A population-based survey of FBN1 variants in Iceland reveals underdiagnosis Marfan syndrome

Patrick Sulem (Patrick.Sulem@decode.is)
decODE Genetics (Iceland)  https://orcid.org/0000-0001-7123-6123

Gudny Amadottir
decODE genetics/Amgen Inc.  https://orcid.org/0000-0001-6571-423X

Brynjar Jensson
decODE genetics/Amgen, Inc., Reykjavik, Iceland  https://orcid.org/0000-0002-2252-4134

Adalbjorg Jonasdottir
decODE genetics / Amgen Inc.

Hildigunnur Katrinardottir
decODE genetics

Run Fridriksdottir
decODE genetics

Aslaug Jonasdottir
decODE genetics / Amgen Inc.

Asgeir Sigurdsson
decODE genetics / Amgen Inc.

Sigurjon Gudjonsson
decODE genetics / Amgen Inc.

Jon Jonsson
National Hospital of Iceland

Vigdis Stefansdottir
Landspitali - The National University Hospital  https://orcid.org/0000-0003-4451-3126

Ragnar Danielsen
National Hospital of Iceland

Astridur Palsdottir
Institution for Experimental Pathology at Keldur, University of Iceland

Hakon Jonsson
decODE genetics/Amgen Inc.

Agnar Helgason
decODE genetics / Amgen Inc.

Olafur Magnusson
Decode Genetics

Unnur Thorsteinsdottir
Hans Björnsson
Landspitali

Kari Stefansson
decODE genetics  https://orcid.org/0000-0003-1676-864X

Elin Klemenzdottir
The National hospital of Iceland
Abstract

Marfan syndrome is an autosomal dominant condition characterized by aortic aneurysm, skeletal abnormalities, and lens dislocation, and is caused by mutations in the FBN1 gene. To explore causes of Marfan syndrome and the prevalence in Iceland we collected samples and information from all living individuals with a clinical diagnosis of Marfan syndrome in Iceland (n = 35) and performed whole-genome sequencing of those who did not have a confirmed genetic diagnosis. Moreover, to assess a potential underdiagnosis of Marfan syndrome in Iceland we attempted a genotype-based approach for identifying individuals with Marfan syndrome. We interrogated deCODE genetics’ database of 35,712 whole-genome sequenced individuals to search for rare sequence variants in FBN1. Overall, we identified 15 pathogenic or likely pathogenic variants in FBN1 in 41 living individuals, only 22 of whom were previously diagnosed with Marfan syndrome.

The most common of these variants, NM_000138.4:c.8038C>T (p.Arg2680Cys), is present in a multi-generational pedigree, and was found to stem from a single forefather born around 1840. The p.Arg2680Cys associates with a form of Marfan syndrome that seems to have an enrichment of abdominal aortic aneurysm, suggesting that this may be a particularly common feature of p.Arg2680Cys-associated Marfan syndrome. Based on these combined genetic and clinical data, we estimate a Marfan syndrome prevalence of at least 1/6,000 in Iceland, compared to 1/10,000 based on clinical diagnosis alone, which indicates underdiagnosis of this actionable genetic disorder.

Introduction

Marfan syndrome (MFS) is an autosomal dominant disorder that typically affects the cardiovascular, ocular and skeletal systems. Other systems commonly involved are the skin, lungs and the dura mater (1). Variation in expressivity is observed both between and within affected families (2). The prevalence has been estimated at approximately 1/5,000–1/10,000, showing no gender, geographic or ethnic bias (3–6). A prior (1996) Icelandic study using clinical criteria (albeit prior to Ghent nosology) estimated prevalence to be 1/15,000 (7).

