population and mean values for Marfan syndrome incidence

especially the diagnosis of quite old individuals, may well illustrate diagnosis of less affected individuals, a factor that could also lead to an increased prevalence of the MFS.

Phenotyping patients can be difficult and time-consuming and clinical manifestations resulting in MFS will sometimes only be evident when the patient reaches

adulthood and thereby "grows into the diagnosis". Clinical manifestations may also vary considerably and some patients have a milder phenotype making it difficult to accurately assess the prevalence of MFS [28]. In theory, *FBN1* genotyping should help solve this problem, but discovery of the *FBN1*-gene did not seem to have any immediate effect on the age at diagnosis (Fig. 2a). However, of the 412 patients diagnosed with MFS in our study cohort approximately half of the population ($n = 196$) had been tested for *FBN1* mutations, even that it is a snapshot it may be the reason why genotyping did not have a major impact in this cohort. It is our impression that access to genetic sequencing is improving and we have not seen the full impact of *FBN1* screening on the prevalence of MFS. *FBN1* genotyping represents a new dimension in diagnosing MFS that could accelerate the process, but still some difficulties remain in the correct interpretation of *FBN1* gene test results [29].

Strength and limitations

The present study is a nationwide register study, covering all subjects ever given a diagnosis of MFS. Furthermore, the study was performed in a uniform public healthcare system making it possible to report precise data on age at diagnosis. The rising incidence, prevalence and age at diagnosis during the study period could be due to information bias in the early time period of the study. Since Danish hospitals are only legally required to keep patient records 10 years after the latest entry, many hospitals have destroyed records. Nonetheless, most Danish hospital records are computerized and kept infinitely. Therefore, data collection from journals may not be as good in the beginning of the study period compared to the latest 10–15 years, resulting in some bias in interpretation of data over time. Many of the elderly persons registered in the first part of the study period are dead before computerization of records and for that reason their records were purely paper files and often not available for evaluation. Consequently, some persons had to be evaluated as "not MFS" due to lack of journal data, while they in reality might have suffered from MFS. This could obviously create a bias in the assessment of the median age at diagnosis and the prevalence early in the study period. However, this problem should not affect our data during the latter part of the study period.

Conclusion

We found a MFS prevalence of 6.5/100,000 in the Danish population but expect a growing prevalence during the next years, since we saw an increasing prevalence and incidence during the study period. We also found a striking time span of patients age at diagnosis of zero to seventy-four years and a median age at diagnosis of 19.0 years emphasizing that diagnosing MFS is a task for both pediatricians as well as other clinicians.

Ethics approval and consent to participate

The study was approved by the Scientific Ethical Committee of Region Midtjylland and the Danish Data Protection Agency.

Abbreviations

CI: Confidence interval; CPR: Danish Central Personal Register; DRCD: The Danish Register of Causes of Death; FBN1: Fibrillin-1 gene; Ghent-I: First revised Ghent nosology [4]; Ghent-II: Second revised Ghent Nosology [5]; ICD: International classification of disease; IRR: Incidence rate ratio; MFS: Marfan Syndrome; NPR: The National Patient Register; RR: Relative risk.

Competing interest

The authors declare that they have no competing interests.

Authors' contribution

All authors have participated in the writing of the manuscript. The following specific areas where each author have an provide extra contribution. KG: Main author of the manuscript, general data collection and evaluation. HH: Provided access to data for patients living in the eastern part of Denmark. Also provided expert knowledge on Marfan Syndrome and phenotyping the patients. KK: Provided access to data from department of cardiology on patients living in the eastern part of Denmark. Also collected data on patients from the eastern part of Denmark. LF: Provided access to patient in the southern part of Denmark as well expert knowledge on evaluation of rare diseases. MG: Provided expert knowledge on genetics and gentypical evaluation. NV: Provided access to data from department of cardiology on patients living in the eastern part of Denmark. Also collected data on patients from the eastern part of Denmark. KS: Provided expert knowledge on statistical analysis and evaluation of rare diseases. JØ: Provided expert knowledge on Marfan Syndrome. Also helped in accessing patient data from patients in the western part of Denmark. NHA: Provided cardiological data for patient from the western part of Denmark as well as expert knowledge on Marfan Syndrome. CHG: Provided expert knowledge on scientific handling of rare diseases. Also provided expert knowledge on Marfan syndrome. All authors read and approved the final manuscript.

Acknowledgements

This study is supported by Department of Clinical Medicine, Aarhus University Hospital and Aarhus University and the Novo Nordisk Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author details

[1]Department of Cardiology, Aarhus University Hospital, DK-8200 Aarhus N, Denmark. [2]Department of Molecular Medicine, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus N, Denmark. [3]Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, DK-2100 Copenhagen, Denmark. [4]The RAREDIS Database, Section of Rare Diseases, Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, DK-2100 Copenhagen, Denmark. [5]Department of Cardiology, Rigshospitalet, DK-2100 Copenhagen, Denmark. [6]Department of Endocrinology, Odense University Hospital, DK-5000 Odense C, Denmark. [7]Institute of Clinical Reasearch, University of Southern Denmark, DK-5000 Odense C, Denmark. [8]Centre for Rare Diseases, Department of Paediatrics, Aarhus University Hospital, DK-8200 Aarhus N, Denmark. [9]Department of Endocrinology and Internal Medicine, Aarhus University Hospital, DK-8000 Aarhus C, Denmark.

