Genetic testing in patients with aortic aneurysms/dissections: a novel genotype/phenotype correlation?

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Received 27 October 2006; received in revised form 26 February 2007; accepted 27 February 2007; Available online 5 April 2007

Abstract

Objective: Mutations in the genes encoding fibrillin-1 (FBN1) and transforming growth factor beta receptor type II (TGFBR2) are known causes of Marfan syndrome (MFS) and related disorders. However, a sound correlation between the genotype and the cardiovascular phenotype has not yet been established. The objective of the present study was to identify novel mutations in FBN1 and TGFBR2 and to assess whether the type of mutation is linked to a particular clinical subtype of the cardiovascular condition. Methods: The clinical records of 36 patients referred to us for molecular genetic diagnosis were reviewed to assess the course and severity of the vascular deterioration. A semiautomatic protocol was established enabling a rapid and cost-effective screening of the genes FBN1 and TGFBR2 by direct sequencing of all coding exons and flanking intronic regions. Results: Novel mutations in FBN1 and TGFBR2 were detected in 12 and 2 patients, respectively. Four individuals carried a recurrent mutation in FBN1. Throughout the study cohort, the incidence of aortic dissections per se did not depend on the type of mutation. However, we found that mutations affecting the calcium-binding epidermal growth factor-like domain were more frequently associated with a dissection of distal parts of the aorta than mutations that lead to a premature termination codon (χ², p = 0.013), suggesting that the spatio-temporal pattern of vascular deterioration may vary with the type of mutation. Conclusions: Detecting a mutation in the genes FBN1 and TGFBR2 proves the genetic origin of vascular findings and allows the identification of family members at risk who should undergo preventive checkups. Routine genetic testing of patients with suspected MFS or thoracic aortic aneurysms/dissections could provide further insight into genotype/phenotype correlations related to aortic dissection.

Keywords: Mutation; FBN1; TGFBR2; Marfan syndrome; Aortic dissection

1. Introduction

With an incidence of 2—3/10,000, Marfan syndrome (MFS) is one of the most frequent inheritable connective tissue disorders. The condition is associated with a high risk of aortic dilatation and dissection. In addition to the cardiovascular system, symptoms may occur in the skeletal system (e.g. scoliosis), in the eye (ectopia lentis) and in the dural sac (ectasia). A correct diagnosis of the Marfan syndrome is hindered by a phenotypic overlap with other conditions (e.g. Ehlers—Danlos syndrome type IV), by an age-dependent penetrance and by a high degree of phenotypic variability among and within affected families. As a consequence, a considerable percentage of patients — in particular young individuals who present the condition de novo (~25% of all cases) — is not aware neither of having MFS nor of carrying an essential component of the extracellular matrix microfibrils (see [2] and references therein). Importantly, MFS cannot be diagnosed solely based on the presence of a FBN1 mutation, since other conditions (e.g. familial thoracic aneurysm/dissection and familial mitral valve prolapse syndrome) have also been traced back to alterations in this gene [3,4].
Conversely, MFS may also be caused by mutations in the gene TGFBR2 encoding the transforming growth factor beta receptor type II [5]. Interestingly, mutations in the same gene (but at different sites) have recently been associated with familial aortic aneurysm and dissection [6] as well as with the Loeys–Dietz aortic aneurysm syndrome (LDS), a condition with specific vascular findings, like arterial tortuosity and dilatation of vessels other than the aorta [Loeys et al. [7]]. Death in LDS patients frequently occurs in childhood, since the aneurysms are prone to dissect at smaller diameters than in MFS. Thus, the position of the genetic alteration within the TGFBR2 gene appears to determine — at least in part — the risk propensity of the vascular phenotype. In FBN1, mutations that lead to a severe neonatal form of MFS are clustered in the central region of the gene [8]. In order to reveal more genotype/phenotype relations, we have recently started a study that will result in the detailed clinicogenetic characterisation of 200 patients suffering from thoracic aortic aneurysm/dissection with or without other signs of MFS or related disorders. Here, we present results from a pilot study on 36 patients referred to hospital for surgical repair of the aorta.

2. Subjects, materials and methods

2.1. Probands and clinical evaluation

The pilot study was conducted according to the regulations of the local advisory board (University of Witten/Herdecke, Germany). All probands underwent vascular and/or valve surgery and were then referred to the Heart and Circulation Institute for mutation detection in the genes FBN1 and TGFBR2. Informed consent was obtained after detailed explanation of the study. Cardiovascular signs were compiled retrospectively from clinical records.