MFS is caused by heterozygous loss-of-function and missense variants in the Fibrinin 1 (FBN1) (8) gene located on chromosome 15 (9). FBN1 has 66 exons and encodes a large protein of 2,871 amino acids. The proprotein is cleaved near its C-terminus and gives rise to fibrillin-1, a fibrous structural protein which is disrupted in MFS, and asprosin, a glucogenic protein hormone associated with Neonatal Progeroid Syndrome (10). Fibrillin-1 is a glycoprotein and a major component of microfibrils that are widely distributed in connective tissue throughout the body. Microfibrils are important in the homeostasis of the extracellular matrix (11, 12). The pathogenesis in MFS is complicated (13) however, one consequence of heterozygous loss of fibrillin-1 is secondary dysregulation of the TGF-beta pathway (14); this insight has led to novel therapeutic strategies (15). Current standard of care involves treatment with beta-blocker and/or ARB inhibitors (Irbesartan/Losartan) as well as specific direction of timing of surgery dependent on size of aortic aneurysm (16). Aorta dilatation, predisposition to aortic dissection and valve deformities are the major cause of morbidity and early mortality in MFS. However, current treatment options have led to life expectancy for individuals with MFS in active care that is similar to that of the general population (17). A clinical diagnosis is currently established according to revised Ghent nosology (18), integrating clinical and genetic information. Identifying a pathogenic variant allows for identification of at-risk family members and early diagnosis for individuals with limited symptoms during childhood and early adult years. Early diagnosis makes it possible to start preventive management and follow-up earlier which may improve outcomes (19, 20). FBN1 is now one of 73 genes that the American College of Medical Genetics (ACMG) recommends for reporting of incidental findings in clinical exome and genome sequencing, since mutations in FBN1 causing MFS are considered to be an actionable genetic diagnosis (21).

Over 2,000 variants have been reported as pathogenic or likely pathogenic in FBN1, 57% of which are predicted loss-of-function variants (i.e. frameshift, nonsense or at essential splice acceptor and donor sites) and 43% are missense (22). Whereas the vast majority of all predicted loss-of-function variants in FBN1 (99%) classify as pathogenic or likely pathogenic, according to ClinVar, less than half (45%) of missense are classified as such, underscoring an interpretation challenge. Consistently, in the Genome Aggregation Database (gnomAD, v2.1.1; n = 141,456, based on multiple ethnicities) one observes less coding sequence diversity in FBN1 than expected (constraint metric scores z = 5.06 for missense; 1609.8 expected versus 1038 observed), pLI (probability of loss of function intolerance) = 1.00 for LoF (158.8 expected versus 3 observed) (23). That is consistent with the fact that sequence variants in the exons and splice sites of FBN1 are under negative selection. In addition to MFS, certain variants in FBN1 can cause other disorders. Some of these disorders have overlapping phenotypes with MFS, for example familial ectopia lentis and MASS syndrome (Mitral
valve prolapse, Aortic root diameter at upper limits of normal for body size, Stretch marks of the skin, and Skeletal conditions similar to Marfan syndrome). Other disorders, for example acromicric dysplasia, gelophysic dysplasia, stiff skin syndrome and Weill-Marchesani, have distinct phenotypes not typically observed in MFS, including short stature. For most of these syndromes, it seems to be the location of the pathogenic variant within \textit{FBN1} that is critical for the phenotypic presentation (24). Mutations in \textit{TGFBRI} and \textit{TGFBRII} have been known to cause Marfan-like phenotypes such as Loeyts-Dietz syndrome (25). Although mutations in \textit{FBN1}, \textit{TGFBRI} and \textit{TGFBRII} have overlapping phenotypes, \textit{TGFBRI} and \textit{TGFBRII} are not currently thought to cause Marfan syndrome and thus are not the focus of this work.

We postulate that there may be a group of MFS cases who have eluded diagnosis in Iceland so we took a two-pronged approach (study design is highlighted in Fig. 1). First, we performed a case-series collecting information on all clinical diagnoses of MFS in the Icelandic healthcare system. Secondly, we took a genotype-based approach, assessing all sequence variants in \textit{FBN1} detected among 35,712 Icelanders whole-genome sequenced (WGS) at deCODE (over 10% of the entire Icelandic population of 330K) to search for MFS patients who have eluded diagnosis. Thirdly, variants detected through WGS were also imputed into a total of 160,112 chip-genotyped Icelanders, increasing the power of detection of genotype-phenotype associations. The second and third approaches, allowed us to identify potentially undiagnosed MFS individuals in a non-biased way through genetic information. Here we present a nationwide study, reporting all \textit{FBN1} variants detected in the Icelandic population, both individuals with a clinical MFS diagnosis and those detected through a genotype-based approach. We provide a combined prevalence of clinically diagnosed and/or genotyped MFS in the Icelandic population, as well as describing a novel MFS-associated phenotype (abdominal aortic aneurysm (AAA)) based on cosegregation of a missense variant NM_000138.4:c.8038C>T (p.Arg2680Cys) in a six generations pedigree.