Received: 21 September 2015 Accepted: 22 November 2015
Published online: 02 December 2015

References

1. von Kodolitsch Y, De Backer J, Schüler H, Bannas P, Behzadi C, Bernhardt AM, et al. Perspectives on the revised Ghent criteria for the diagnosis of

Marfan syndrome. Appl Clin Genet. 2015;8:137–55. doi:10.2147/TACG.S60472. eCollection@2015.:137–55.
2. Beighton P, de Paepe A, Danks D, Finidori G, Gedde-Dahl T, Goodman R, et al. International nosology of heritable disorders of connective tissue, Berlin, 1986. Am J Med Genet. 1988;29(3):581–94.
3. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature. 1991;352(6333):337–9.
4. De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet. 1996;62(4):417–26.
5. Loeys BL, Dietz HC, Braverman AC, Callewaert BL, De Backer J, Devereux RB, et al. The revised Ghent nosology for the Marfan syndrome. J Med Genet. 2010;47(7):476–85.
6. Pepe G, Lapini I, Evangelisti L, Attanasio M, Giusti B, Lucarini L, et al. Is ectopia lentis in some cases a mild phenotypic expression of Marfan syndrome? Need for a long-term follow-up. Mol Vis. 2007;13:2242–7.
7. Gillis E, Kempers M, Salemink S, Timmermans J, Cheriex EC, Bekkers SC, et al. An FBN1 deep intronic mutation in a familial case of Marfan syndrome: an explanation for genetically unsolved cases? Hum Mutat. 2014;35(5):571–4.
8. Pyeritz RE, Pye. Marfan Syndrome and Related Disorders. Sixthth ed. 2013. p. 1–51.
9. Lynas MA. Marfan's syndrome in Northern Ireland; an account of thirteen families. Ann Hum Genet. 1958;22(4):289–309.
10. Sun QB, Zhang KZ, Cheng TO, Li SL, Lu BX, Zhang ZB, et al. Marfan syndrome in China: a collective review of 564 cases among 98 families. Am Heart J. 1990;120(4):934–48.
11. Gray JR, Bridges AB, Faed MJ, Pringle T, Baines P, Dean J, et al. Ascertainment and severity of Marfan syndrome in a Scottish population. J Med Genet. 1994;31(1):51–4.
12. Fuchs J. Marfan syndrome and other systemic disorders with congenital ectopia lentis. A Danish national survey. Acta Paediatr. 1997;86(9):947–52.
13. Chiu HH, Wu MH, Chen HC, Kao FY, Huang SK. Epidemiological profile of Marfan syndrome in a general population: a national database study. Mayo Clin Proc. 2014;89(1):34–42.
14. Faivre L, Collod-Beroud G, Adès L, Arbustini E, Child A, Callewaert BL, et al. The new Ghent criteria for Marfan syndrome: what do they change? Clin Genet. 2012;81(5):433–42.
15. Cañadas V, Vilacosta I, Bruna I, Fuster V. Marfan syndrome. Part 1: pathophysiology and diagnosis. Nat Rev Cardiol. 2012;7(5):256–65.
16. Andersen TF, Madsen M, Jørgensen J, Mellemkjoer L, Olsen JH. The Danish National Hospital Register. A valuable source of data for modern health sciences. Dan Med Bull. 1999;46(3):263–8.
17. Helweg-Larsen K. The Danish Register of Causes of Death. Scand J Public Health. 2011;39(7 Suppl):26–9.
18. Jondeau G, Detaint D, Tubach F, Arnoult F, Milleron O, Raoux F, et al. Aortic event rate in the Marfan population: a cohort study. Circulation. 2012;125(2):226–32.
19. Silverman DI, Burton KJ, Gray J, Bosner MS, Kouchoukos NT, Roman MJ, et al. Life expectancy in the Marfan syndrome. Am J Cardiol. 1995;75(2):157–60.
20. Stochholm K, Juul S, Juel K, Naeraa RW, Gravholt CH. Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. J Clin Endocrinol Metab. 2006;91(10):3897–902.
21. Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. J Clin Endocrinol Metab. 2013;88(2):622–6.
22. Milewicz DM, Dietz HC, Miller DC. Treatment of aortic disease in patients with Marfan syndrome. Circulation. 2005;111(11):e150–7.
23. Januzzi JL, Isselbacher EM, Fattori R, Cooper JV, Smith DE, Fang J, et al. Characterizing the young patient with aortic dissection: results from the International Registry of Aortic Dissection (IRAD). J Am Coll Cardiol. 2004; 43(4):665–9.
24. McKusick VA. The cardiovascular aspects of Marfan's syndrome: a heritable disorder of connective tissue. Circulation. 1955;11(3):321–42.
25. Cameron DE, Alejo DE, Patel ND, Nwakanma LU, Weiss ES, Vricella LA, et al. Aortic root replacement in 372 Marfan patients: evolution of operative repair over 30 years. Ann Thorac Surg. 2009;87(5):1344–9.
26. Stheneur C, Tubach F, Jouneaux M, Roy C, Benoist G, Chevallier B, et al. Study of phenotype evolution during childhood in Marfan syndrome to improve clinical recognition. Genet Med. 2014;16(3):246–50.
27. Grosse SD, Rogowski WH, Ross LF, Cornel MC, Dondorp WJ, Khoury MJ. Population screening for genetic disorders in the 21st century: evidence, economics, and ethics. Public Health Genomics. 2010;13(2):106–15.
28. Dietz HC, Pyeritz RE. Mutations in the human gene for fibrillin-1 (FBN1) in the Marfan syndrome and related disorders. Hum Mol Genet. 1995;4 Spec No:1799–809. 1799–809.
29. Groth KA, Gaustadnes M, Thorsen K, Østergaard JR, Jensen UB, Gravholt CH et al. Difficulties in diagnosing Marfan syndrome using current FBN1 databases. Genet Med. 2015;10.