2.2. Mutation detection

Total genomic DNA was isolated from peripheral blood lymphocytes using the EZ1 DNA Blood kit and the EZ1 BioRobot (Qiagen, Hilden, Germany). PCR reactions were set up manually using GoTaq® Flexi DNA Polymerase (Promega, Mannheim, Germany). Proprietary primers were used to generate 64 FBN1 amplicons (65 exons, plus ~80 bp at both ends of each intron) and two TGFBR2 amplicons (exon 1 and the 3’ portion of exon 4), while the remaining exons were amplified using the VariantSEQ® Resequencing primer set for TGFBR2 (Applied Biosystems, Darmstadt, Germany). Cycling conditions were as follows: one cycle of 4 min at 95 °C/40 cycles of 30 s at 95 °C, 30 s at 58 °C and 60 s at 72 °C/one cycle at 72 °C for 10 min. Proprietary methods were developed to run all post-PCR sequencing steps on a MICROLAB STAR IVD liquid handling robot (Hamilton, Bonaduz, Switzerland). Reagent kits used were Ampure®, CleanSEQ® (for PCR and sequencing reaction clean up, respectively; Agencourt, Beverly, USA) and GenomeLab™ DTCs with quick start (Beckman Coulter, Krefeld, Germany). Sequencing products were separated in a 33 cm 8-capillary array on a CEQ 8000 instrument (Beckman Coulter). For mutation detection, alignments of forward and reverse sequencing traces were visually inspected using Sequence Investigator software (Beckman Coulter). With regard to the detection of heterozygous single base substitutions, the sensitivity of the bidirectional sequencing assay was found to be >99% (1 out of 146 reference mutations/single nucleotide polymorphisms remained undetected). False positive/negative rates were <1%, respectively (data not shown). Mutations were named according to recommendations by Antonarakis and coworkers [9,10]. Intron–exon boundaries were derived from GenBank files (see below). All primer sequences, protocols and liquid handling methods are available upon request (waldmueller@herzkreislaufforschung.de).

2.3. GenBank accession numbers

FBN1 cDNA, NM_000138.2; FBN1 genomic DNA, NT_010194.16; TGFBR2 cDNA, NM_003242.4; TGFBR2 genomic DNA, NT_022517.17.

2.4. Statistical analysis

Using SigmaStat 3.1 software, a Chi square analysis was performed (degree of freedom: 1; $\chi^2$). A p-value <0.05 indicates that the genotype/phenotype correlation in question is significant.

3. Results

3.1. Cardiovascular symptoms and interventions

3.1.1. Primary complications

The study group comprised of 28 patients (F = 9, 32%; M = 19, 68%) for whom clinical records were available. The mean age was 35 ± 13 years at time of initial hospitalisation (see Table 1). Seventeen probands (61%) were suspected having Marfan syndrome (MFS) and eight individuals had a family history of thoracic aortic aneurysm/dissection. The most common vascular sign among the patients was dilatation of the aortic root (60.7%; mean maximal diameter: 59.4 ± 18.7 mm) followed by ectasia of the ascending aorta (32.1%; 75.4 ± 23.4 mm). Dissection of the aortic wall was reported for the ascending aorta (32.1% of all patients), for the aortic arch (10.7%), for the descending thoracic aorta (21.4%) and for the descending abdominal aorta (14.3%). The function of the aortic valve (AV) was compromised in roughly two thirds of the cohort. Numerous patients suffered from mitral valve (MV) insufficiency (25%), mitral valve prolapse (MVP; 17.9%) or both (10.7%). The tricuspid valve (TV) was only mildly affected in five patients (17.9%). Dilatation or hypertrophy of the left ventricle was reported in 32.1% and 14.3% of the cases, respectively. As to the surgical treatment of the primary manifestations, the composite valved graft (CVG) procedure slightly prevailed over the valve-sparing repair of the ascending aorta (32.1% and 25.0%, respectively). In three patients (10.7%), the primary surgical intervention exclusively concerned distal (i.e. descending) parts of the aorta.

3.1.2. Secondary complications

Nearly one third of the patients presented for additional surgical (eight patients) or conservative intervention (one
patient) after a mean time span of 35.1 ± 32.0 months. In the remainder of the probands, the time span between hospitalisation and analysis of the clinical records was 25 ± 13 months. Of the 19 patients who initially underwent repair of the ascending aorta, 3 required subsequent distal aortic surgery. Repair of a secondary type A dissection (Stanford) or ex post replacement of the aortic valve became mandatory in two individuals, respectively.