Materials And Methods

Case series

Discharge diagnoses from Landspitali University Hospital and the only other major hospital system in Iceland (Akureyri hospital) were collected to find all Icelandic patients with a clinical diagnosis of MFS (date of evaluation 04/07/2015). These two hospitals serve the vast majority of patients requiring complicated care. These diagnoses were achieved in many different ways and some predate the Ghent criteria. Further phenotypic data for the patients identified were obtained from family members or medical records. Data were analyzed for all of those with a diagnosis as well as individuals suspected to have the disorder. In addition, data from Landspitali University Hospital, death certificates and autopsy records were collected for ancestors and other family members to look for clues of the disorder. The oldest medical records viewed were from the late 19th century. Diagnosed patients were contacted, one from each family, and called in for an interview and a blood sample although a detailed physical examination was not performed. Blood was also drawn from a few other undiagnosed family members. Data were collected on deceased patients known to have had MFS when available.

deCODE's genealogical database

The book of Icelanders is a database that contains information about over 900,000 Icelanders. It's estimated to contain data on majority of the population since the settlement of the island and 95% of Icelanders born after the year 1700. It has information on parents, date of birth and death. Anonymous data from the database was used in this study, to establish pedigrees (26).

Identification of pathogenic and likely pathogenic \textit{FBN1} variants

deCODE genetics has collected DNA samples for 160,112 Icelanders and 35,712 of those have undergone WGS (August 2017). The sequence diversity over exons and splice sites of the \textit{FBN1} gene was derived for all WGS individuals in the deCODE database. Variants with a minor allele frequency greater than 0.01% were excluded. On the individual basis, on WGS calls, limits were set on depth > 12 and allelic ratio > 0.25. Sanger sequencing was performed on DNA for close relatives of a single large family with a known pathogenic variant (p.Arg2680Cys).

Pathogenicity of variants was predicted based on the ACMG's guidelines for interpretation of sequence variants (27). The guidelines recommend using the following terminology for classification of sequence variants; „pathogenic“, „likely pathogenic“, „variant of uncertain significance (VUS)“, „likely benign“ and „benign“. As previously described, variants in \textit{FBN1} can cause other syndromes. If
variants were reported to cause other syndromes in the ClinVar database they were excluded from the study. An association between the FBN1 variants and common MFS clinical features was tested using logistic regression. The clinical features included height, severe thoracic aneurysm, mitral valve prolapse and congenital lens malformation. An association to these clinical features at a p-value < 0.05 was considered to be supporting pathogenic evidence and was taken into account when following the ACMG guidelines for classification of sequence variants. All variants reaching "likely pathogenic" or "pathogenic" based on ACMG guidelines for MFS were included in the study. Association data were assessed in April 2021.

Whole genome sequencing

The methods used for WGS in deCODE have previously been described in detail (28). Genotypes of close relatives of known MFS patients were predicted by imputation and then validated by targeted Sanger sequencing. Imputation has been described thoroughly in prior publications (29, 30).

Calculating prevalence

When calculating the new prevalence, we used the total population of Iceland on January 1st 2015 (329,100) and variants predicted to be likely pathogenic or pathogenic for MFS in living individuals.

Results

Our examination of medical records in the two major hospitals that treat all complex diseases in Iceland yielded 35 living individuals with a clinical diagnosis of MFS, making the prevalence at least 1/10,000 in Iceland based on clinical diagnosis alone. We acquired biological samples from 30 of these 35 affected individuals and performed WGS. WGS yielded nine rare coding sequence variants in FBN1 in 22/30 (73%) of these individuals (Table 1). In the remaining eight individuals no pathogenic or likely pathogenic variant was identified, including in FBN1, TGFBR1 and TGFBR2. Out of the nine FBN1 variants identified in our case-series, three are previously reported pathogenic or likely pathogenic variants and six are novel in the Icelandic population and classify as pathogenic based on the ACMG criteria (Methods). Five of the variants were private (i.e., carried by a single individual), three were carried by two closely related individuals, and one variant, p.Arg2680Cys, was carried by 10 individuals, all of whom are part of the same extended family. There is one additional individual in the p.Arg2680Cys family with a MFS diagnosis for whom a DNA sample was not available to confirm the presence of p.Arg2680Cys (Fig. 2). The p.Arg2680Cys variant is a known pathogenic variant, previously described as a recurrent de novo Marfan syndrome variant (31–33).
Table 1