Study 4

Clin Res Cardiol
DOI 10.1007/s00392-016-1028-3

ORIGINAL PAPER

Aortic events in a nationwide Marfan syndrome cohort

Kristian A. Groth[1,2] · Kirstine Stochholm[3,4] · Hanne Hove[5,6] · Kasper Kyhl[7] · Pernille A. Gregersen[3,8] · Niels Vejlstrup[7] · John R. Østergaard[3] · Claus H. Gravholt[2,4] · Niels H. Andersen[1]

Received: 25 April 2016 / Accepted: 16 August 2016

Abstract
Background Marfan syndrome is associated with morbidity and mortality due to aortic dilatation and dissection. Preventive aortic root replacement has been the standard treatment in Marfan syndrome patients with aortic dilatation. In this study, we present aortic event data from a nationwide Marfan syndrome cohort.
Method The nationwide cohort of Danish Marfan syndrome patients was established from the Danish National Patient Registry and the Cause of Death Register, where we retrieved information about aortic surgery and dissections. We associated aortic events with age, sex, and Marfan syndrome diagnosis prior or after the first aortic event.
Results From the total cohort of 412 patients, 150 (36.4 %) had an aortic event. Fifty percent were event free at age 49.6. Eighty patients (53.3 %) had prophylactic surgery and seventy patients (46.7 %) a dissection. The yearly event rate was 0.02 events/year/patient in the period 1994–2014. Male patients had a significant higher risk of an aortic event at a younger age with a hazard ratio of 1.75 (CI 1.26–2.42, $p = 0.001$) compared with women. Fifty-three patients (12.9 %) were diagnosed with MFS after their first aortic event which primarily was aortic dissection [$n = 44$ (83.0 %)].
Conclusion More than a third of MFS patients experienced an aortic event and male patients had significantly more aortic events than females. More than half of the total number of dissections was in patients undiagnosed with MFS at the time of their event. This emphasizes that diagnosing MFS is lifesaving and improves mortality risk by reducing the risk of aorta dissection.

Keywords Epidemiology · Rare diseases · Aortic aneurism · Aortic dissection · Genetic disease

Abbreviations

CI	Confidence interval
CPR	Danish Central Personal Register
CDR	The Cause of death Register
DNPR	The Danish National Patient Register
FBN1	Fibrillin-1 gene
Ghent-I	First revised Ghent nosology [31]
Ghent-II	Second revised Ghent nosology [14]
ICD	International classification of disease
MFS	Marfan syndrome
RR	Relative risk

Electronic supplementary material The online version of this article (doi:10.1007/s00392-016-1028-3) contains supplementary material, which is available to authorized users.

✉ Kristian A. Groth
kristian.groth@ki.au.dk

[1] Department of Cardiology, Aarhus University Hospital, 8200 Aarhus N, Denmark

[2] Department of Molecular Medicine, Aarhus University Hospital, 8200 Aarhus N, Denmark

[3] Centre for Rare Diseases, Department of Pediatrics, Aarhus University Hospital, 8200 Aarhus N, Denmark

[4] Department of Endocrinology and Internal Medicine, Aarhus University Hospital, 8000 Aarhus C, Denmark

[5] Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, 2100 Copenhagen, Denmark

[6] The RAREDIS Database, Section of Rare Diseases, Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, 2100 Copenhagen, Denmark

[7] Department of Cardiology, Rigshospitalet, 2100 Copenhagen, Denmark

[8] Department of Clinical Genetics, Aarhus University Hospital, 8200 Aarhus N, Denmark

Published online: 22 August 2016

Background

Marfan syndrome (MFS) is associated with morbidity and mortality due to the natural history of the disease with risk of progressive aortic dilatation, dissection, and sudden cardiac death [1, 2]. Preventive surgery with aortic root replacement has been the standard treatment in MFS patients with aortic dilatation [3–5]. Initially, patients received a composite aortic valve and root replacement, but over the years, aortic valve-sparing surgery has become the preferred surgical procedure to treat aortic root dilatation in patients with MFS [3, 6]. The long-term prognosis after prophylactic surgery of the ascending aorta seems excellent [7–9] with a mortality rate less than 10 % over 15 years [7]. However, recent data suggest that type B aortic dissections can occur even in patients who have undergone prophylactic aortic root surgery [6, 10]. MFS patients who experience an acute Stanford type A aortic dissection have a high risk of death or reoperation [11, 12], which emphasizes that MFS patients need to be diagnosed, followed-up in tertiary specialist units and operated according to guidelines to prevent detrimental acute aortic events [4]. As prophylactic aortic root surgery minimizes the risk of aortic dissection both aortic events represent life-threatening aortic disease in the MFS patient. In a significant number of patients, the acute aortic event may lead to the MFS diagnose in a patient. The risk of death or aortic dissection in patients with known MFS monitored in a specialized and centralized outpatients' clinic is reported to be 0.17 % events per year [13], however, there are no reports on aortic event rates outside specialized settings and no reports with data from an entire nation.

Recently, we identified all known MFS patients in Denmark according to the Ghent II criteria [14] in the time period between 1977 and 2014 [15]. We retrieved all registered aortic events during this period. We set out to investigate the burden of aortic events in the total Danish MFS cohort and to examine the disease burden of patients diagnosed with MFS after their first aortic event.

Materials and methods

All Danish citizens have a unique personal identification number (CPR-number) in the Danish Central Person Register (www.cpr.dk), thus providing a unique opportunity for record linkage, including The Danish National Patient Registry (DNPR) [16] and The Cause of Death Register (CDR) [17]. From 1977 onward, the DNPR registered all in-patient contacts with the Danish healthcare system, and from 1995, also registered all outpatient contacts. All contacts were given an International Classification of Diseases (ICD) code (ICD-8 until 1993 and ICD-10 from 1994 onward). For operations, the ICD-8 coding system was used until 1996 and NOMESCO Classification of Surgical Procedures (NCSP) was used onward. CDR records all death certificates since 1973 according to the ICD system, and used ICD-8 in 1973–1983, and ICD-10 from 1984 onward. CDR was updated through 2014. Follow-up ended on the 30th of December 2014.