3.2. Mutations in FBN1

For the genetic analysis, the study group comprised of 36 patients, the 28 patients described above and a group of 8 patients for whom clinical records were not available. A unique non-synonymous FBN1 mutation (i.e. a mutation that changed the coding sense of the FBN1 mRNA) was found in 44% of the patients (16/36, see Table 2). Twelve patients (33%) carried a novel mutation whereas the genetic defect in the remaining mutations (5/16, 31%) led to a premature termination codon (PTC) that occurred either due to a frameshifting insertion/deletion (19% and 13% of the mutations, respectively). One third of the patients (10/36) carried a mutation in FBN1 which was not associated with clinical symptoms. The mutations found in 16 patients (44%) were scattered over the entire coding sequence. Of the 20 patients with no exonic mutations (10/16), 7 were not tested for mutations in the coiled-coil domain (non-cbEGF, 6%) or in a non-Ca$^{2+}$-binding EGF-like domain (cbEGF, 56%). Six mutations (6/16, 38%) affected highly conserved amino acids that are involved in Ca$^{2+}$-binding. A less conserved residue of a cbEGF-like domain was substituted in three other patients (3/16, 19%). It is worth noting that one missense mutation (c.6871G>C) might also be associated with the synthesis of an aberrant FBN1 mRNA, since it affects the splice donor site of exon 55. Five of the six remaining mutations (5/16, 31%) led to a premature termination codon (PTC) that occurred either due to a nonsense mutation or following a frameshifting insertion/deletion (19% and 13% of the mutations, respectively). One patient carried an in-frame deletion of 3 bp that caused the loss of an arginine (R1692) in a latent transforming growth factor-$\beta$1 binding protein (LTBP)-like module of fibrillin-1. Three out of 16 FBN1 mutations (19%) involve a CpG dinucleotide. The genetic variations detected in this study are scattered over the entire FBN1 gene (Fig. 1) and show a considerable degree of clustering between domains cbEGF3/exon 12 and cbEGF8/exon 19 (31% of the mutations in 10% of the coding sequence). Of the 20 patients with no exonic FBN1 mutation, 9 had a mutation in TGFB2 (see below) and 4

Table 1

Clinical findings at primary and secondary hospitalisation

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<th>Dissection type</th>
<th>Cardiac manifestations</th>
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* Data in parenthesis refer to the secondary hospitalisation.

** Numbers refer to Table 2.

* FBN1 mutations leading to either a substitution in a cbEGF domain (1) or to a premature termination codon (PTC; 2). 3, no mutation detected in either FBN1 or TGFB2. F. mutation in FBN1; T. mutation in TGFB2; ?, mutations with uncertain molecular consequences.

** AR, aortic root; ASC, ascending aorta; ARC, aortic arch; DTA, descending thoracic aorta; DAA, descending abdominal aorta. If determined, the maximal diameter of an aneurysm (A) is given in millimetre. D indicates the presence of a dissection.

* Acute (a) or chronic (c) dissections according to the Stanford (A, B) or De Bakey (I) classification.

* AI, aortic insufficiency. MI, mitral valve insufficiency; MVP, mitral valve prolapse; TI, tricuspid valve insufficiency; LV D, left ventricular dilatation; LVH, left ventricular hypertrophy.

* sMFS, suspected Marfan Syndrome.
carried intronic FBN1 mutations which have been excluded in 192 control chromosomes. However, we cannot exclude that these intronic mutations represent rare neutral variants rather than disease causing mutations.

Fig. 1. Localisation of various mutations detected in FBN1 and TGFBR2. (A) The mutations detected in the present work were plotted on a scheme showing the domain structure of fibrillin-1. Fibr-cys, the domain resembles a 4-cysteine domain found in N-terminal extended forms of latent transforming growth factor (TGF)-β-binding proteins (LTBPs). EGF, epidermal growth factor-like domain; Pro, proline-rich region; Hybrid, hybrid module; cbEGF, calcium binding EGF-like module; TGFβ-BP, TGFβ1-binding protein-like module frequently referred to as 8-cysteine module. Mutations that have been described previously are marked by asterisks. (B) Known and novel mutations are shown on a map of the exon structure of TGFBR2. The exon numbers are given and the encoded domains are indicated. The mutations detected in the present work are displayed below the exon map and are marked either in green (missense) or black (uncertain consequence). Several adjacent mutations previously described by others [5–7,14–16] are shown above the gene scheme.

3.3. Mutations in TGFBR2

Two patients harboured a substitution/missense mutation that affected the protein kinase domain of TGFBR2. The substitution was either conservative (p.Tyr470Ser) or non-conservative (p.His360Pro). In both cases, the substituted amino acid is invariant in pig, mouse and frog. In addition to the two substitutions, two mutations with unknown molecular consequences were detected in three affected individuals. Both the intronic mutation c.1524+10G>C and the silent exonic mutation c.1167C>T were excluded in 192 control chromosomes, though the latter one occurred in two (apparently) unrelated patients with distinct phenotypes (see below).