A summary of molecular and clinical data from patients with prior clinical diagnosis out of 30 cases with genetic material or existing diagnosis. In addition the case of neonatal MFS is included. All variants are in FBN1 ref. seq. NM_000138.4. FH = family history; CVS = cardiovascular system; dup = duplication; fs = frameshift; sub = substitution; miss = missense; splice = splice site; del = deletion; if = inframe; AD = aorta dissection; Ao = aorta dilation; AoR = aorta valve regurgitation; MVP = mitral valve prolapse; MR = mitral valve regurgitation; Hf = heart failure; NA = not available; ASA = abdominal aortic aneurysm; EL = ectopia lentis; ff = facial features; jh = joint hypermobility; pe = pectus excavatum; sc = scoliosis; pp = pes planus. Family history: "+"=positive, "-"=negative, de novo = parents were tested and are not carriers.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Consequence</th>
<th>Exon</th>
<th>N cases</th>
<th>Type</th>
<th>FH</th>
<th>CVS</th>
<th>Ocular</th>
<th>Skeletal</th>
<th>Published</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.1464dupT</td>
<td>p.Ile489TyrfsX2</td>
<td>12</td>
<td>1</td>
<td>dup/fs</td>
<td>de novo</td>
<td>AD, Ao, AoR, MVP, MR</td>
<td>-</td>
<td>tall, ff, jh, pe</td>
<td>no</td>
</tr>
<tr>
<td>c.1850G &gt; A</td>
<td>p.Cys617Tyr</td>
<td>16</td>
<td>1</td>
<td>sub/miss</td>
<td>+/de novo</td>
<td>AD, Ao, MR, AR</td>
<td>EL</td>
<td>tall, ff</td>
<td>(22)</td>
</tr>
<tr>
<td>c.2855-2A &gt; G</td>
<td>p.IVS24-2</td>
<td>25</td>
<td>1</td>
<td>sub/splice</td>
<td>de novo</td>
<td>MVP</td>
<td>-</td>
<td>sc, tall, pe</td>
<td>no</td>
</tr>
<tr>
<td>c.4211A &gt; G</td>
<td>p.Asp1404Gly</td>
<td>35</td>
<td>1</td>
<td>sub/miss</td>
<td>+</td>
<td>Ao, MVP, MR</td>
<td>-</td>
<td>tall, ff</td>
<td>no</td>
</tr>
<tr>
<td>c.6446A &gt; G</td>
<td>p.Tyr2149Cys</td>
<td>52</td>
<td>1</td>
<td>sub/miss</td>
<td>de novo</td>
<td>Ao, MVP</td>
<td>-</td>
<td>na</td>
<td>(46, 47)</td>
</tr>
<tr>
<td>c.5788 + 5G &gt; A</td>
<td>p.IVS47 + 5</td>
<td>47</td>
<td>2</td>
<td>sub/splice</td>
<td>+/de novo</td>
<td>NA</td>
<td>NA</td>
<td>sc, pp, tall, ff</td>
<td>(8, 36–38)</td>
</tr>
<tr>
<td>c.6744_6746delGGA</td>
<td>p.Glu2248del</td>
<td>56</td>
<td>2</td>
<td>del/if</td>
<td>+</td>
<td>Ao, AoR, MVP, MR</td>
<td>EL</td>
<td>sc, tall, ff</td>
<td>no</td>
</tr>
<tr>
<td>c.7699 + 2T &gt; C</td>
<td>p.IVS62 + 2</td>
<td>62</td>
<td>2</td>
<td>sub/splice</td>
<td>+</td>
<td>AD, Ao, MVP, MR, AoR</td>
<td>m</td>
<td>ff, tall</td>
<td>no</td>
</tr>
<tr>
<td>c.8038C &gt; T</td>
<td>p.Arg2680Cys</td>
<td>64</td>
<td>10</td>
<td>sub/miss</td>
<td>+</td>
<td>AD, Ao, AoR, MVP, ASA, AAA</td>
<td>EL</td>
<td>tall, ff, pe, pp, jh</td>
<td>(31–33)</td>
</tr>
<tr>
<td>c.3290G &gt; A</td>
<td>p.Cys1097Tyr</td>
<td>27</td>
<td>1</td>
<td>sub/miss</td>
<td>de novo</td>
<td>Ao, MVP, MR, Hf, enlarged heart</td>
<td>EL</td>
<td>tall, ff</td>
<td>no</td>
</tr>
</tbody>
</table>

In addition to the 35 patients with a clinical diagnosis, there were two individuals with a clinical diagnosis that were deceased at the time of the study. One of them, a male infant, had a neonatal MFS presentation (nMFS) with severe symptoms, including a severe mitral valve defect which was detected prenatally and a Marfan diagnosis that was made soon after birth. He was noted to have long extremities, thin stature, arachnodactyly among other typical skeletal features. He had ectopia lentis and early myopia. He also had pulmonary bullae and had spontaneous pneumothorax. His most severe signs included dilation of the aorta, enlarged heart and mitral valve prolapse. He passed away at 18 months of age, due to heart failure because of severe mitral valve regurgitation. A pathogenic variant was detected in this patient via genetic testing of FBN1 prior to this study, NM_000138.4c.3290G > A (p.Cys1097Tyr) (Table 1). The second case was a 22-year-old male who died in an accident, his genetic information was unavailable but he had two family members in the diagnosed case-series group.