Our main objective was to evaluate the number of aortic event in the Danish MFS population. We retrieved codes of diagnosis and operations associated with aortic events in our MFS cohort from DNPR and CDR (for details, see table S1 in supplemental material), as well as the relevant dates. We discriminated between two types of first aortic events: (1) prophylactic aortic surgery, i.e., aortic surgery before aortic dissection, or (2) acute aortic dissection without prior prophylactic surgery. We also registered aortic dissection subsequent to prophylactic surgery as a secondary aortic event. In addition, as a secondary event, we registered surgery after aortic dissection as either acute within 30 days of dissection, or late surgery as after 30 days after dissection. We defined the date of the aortic event as the first date of a registration of the surgery code of aortic intervention or first date of a registration of dissection. If the MFS diagnosis was registered up to 15 days before the aortic event, it was still considered an event prior to the MFS diagnosis, since a diagnosis that close to the aortic event would hardly affect the management of the patient.

The study was approved by the Scientific Ethical Committee (31422) and the Danish Data Protection Agency (2011-41-6986). The article is structured according to the STROBE guidelines for cohort studies.

Statistical analysis

Since MFS is a genetic disorder, we regarded the disease as a congenital condition and, therefore, risk time started at birth, and exposure time was calculated from date of birth to date of first registered aortic event, emigration, or death, whichever came first. We present data on annual aortic event rate, which is the cumulative incidence of events. Kaplan–Meier estimates were constructed for first aortic event. Hazard ratios (HR) and p values were calculated using Cox regression using aortic events as primary variable and sex and person years as co-variables. p values lower than 0.05 were considered statistically significant.

Stata 12.1 for Windows (StataCorp LP, College Station, TX, USA) was used for all calculations.

Results

Cohort data

Previously, we identified 412 MFS out of 1628 patients suspected of having MFS (Table 1; Fig. 1) (men $n = 215$) [15]. We updated registry data with aortic events and deaths until the end of 2014 and identified that 364/412 (88.3 %) were alive and 48/412 (11.7 %, men $n = 27$) dead (Table 1). The exposure time was 13,932 person years.

Aortic events

Of the total MFS cohort, 150/412 patients (36.4 %) had an aortic event during the study period (Table 1; Fig. 1). We found that at an age of 20, less than 5 % had experienced an aortic event, and at 49.6 years, 50 % were still free of an aortic event (Fig. 2). The annual aortic event rate for the period 1994–2014 was 0.02 events/year/patient. Among those who had an aortic event, 80/150 (53.3 %) had prophylactic aortic surgery as their first event, while 70/150 (46.7 %) patients (Fig. 1) experienced aortic dissection as their first aortic event.

Males had a significantly increased risk of an aortic events (89/215, 41.4 %) compared with (61/197, 31.0 %) women. Further, the aortic events happened earlier in men at median age of 36.5 years (range 4–74 years) compared with a median age of 39.2 years (range 4–69 years) in females. Thus, we identified a significantly increased risk of aortic event in men, corresponding to a hazard ratio (HR) of 1.75 (CI 1.26–2.42, $p = 0.001$) (Fig. 3). Of the 48 (men = 27) deceased patients, 10 patients (20.8 %, men = 4) did not experience an aortic event before they died.

Prophylactic aortic surgery

Eighty patients had prophylactic aortic surgery. The median age at surgery was 33.3 years (range 4–66 years), and at an age of 45 years, 75 % were not operated (Figs. 2, 4). The youngest individual who had prophylactic surgery was a 4-year-old boy with verified MFS, aortic dilatation, and a bicuspid aortic valve with severe aortic regurgitation. At follow-up, 12 prophylactically operated patients were deceased. Only one patient died within 30 days of surgery. The median survival time for deceased patients after the prophylactic surgery was 8.9 years (range 4 days–25 years). Numerically, more males ($n = 47/80$, 21.9 %) than females ($n = 33/80$, 16.8 %) underwent prophylactic aortic surgery. Men had prophylactic surgery at a younger age at a median age of 32.9 years (range 4–58 years) compared with women with a median age of 34.7 years (range 4–66 years). This resulted in a significant HR of 1.60 (CI 1.02–2.50, $p = 0.04$).

Prophylactic aortic surgery followed by aortic dissection

Among patients who had prophylactic aortic surgery, 14/80 patients (17.5 %, men = 7) (Fig. 1) experienced aortic dissection later on in life, of which three dissections happened within 30 days of the initial prophylactic aortic surgery. Dissection occurred on a median 11.4 years (range 7 days–28.4 years) after the prophylactic surgery.

Aortic dissection

Eighty-four experienced aortic dissection of which seventy patients had the aortic dissection as their first aortic event. Examining the group who experienced aortic dissection as the first aortic event, 50 % of the patients living to the age of 74 years (Fig. 2) were free of aortic dissection and that the aorta dissected at an age range from 17 to 74.5 years (Fig. 4). We found that more men ($n = 42/215$, 19.5 %) compared with women ($n = 28/197$, 14.2 %) experienced aortic dissection as their first aortic event. Men also

Table 1 Aortic event data

	All	Men	Women	p value
Number of patients (%)	412	215 (52.2)	197 (47.8)	0.4
Deceased (%)	48	27 (56.3)	21 (43.8)	0.5
Deceased with aortic events (%)	38	23 (60.5)	15 (39.5)	0.3
First aortic event (%)*	150	89 (59.3)	61 (40.7)	0.03
Prophylactic aortic surgery (%)	80	47 (58.8)	33 (41.3)	0.1
All aortic dissection (%)	84	49 (58.3)	35 (41.7)	0.2
Aortic dissection without prophylactic aortic surgery (%)	70	42 (60.0)	28 (40.0)	0.1
With dissection and subsequent surgery (%)	56	32 (57.1)	24 (42.9)	0.4
With dissection and no subsequent surgery (%)	28	17 (60.7)	11 (34.3)	0.3
With dissection after prophylactic surgery (%)	14	7 (50.0)	7 (50.0)	1.00