3.4. Genotype/phenotype correlations

In order to detect mutation class-specific cardiovascular subtypes, the frequency of a number of clinical signs was determined for the following three groups: (1) carriers of a FBN1 mutation that affects a cbEGF domain (7 probands); (2) carriers of a mutation associated with a premature termination codon, PTC, in FBN1 (5 probands); (3) individuals with no mutation detected in either FBN1 or TGFBR2 (7 probands). As shown in Fig. 2, the mutation detection rate was higher in patients suspected of having MFS than in patients with no obvious signs of this condition (11/17, 64.7% vs 3/11, 27.3%; x²: p = 0.053). At time of primary hospitalisation, two patients from each group presented with a dissection of the proximal aorta (aortic root and/or ascending aorta). In
contrast, a significant difference was observed in the frequency of dissections in the distal aorta (descending thoracic and/or abdominal parts): a primary dissection of the descending aorta was exclusively observed in carriers of a cbEGF mutation (5 out of 7 patients, 71.4%, compared to 0/5, 0%, for the carriers of a PTC mutation; $\chi^2$: $p = 0.013$). In patients with a PTC mutation (group 2), distal dissections exclusively occurred as a secondary event (2/5, 40%). The frequency of cardiac findings (e.g. aortic and mitral valve insufficiency, left ventricular dilatation or hypertrophy) did not differ substantially throughout the patient groups. As to the type of surgical intervention, no significant difference was observed between group 1 (cbEGF) and group 2 (PTC) patients, which in the majority of cases received a valved graft (4/7, 57.1% and 3/5, 60%, respectively). Interestingly, patients in which no mutation could be detected underwent a valve-sparing procedure more often (4/7, 57.1%) and rarely received a valved graft (1/7, 14.3%). However, rational arguments for the choice of the individual surgical measure could not be elucidated retrospectively.

4. Discussion

We here report on the semiautomatic detection of mutations associated with aortic aneurysms and dissections. An overall detection rate of 50% was achieved, with 44% of the patients carrying a mutation in FBN1 and 6% of the patients carrying a mutation in TGFBR2. In addition, six Patients (17%) were found to carry a synonymous mutation with unknown consequences. The efficacy of mutation detection was ~65% in probands with suspected MFS and was thus lower than the 90% previously reported for a cohort that fully matched the Gent criteria [11], suggesting that some of the patients with suspected MFS in our cohort had conditions other than MFS.

Despite the vast number of FBN1 mutations reported to date, only a few genotype/phenotype relationships could firmly be established so far. For example, mutations leading to premature termination codons (PTC) are less frequently associated with ectopia lentis and retinal detachment than mutations that cause the substitution of a cysteine residue in a calcium-binding epidermal growth factor-like (cbEGF) domain [12]. As to the development of cardiovascular manifestations, Shrijver et al. [13] found that cysteine substitutions in cbEGF domains encoded by FBN1 exons 26–32 are associated with early dilatation of the aorta in childhood. Controversy exists regarding the correlation of both cysteine substitutions and PTC mutations and the risk of dissection of the aorta [11,12]. For example, Shrijver et al. [12] found a higher incidence of aortic dissections in patients carrying a PTC mutation compared to those with a cysteine substitution, although the difference observed was not statistically significant. In agreement with work by Loeys et al. [11], our data do not suggest that the type of mutation is associated with the over-all risk of dissection in the ascending thoracic aorta. However, most of the patients who presented at initial hospitalisation with a dissection that either originated in or extended to the descending aorta were found to harbour a substitution in one of the cbEGF-like domains of fibrillin. In contrast, probands with a different genotype generally developed such a condition ‘later’, i.e. following the initial intervention. Thus, it is tempting to speculate that a link exists between the type of mutation and the spatio-temporal pattern of vascular deterioration. In order to further substantiate this issue, we have recently started to enrol in a large-scale study aiming to investigate 200 patients suffering from thoracic aortic aneurysms and dissections.

In conclusion, our data suggest that the incidence of few vascular complications may be related to the FBN1 genotype. Despite novel insights into genotype/phenotype correlations, detailed knowledge of the FBN1 and TGFBR2 genotype does not allow (at present) to foresee the individual risk of a patient or to adjust therapeutic measures. However, testing of both genes can prove the genetic origin of the vascular risk. In the future, with the discovery of novel disease genes in genome-wide studies and through candidate gene analysis, the mutation detection rate may rise beyond 50%. The knowledge of the presence of a disease-causing mutation provides the basis to identify (presymptomatic) relatives at risk in a straightforward and low-cost assay, and to relieve the burden of regular checkups from non-carriers. Further studies are needed to assess whether the routine genetic screening of patients that undergo repair of a thoracic aortic aneurysm/dissection can help to prevent multiple cases of emergency interventions within families.

Acknowledgements

We thank the probands for their interest and cooperation; Petra Gehle, Johannes Frömke and Anastassia Dermintzoglou for referring affected individuals; Priska Binner for management of the study; and Joanne Davies for critical reading of the manuscript.

References