We next expanded our study to the deCODE database of 35,712 WGS Icelanders and identified 63 rare, coding or splice sequence variants in FBN1 (Supplementary Table 1). Eight of the 63 variants classify as pathogenic or likely pathogenic based on the ACMG criteria, two of which were detected in the case-series, p.Arg2680Cys and NM_000138.4c.4211A > G (p.Asp1404Gly) (Table 2). In total, the eight pathogenic or likely pathogenic variants are carried by 20 living individuals, amounting to an MFS prevalence of 1/16,000 considering a genotype-based approach only. Two of these eight variants have previously been reported as pathogenic by other groups, NM_000138.4c.2860C > T (p.Arg954Cys) and p.Arg2680Cys (Supplementary table 2). From the genotype-based approach we identified 11 additional carriers of Arg2680Cys, as well as an additional carrier of p.Asp1404Gly, that is a brother of the original carrier.
of p.Asp1404Gly from the case series. Four pathogenic or likely pathogenic variants showed an association with one or more clinical MFS features, providing further phenotypic support of the pathogenic nature of these variants.

Table 2

<table>
<thead>
<tr>
<th>Nt. change</th>
<th>Protein change</th>
<th>N carriers deCODE</th>
<th>N carriers in case-series</th>
<th>Mean height (SD)*</th>
<th>Previously reported</th>
<th>Classification</th>
<th>ClinVar variation ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.8405G&gt;T</td>
<td>p.Gly2802Val</td>
<td>1</td>
<td>-</td>
<td>2,183645</td>
<td>No</td>
<td>Likely pathogenic</td>
<td>-</td>
</tr>
<tr>
<td>c.8038C&gt;T</td>
<td>p.Arg2680Cys</td>
<td>11</td>
<td>10</td>
<td>1,859574</td>
<td>(31–33)</td>
<td>Pathogenic</td>
<td>200127</td>
</tr>
<tr>
<td>c.7699+2T&gt;C</td>
<td>p.IVS62+2</td>
<td>2</td>
<td>2</td>
<td>1,244039</td>
<td>No</td>
<td>Pathogenic</td>
<td>-</td>
</tr>
<tr>
<td>c.6744-6746delGGA</td>
<td>p.Glu2248del</td>
<td>2</td>
<td>2</td>
<td>2,117884</td>
<td>No</td>
<td>Pathogenic</td>
<td>-</td>
</tr>
<tr>
<td>c.6446A&gt;G</td>
<td>p.Tyr2149Cys</td>
<td>1</td>
<td>1</td>
<td>NA</td>
<td>(46, 47)</td>
<td>Pathogenic</td>
<td>42402</td>
</tr>
<tr>
<td>c.5788+5G&gt;A</td>
<td>p.IVS47+5</td>
<td>2</td>
<td>2</td>
<td>NA</td>
<td>(36–38)</td>
<td>Pathogenic</td>
<td>42394</td>
</tr>
<tr>
<td>c.4211A&gt;G</td>
<td>p.Asp1404Gly</td>
<td>1</td>
<td>1</td>
<td>NA</td>
<td>No</td>
<td>Pathogenic</td>
<td>-</td>
</tr>
<tr>
<td>c.4087+6T&gt;C</td>
<td>p.IVS33+6</td>
<td>2</td>
<td>-</td>
<td>1,306860</td>
<td>No</td>
<td>Likely pathogenic</td>
<td>-</td>
</tr>
<tr>
<td>c.4085C&gt;G</td>
<td>p.Thr1362Ser</td>
<td>1</td>
<td>-</td>
<td>NA</td>
<td>No</td>
<td>Likely pathogenic</td>
<td>-</td>
</tr>
<tr>
<td>c.3290G&gt;A</td>
<td>p.Cys1097Tyr</td>
<td>1</td>
<td>1</td>
<td>NA</td>
<td>No</td>
<td>Pathogenic</td>
<td>-</td>
</tr>
<tr>
<td>c.2860C&gt;T</td>
<td>p.Arg954Cys</td>
<td>4</td>
<td>-</td>
<td>0,149175</td>
<td>(19, 48–50)</td>
<td>Pathogenic</td>
<td>495582</td>
</tr>
<tr>
<td>c.2855-2A&gt;G</td>
<td>p.IVS24-2</td>
<td>1</td>
<td>1</td>
<td>NA</td>
<td>No</td>
<td>Pathogenic</td>
<td>-</td>
</tr>
<tr>
<td>c.1850G&gt;A</td>
<td>p.Cys617Tyr*</td>
<td>1</td>
<td>1</td>
<td>1,902062</td>
<td>Clinvar</td>
<td>Pathogenic</td>
<td>495563</td>
</tr>
<tr>
<td>c.1616G&gt;A</td>
<td>p.Arg539Gln</td>
<td>4</td>
<td>-</td>
<td>0,730187</td>
<td>No</td>
<td>Likely pathogenic</td>
<td>-</td>
</tr>
<tr>
<td>c.1464dupT</td>
<td>p.Ile489TyrfsTer2</td>
<td>1</td>
<td>1</td>
<td>NA</td>
<td>No</td>
<td>Pathogenic</td>
<td>-</td>
</tr>
</tbody>
</table>