* First aortic event defined as either aortic surgery or aortic dissection

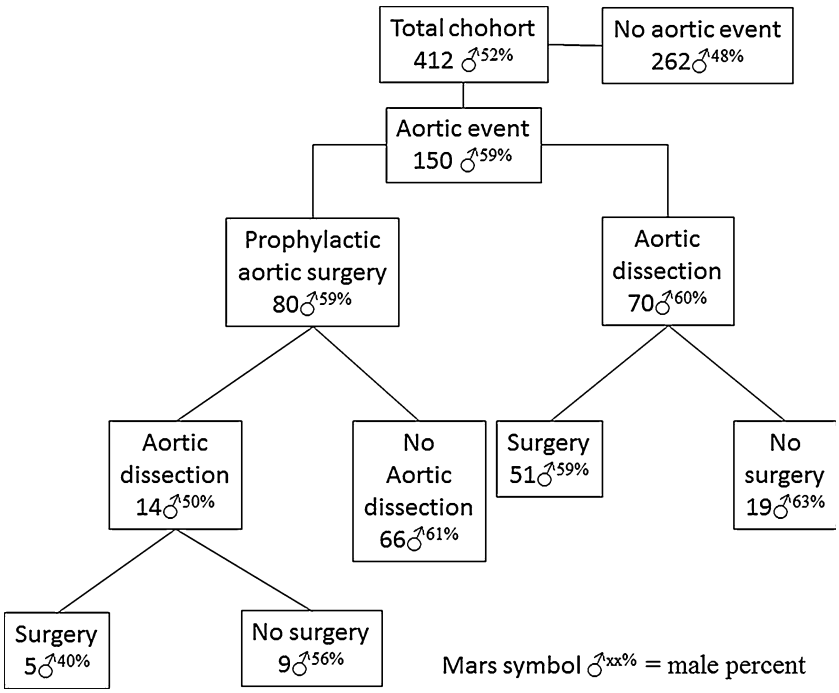

Fig. 1 Flowchart of the cohort showing the numbers in subgroups

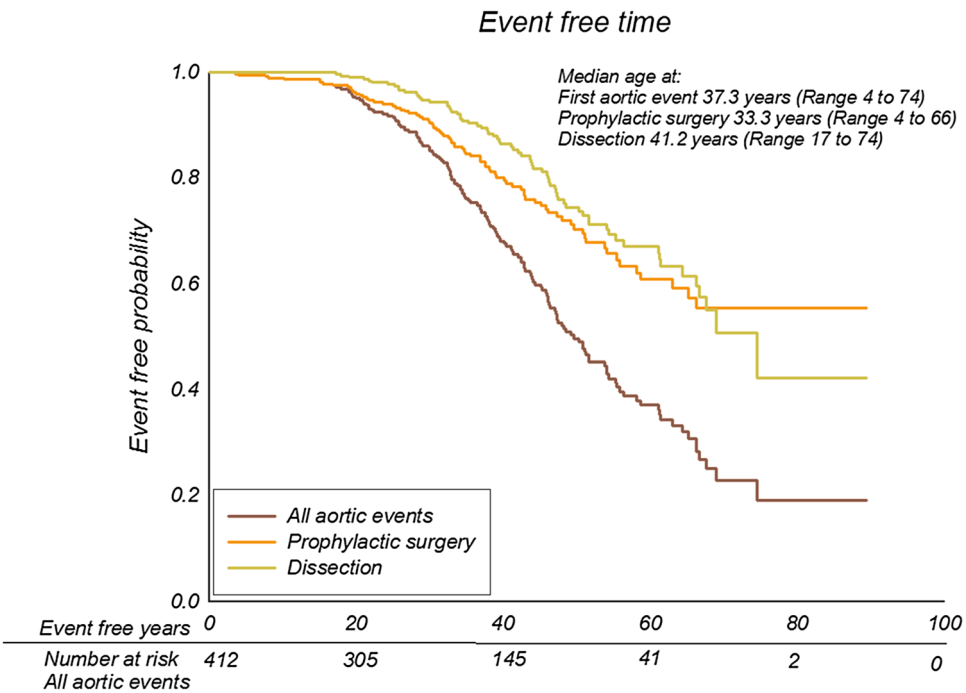

Fig. 2 *Curve* of event free time to first aortic event divided on all aortic event, prophylactic aortic surgery, and aortic dissection. The *curves* show the percentage of MFS patients free of aortic events at a given age. Patient years are calculated from birth date to date of first registered aortic event. Patients are censored at death or at follow-up on 31 December 2014. Numbers at risk only stated for all aortic events

experienced aortic dissection at a younger age with a median age of 40.3 years (range 18–74 years) compared with female with a median age of 43.2 (range 17–69 years) resulting in a significant HR of 1.66 (CI 1.03–2.67, $p = 0.04$).

Fifty-six MFS patients with aortic dissection underwent subsequent aortic surgery either acutely ($n = 46$), defined as surgery within 30 days of dissection, or at a later stage ($n = 10$). Of the patients who had acute surgery, 14 patients died, of who 4 died within 30 days of surgery. The median survival time after surgery was 3.3 years (range 1 day–15.2 years). In the group with dissection and late surgery (surgery after 30 days of dissection; $n = 10$), six patients died with a median survival time after surgery of 4.7 years (range 3 days–17.7 years). Two of these patients died within 30 days of surgery and one died 47 days after

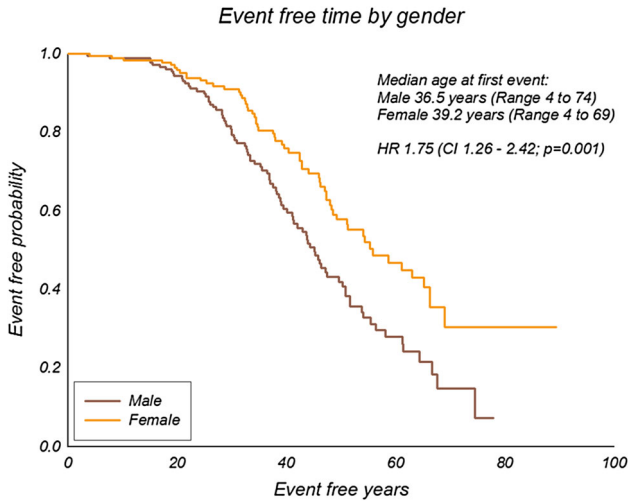

Fig. 3 *Curve* of event free time to first aortic event divided on sex. The *curves* show the percentage of MFS patients free of aortic events at a given age. Patient years are calculated from birth date to date of first registered aortic event. Patients are censored at death or at follow-up on 31 December 2014

surgery. Eleven of the patients who had acute aortic dissection without subsequent surgery died during the observation period. Their median survival time after dissection was 4 days (range 0 days–13.6 years). Six died within 5 days of the aortic dissection, while the remaining five patients survived at least 408 days.

Diagnosed with MFS after the first aortic event

Fifty-three patients were diagnosed with MFS after their first aortic event which primarily was an aortic dissection [$n = 44/53$ patients (83.0 %)]. This subgroup represented 52.4 % (44/84) of all registered aortic dissections in the total MFS cohort. There was no sex differences (males $n = 33$, females $n = 20$, $p = 0.12$). The median age at the first aortic event in this subgroup was 39.2 years (range 18–74 years).

Discussion

This paper presents outcome data from the first nationwide study of MFS. Every patient in Denmark diagnosed with MFS was identified, and a subsequent reevaluation of the diagnosis was performed. Since this cohort represents the entire national MFS population, our data represent a credible status of aortic events in a general MFS population in a uniform healthcare system, and thus, the results in this paper can generally be extrapolated to other developed countries.

More than one-third of the MFS population experienced an aortic event. The probability of staying event free through life decreased from almost 100 % at age 20 to around 20 % at age 70. Around 80 % of all deceased patients had experienced an aortic event in their lifetime.

We found a yearly event rate of 0.02 per patient which is around the same level reported by Jondeau et al. in a one-center specialist setup [13]. We chose to only evaluate the event rate during the latest 20 years, since our data are based on MFS patients' records evaluated according to the revised Ghent II diagnostic criteria [14], because older patient records are less accessible and this could easily underestimate the prevalence of MFS, thus affecting the event rate estimate [15].

We found a significantly higher proportion of MFS males with aortic events compared with females with a significantly increased HR of 1.75, indicating that male patients have a 75 % higher risk of an aortic event than female patient at any given age. The increased hazard for men was seen for both prophylactic aortic surgery and aortic dissection. There are no data to support that aortic disease is more prevalent in male children or adolescents compared with females [18], and men and women are diagnosed having MFS at the same age in Denmark [15]. In a study from 2005, the authors suggested aortic intervention a lower aortic diameter in female MFS patients due to more dissections at an lower diameter in female patients [19]. Our study does not support this finding. Intuitively, more aortic events should be expected among women, since the current international guidelines recommend early prophylactic surgery before intended pregnant [4]. It is also well known that pregnancy increases the risk of aortic dissection or rupture [20], nevertheless, the risk for developing an aortic event was much higher in men. In theory, the general health status for the female MFS patient is probably better, since Danish women generally use the healthcare system more than men [21], but this assumption is purely speculative. In addition, a protective effect by estrogen could be hypnotized, as it is well known that men have an increased risk of abdominal aortic dilatation and dissection [22], and studies have shown a protective effect of estrogen on this part of aorta [23].

Prophylactic medical therapy to prevent aortic events has been considered over the last 20 years. Initially, the recommendation was to treat MFS patients with beta-blockers based on a randomized study from the nineties [24]. However, in the recent years, treatment with angiotensin II receptor blockers has gained interest due to the inhibition of transforming growth factor (TGF)-β receptors [25]. Even so, a recent placebo controlled study did not prove any significant benefit comparing losartan to placebo [26]. Instead, preventive aortic root replacement has been the best treatment option in MFS patients with an aortic size above 4.9 cm or with progressive aortic dilatation [4, 27]. In the present cohort, 80 patients had prophylactic aortic surgery and only one patient died and three experienced aortic dissection