Our study revealed a six-generation family with a single pathogenic variant in FBN1, p.Arg2680Cys. Among this family, 11 were diagnosed or suspected to have MFS and an additional 11 were found through a genotype-based approach, 8 of whom were alive in 2017, at the time of the study. The age of living family members that are carriers of Arg2680Cys ranges from 6 to 90 years. All 22 individuals are descended from a common ancestor couple, born in 1838 and 1841 (Fig. 2). Affected family members have a wide range of clinical features ranging from isolated skeletal abnormalities to aortic dissection needing emergency surgery. As far as we know, no carriers have died due to MFS complications suggesting that heterozygosity for p.Arg2680Cys causes a relatively mild form of MFS. We have summarized available clinical features of individuals clinically diagnosed and/or carriers of this variant (Table 3). This large kindred appears to have a notable predisposition to abdominal aneurysms. Four family members, including one carrier and one obligate carrier, had abdominal aneurysm either diagnosed during the life of the individual or based on post-mortem examination. On average the carriers’ height (n = 12) was 1,46 standard deviations over the population mean (around 10 cm taller), adding further support to the notion that this is a pathogenic sequence variant causing MFS (34).
Table 3
Clinical data of diagnosed MFS patients in the p.Arg2680Cys family.

<table>
<thead>
<tr>
<th>Pedigree no.</th>
<th>Age</th>
<th>Sex</th>
<th>Ectopia lentis</th>
<th>Joint laxity</th>
<th>Vertebral deformity</th>
<th>Chest deformity</th>
<th>Height SD</th>
<th>Facial features</th>
<th>Aortic dilation</th>
<th>Aortic dissection</th>
<th>Valve deformities</th>
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<tr>
<td>III-3</td>
<td>90</td>
<td>M</td>
<td>+</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>2.58</td>
<td>na</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IV-4</td>
<td>57</td>
<td>F</td>
<td>+</td>
<td>na</td>
<td>na</td>
<td>-</td>
<td>2.11</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AoR</td>
</tr>
<tr>
<td>V-2</td>
<td>35</td>
<td>F</td>
<td>-</td>
<td>na</td>
<td>-</td>
<td>na</td>
<td>na</td>
<td>-</td>
<td>-</td>
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<tr>
<td>V-3</td>
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<td>na</td>
<td>na</td>
<td>na</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V-4</td>
<td>28</td>
<td>M</td>
<td>+</td>
<td>na</td>
<td>-</td>
<td>na</td>
<td>na</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>MVP</td>
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</table>

Numbers of individuals refers to position in pedigree in Fig. 2.

M = male; F = female; “+”=indicates that clinical feature is present; “-”=indicates that clinical feature hasn’t been observed; NA = data not available; SD = standard deviation; AoR = Aorta valve regurgitation; MVP = Mitral valve prolapse.