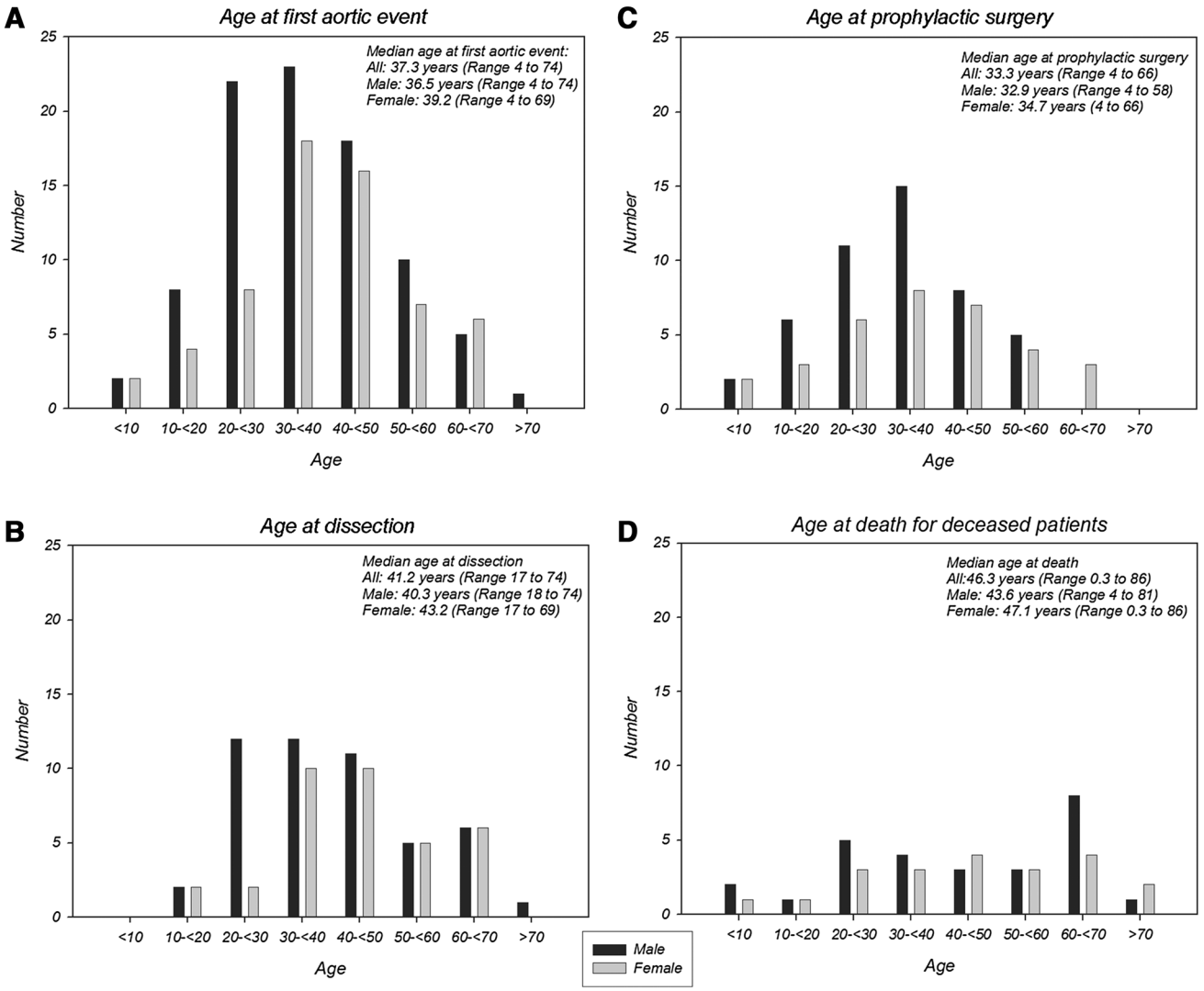

Fig. 4 **a** Age at first aortic event (aortic dissection or aortic surgery) divided in 10-year age intervals. **b** Age at dissection divided in 10-year age intervals. **c** Age at operation divided in 10-year age intervals. **d** Age at death divided in 10-year intervals

within 30 days after surgery. This indicates that prophylactic surgery is a good treatment with low mortality and the current guidelines for monitoring and providing surgery in MFS, improves life expectancy significant [4].

This is also emphasized by the mortality data from the patients who suffered from aortic dissection. For patients with subsequent acute surgery, the 30 days mortality was 4/46 (8.7 %), and patients who for some reason were not offered surgery, the 30 days mortality was 6/28 (21.4 %).

Late dissections after prophylactic surgery have previously been reported [10] and were also seen in this cohort. On average, it happened 10 years after the first aortic surgery which emphasizes the need for lifelong follow-up for MFS patients and necessitating preventive measures with good blood pressure control and the use of advanced imaging.

Interestingly, we found a high proportion of aortic dissections in our cohort among MFS patients, who were not diagnosed as having MFS at the time of their event. Of the deceased patients, more than a third were undiagnosed at the time of their first aortic event, and of these, more than 90 % had aortic dissection as their first event, while the remaining patients received prophylactic aortic surgery before they were diagnosed MFS. These findings highlight that diagnosing MFS and caretaking of MFS patients are lifesaving, but it also shows that diagnosis of MFS today is still difficult and shows that continued efforts to increase the diagnostic vigilance among clinician is necessary [15, 28, 29].

Strength and limitations

Our study is a cohort study and includes only diagnosed MFS patients. Due to the above-mentioned discussion of diagnosis, it is likely that we primarily include the patients

with the worst phenotype and, therefore, overestimate the event rate. Due to the epidemiological nature of the study, we cannot ascertain how many MFS with a more discrete phenotype were missing either due to non-diagnosis or late diagnosis; however, we describe a cohort of MFS patients that is seen in the current clinical setting. Diagnostic criteria for MFS have changed several times over the years and this could have altered the prevalence of MFS in the cohort. However, we recently showed that the implementation of the Berlin, the Ghent I, and the Ghent II criteria [14, 30, 31] did not have a large impact on incidence and prevalence of MFS [15].

A key risk factor for aortic events is aortic size, but this kind of information was not accessible in this study. However, all patients were treated according to the current guidelines at any time, so we do not believe that the size of the aorta could have influenced the incidence of events, except from the cases of acute aortic dissection in undiagnosed MFS.

Conclusion

A high proportion of MFS patients will experience an aortic event of either aortic dissection or prophylactic aortic surgery. Furthermore, male patients have significantly more aortic events than females, and they occur at an earlier age. Over half of the total number of dissections was in patients undiagnosed with MFS at the time of the event. This emphasizes that diagnosing MFS is lifesaving and improves mortality risk by reducing the risk of aorta dissection, and that the diagnostic vigilance should be increased, possibly leading to fewer deaths among MFS.

Acknowledgments This study is supported by the Department of Clinical Medicine, Aarhus University Hospital and Aarhus University and the Novo Nordisk Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Compliance with ethical standards

Ethics approval and consent to participate The study was approved by the Scientific Ethical Committee of Region Midtjylland and the Danish Data Protection Agency.