The sequence variants found in this study are spread throughout the FBN1 gene from exon 1 to 65 (Fig. 3). All in all, we have identified 15 pathogenic or likely pathogenic variants in FBN1, nine of which are missense variants (60%), four are predicted to affect splicing (27%), one is an in-frame deletion (3 bp), and one is a single base-pair duplication resulting in a premature termination codon. Seven variants were confirmed to be de novo variants, five of which were found in the case-series, NM_000138.4c.1464dupT (p.I489YfsX2), NM_000138.4c.1850G > A (p.Cys617Tyr), NM_000138.4c.2855-2A > G (p.IVS24-2), NM_000138.4c.5788 + 5G > A (p.IVS47 + 5) and NM_000138.4c.4085C > G (p.Thr1362Ser), found through the genotype-based approach, and later determined to be de novo. The splice region variant p.IVS47 + 5 is de novo, shared by two siblings but absent from both of their parents (based on WGS of blood). The sixth de novo variant is NM_000138.4c.4085C > G (p.Thr1362Ser), found through the genotype-based approach, and later determined to be de novo. Lastly, the nMFS case is a confirmed de novo case. The pathogenic and likely pathogenic FBN1 variants identified in this study are carried by 41 living individuals, 22 of whom were previously diagnosed with MFS (Table 2). In addition, we have knowledge of 13 individuals with a clinical diagnosis of MFS from whom either no DNA was available, or no FBN1 mutation was detected. Based on these numbers, we estimate the overall prevalence of MFS in Iceland to be at least 1/6000 (41 + 13/330,000).

Discussion

We present a nationwide genetic study on MFS in Iceland, including all MFS patients with a clinical diagnosis and individuals identified through a genotype-based approach. We identified 15 pathogenic or likely pathogenic variants in FBN1. Four are previously reported pathogenic or likely pathogenic variants and 11 are novel variants from our study. Our study thus expands the list of known Marfan causing variants and provides the healthcare system with a comprehensive list of variants in Iceland with emphasis on one highly represented variant (p.Arg2680Cys).

In this study we observe both inherited and de novo variants that span the entire region of the gene and cause both early and late presentations. In total, seven of the variants identified were confirmed to be de novo variants. One of these is p.IVS47 + 5G > A, shared by two siblings in our sample set (35). This variant is one of the most frequent recurrent variants reported to cause classical MFS, with involvement of cardiovascular, ocular and skeletal systems (8, 36–38). It substitutes a conserved residue within the mammalian splice donor consensus and causes in-frame exon skipping (8). PCys617Tyr is another confirmed de novo variant and was detected in one woman in our sample set. The woman has descendants (at least a son and granddaughter) diagnosed with the disorder,
however, their DNA was not available for this current study. This is an obvious limitation of our study which likely lowers the prevalence. Four other variants were confirmed to be de novo. The frameshift variant p.Ile489TyrfsX2 was detected in a tall male with aortic dilation and previous surgery because of aortic dissection. The splice essential variant p.IVS24-2 was detected in a young girl with a tall stature, scoliosis, pectus excavatum and MVP. The missense variant p.Tyr2149Cys was detected in a male that has needed repair surgery of his ascending aorta. Lastly, the missense variant p.Thr1362Ser was detected in a male who died of an unknown cause at the age of 25. He was the only confirmed de novo case that was not included in the case series. Clinical data were not available for that individual but the variant classifies as likely pathogenic using the ACMG criteria (39).

We also describe a single case of neonatal onset MFS in an infant with a de novo variant p.Cys1097Tyr. The boy had a very serious clinical picture that led to his death at 18 months of age. This particular variant is novel but a known pathogenic variant involving the same amino acid has similarly been reported to cause nMFS (40). This variant is located in exon 27 which belongs to a region with well-established genotype-phenotype correlation. Missense variants in exons 24–32 and variants causing exon skipping of exon 31 or 32 have been connected to severe phenotype in all systems and variants in this region are the best predictive factor in early onset dilation of the aorta. It applies even when nMFS is excluded (41).

One of the variants we identified, NM_000138.4c.6724C>T (p.Arg2242Cys), is a previously reported pathogenic variant (42), but we observe no significant association with MFS phenotypes among 63 imputed heterozygous carriers in Iceland. Based on the evidence available to us, we classify p.Arg2242Cys as a likely benign variant and do not believe it has a role in MFS.