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Murdoch JL, Walker BA, Halpern BL et al (1972) Life expectancy and causes of death in the Marfan syndrome. N Engl J Med 286(15):804–808
2. Schaeffer BN, Rybczynski M, Sheikhzadeh S et al (2015) Heart rate turbulence and deceleration capacity for risk prediction of serious arrhythmic events in Marfan syndrome. Clin Res Cardiol 104(12):1054–1063
3. Benedetto U, Melina G, Takkenberg JJ et al (2011) Surgical management of aortic root disease in Marfan syndrome: a systematic review and meta-analysis. Heart 97(12):955–958
4. Erbel R, Aboyans V, Boileau C et al (2014) 2014 ESC Guidelines on the diagnosis and treatment of aortic diseases: document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. The Task Force for the Diagnosis and Treatment of Aortic Diseases of the European Society of Cardiology (ESC). Eur Heart J 35(41):2873–2926
5. Treasure T, Takkenberg JJ, Pepper J (2014) Surgical management of aortic root disease in Marfan syndrome and other congenital disorders associated with aortic root aneurysms. Heart 100(20):1571–1576
6. David TE, David CM, Manlhiot C et al (2015) Outcomes of aortic valve-sparing operations in Marfan syndrome. J Am Coll Cardiol 66(13):1445–1453
7. Price J, Magruder JT, Young A et al (2015) Long-term outcomes of aortic root operations for Marfan syndrome: a comparison of Bentall versus aortic valve-sparing procedures. J Thorac Cardiovasc Surg 15:10
8. Kunihara T, Aicher D, Asano M et al (2014) Risk factors for prophylactic proximal aortic replacement in the current era. Clin Res Cardiol 103(6):431–440
9. Koene BM, Soliman Hamad MA, Bouma W et al (2014) Can postoperative mean transprosthetic pressure gradient predict survival after aortic valve replacement? Clin Res Cardiol 103(2):133–140
10. den Hartog AW, Franken R, Zwinderman AH et al (2015) The risk for type B aortic dissection in Marfan syndrome. J Am Coll Cardiol 65(3):246–254
11. Rylski B, Bavaria JE, Beyersdorf F et al (2014) Type A aortic dissection in Marfan syndrome: extent of initial surgery determines long-term outcome. Circulation 129(13):1381–1386
12. Bachet J, Goudot B, Dreyfus G et al (2000) Surgery of acute type A dissection: what have we learned during the past 25 years? Z Kardiol 89(Suppl 7):47–54
13. Jondeau G, Detaint D, Tubach F et al (2012) Aortic event rate in the Marfan population: a cohort study. Circulation 125(2):226–232
14. Loeys BL, Dietz HC, Braverman AC et al (2010) The revised Ghent nosology for the Marfan syndrome. J Med Genet 47(7):476–485
15. Groth KA, Hove H, Kyhl K et al (2015) Prevalence, incidence, and age at diagnosis in Marfan Syndrome. Orphanet J Rare Dis 2(10):153. doi:10.1186/s13023-015-0369-8.:153-0369
16. Schmidt M, Schmidt SA, Sandegaard JL et al (2015) The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol 17(7):449–490. doi:10.2147/CLEP.S91125.eCollection@2015.:449-90
17. Helweg-Larsen K (2011) The Danish Register of causes of death. Scand J Public Health 39(7 Suppl):26–29
18. Mueller GC, Stark V, Steiner K et al (2013) Impact of age and gender on cardiac pathology in children and adolescents with Marfan syndrome. Pediatr Cardiol 34(4):991–998
19. Meijboom LJ, Timmermans J, Zwinderman AH et al (2005) Aortic root growth in men and women with the Marfan's syndrome. Am J Cardiol 96(10):1441–1444
20. Wanga S, Silversides C, Dore A et al (2016) Pregnancy and thoracic aortic disease: managing the risks. Can J Cardiol 32(1):78–85

21. Vedsted P (2007) Gender differences in the use of health care system. Ugeskr Laeger 169(25):2403–2408
22. Lo RC, Schermerhorn ML (2016) Abdominal aortic aneurysms in women. J Vasc Surg 63(3):839–844
23. Wu XF, Zhang J, Paskauskas S et al (2009) The role of estrogen in the formation of experimental abdominal aortic aneurysm. Am J Surg 197(1):49–54
24. Shores J, Berger KR, Murphy EA et al (1994) Progression of aortic dilatation and the benefit of long-term beta-adrenergic blockade in Marfan's syndrome. N Engl J Med 330(19):1335–1341
25. Habashi JP, Judge DP, Holm TM et al (2006) Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science 312(5770):117–121
26. Milleron O, Arnoult F, Ropers J et al (2015) Marfan Sartan: a randomized, double-blind, placebo-controlled trial. Eur Heart J 36(32):2160–2166
27. Doenst T, Struning C, Moschovas A et al (2015) Cardiac surgery 2014 reviewed. Clin Res Cardiol 104(12):1006–1020
28. Groth KA, Gaustadnes M, Thorsen K et al (2016) Difficulties in diagnosing Marfan syndrome using current FBN1 databases. Genet Med 18(1):98–102
29. von Kodolitsch Y, De BJ, Schuler H et al (2015) Perspectives on the revised Ghent criteria for the diagnosis of Marfan syndrome. Appl Clin Genet 16(8):137–155. doi:10.2147/TACG.S60472.eCollection@2015.:137-55
30. Beighton P, de Paepe A, Danks D et al (1988) International nosology of heritable disorders of connective tissue, Berlin, 1986. Am J Med Genet 29(3):581–594
31. De Paepe A, Devereux RB, Dietz HC et al (1996) Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet 62(4):417–426