Because of the modest size of our group of carriers of pathogenic variants it is challenging to get significant predictions of genotype-phenotype correlations. Interestingly, the most common pathogenic variant in our study (p.Arg2680Cys) leads to relatively mild symptoms and doesn’t appear to decrease fitness in this 6 generation pedigree, unlike many of the sporadic ones. P.Arg2680Cys is a known pathogenic variant published multiple times previously (31–33). It has usually been described as mild, affecting skeletal and ocular systems although one carrier presented with mitral valve prolapse without further involvement of the CVS (32). In 2016 a case was reported where the individual had aorta dilation, however the affected individual was compound heterozygous also carrying the variant NM_000138.4c.4270C>G (p.Pro1424Ala) in exon 35. Another family member who carried only p.Arg2680Cys presented with ectopia lentis without other symptoms at the age of two (31). Here we report individuals, heterozygous for the p.Arg2680Cys variant, who have severe cardiovascular symptoms. In the Icelandic sample set we detected three patients carrying the variant who had dilation of the ascending aorta with one having had emergency surgery after aortic dissection. Four individuals in this family have had AAA, including one known carrier and one obligate carrier. The other two are close relatives where AAAs were observed at autopsies. At least one of them was suspected to have the syndrome by family members, however, their genotype is not certain. Thus, our observed frequency of AAAs in four individuals is an apparent overrepresentation as prevalence in population has been estimated to be around 1% (43). These data suggest that heterozygous p.Arg2680Cys carriers should be screened for AAAs as part of their MFS workup. AAA is a known albeit rare clinical presentation in MFS. AAAs associated with MFS are usually seen in younger individuals than AAAs found in the general population (mostly in males after 65). The aneurysms also have different features, they usually don’t have mural thrombi, atherosclerotic changes are less frequent and abdominal aneurysms in MFS patients are more prone to rupture or dissection with a high mortality rate (44). One of our p.Arg2680Cys carriers has an atrial septal aneurysm (ASA) without other cardiovascular signs, but ASA have been associated with MFS before (45) so this may be another rare complication of p.Arg2680Cys.

In summary, we describe a nationwide study on MFS in Iceland and provide a comprehensive list of disease-causing variants in FBN1 in the Icelandic population. We describe a large kindred with mild MFS symptoms but a strong predisposition to the development of abdominal aortic aneurysms. We identified 35 individuals with a clinical diagnosis of MFS in Iceland, corresponding to a prevalence of approximately 1 in 10,000, which matches reported prevalence for MFS around the world (3–6). By combining clinical data with genetic information, we show that the prevalence of MFS in Iceland is much higher than based on clinical diagnosis alone, at least 1/6,000. Our data demonstrate that MFS is underdiagnosed in the Icelandic population with at least 35% MFS cases in Iceland without a clinical diagnosis. Importantly, as we only have genetic information for less than half of the population, that number is still an underestimate. Since MFS is a treatable genetic disorder that is life threatening if left untreated, it is important to find these carriers.

Declarations

Data availability
All data supporting the findings of this study are available within the main text of the article, and in the supplementary files.

Acknowledgements

We thank all the patients that participated in this study.

Author contributions


Funding

H.T.B. is supported by a grant from the Louma G. Foundation. H.T.B. is also supported by grants from the Icelandic Research Fund (#195835, #206806) and the Icelandic Technology Development Fund (#2010588). G.A.A., B.O.J., Ad.J., H.K., R.F., As.J., A.S., S.A.G., H.J., A.H., O.Th.M., U.Th., P.S. and K.S. are employed by deCODE genetics, which is owned by Amgen, Inc.

Ethical approvals

Consenting process. The case series study (patient identifiers) was approved by the Icelandic Ethics Committee (Visindasiðanefnd). The database study (no patient identifiers) has prior approval by Icelandic Ethics Committee (Visindasiðanefnd) but all participating individuals, or their guardians, have given informed consent prior to participation. All sample identifiers are encrypted in accordance with the regulations of the Icelandic Data Protection Authority. No single person was simultaneously able to access data with or without identifiers. Approval for these studies was provided by the National Bioethics Committee and the Icelandic Data Protection Authority.

Competing interests

Authors affiliated with deCODE Genetics/Amgen declare competing interests as employees. H.T.B.is a Consultant for Mahzi therapeutics. The remaining authors declare no competing financial interest.

References


Figures
Figure 1

Study design to explore the prevalence of Marfan syndrome in the Icelandic population. A diagram showing the study design, we have individuals with a found with a clinical diagnosis and individuals found after screening of the FBN1 gene at deCODE.
Figure 2

Pedigree presenting the family carrying the p.(Arg2680Cys) variant in FBN1.

Circle=female, Square=male. Full circle/square=Affected; Circle/square with a dot=Obligate carrier; Marfan=MFS clinical diagnosis; +/-=Heterozygous carrier of NM_000138.4:c.8038C>T p.(Arg2680Cys).

Figure 3

Domain organization of FBN1 with variants detected in the Icelandic sample set. Likely pathogenic and pathogenic variants are in red. Variants found in individuals with clinical diagnosis are underlined.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarytables.pdf