SHORT REPORT

A 1-bp duplication in *TGFB2* in three family members with a syndromic form of thoracic aortic aneurysm

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A number of autosomal dominantly inherited disorders, such as Marfan syndrome (MFS) and Loeys–Dietz syndrome (LDS), are associated with predisposition to thoracic aortic aneurysms and dissections (TAADs). In the majority of cases, mutations in genes encoding components of the transforming growth factor- β (TGF- β) signaling pathway, such as *FBN1*, *TGFBR1*, *TGFBR2* and *SMAD3*, underlie the disease. Recently, a familial syndromic form of TAAD with other clinical features that overlap the MFS–LDS spectrum has been described to be caused by heterozygous loss-of-function mutations in *TGFB2*, encoding the TGF- β 2 ligand of TGF- β serine/threonine kinase receptors (TGFBRs). We analyzed the *TGFB2* gene by sequencing in a cohort of 88 individuals with a Marfan-like phenotype and/or TAAD, who did not have mutations in known genes causing thoracic aortic disease. We identified the novel heterozygous c.1165dupA mutation in exon 7 of *TGFB2* in three members of a family, a 51-year-old male, his brother and nephew with aortic aneurysms, cervical arterial tortuosity and/or skeletal abnormalities as well as craniofacial dysmorphisms. The 1-bp duplication causes a frameshift leading to a stable transcript with a premature stop codon after seven TGF- β 2-unrelated amino acids (p.Ser389Lysfs*8). As the resulting protein is unlikely functional and by considering data from the literature, we support the notion that functional haploinsufficiency for TGF- β 2 predisposes to thoracic aortic disease. Taken together, *TGFB2* is a rarely mutated gene in patients with syndromic TAAD, and the clinical features of our *TGFB2* mutation-positive individuals fit in the scheme of LDS, rather than MFS-related disorders.

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INTRODUCTION

Thoracic aortic aneurysm and dissection (TAAD) is a genetically heterogeneous disease that can occur in conjunction with several genetic syndromes, including Marfan syndrome (MFS; MIM 154700), Loeys–Dietz syndrome (LDS; MIM 609192, 608967, 610168, 613795 and 610380), vascular type of the Ehlers–Danlos syndrome (MIM 130050 and 225400) and others.¹ As pathogenic mutations in various genes encoding proteins of the transforming growth factor- β (TGF- β) signaling pathway, such as *FBN1*, *TGFBR1*, *TGFBR2* and *SMAD3*, have been identified,^{2–7} dysregulated TGF- β signaling likely contributes to the pathogenesis of thoracic aortic diseases.^{1,8} Indeed, several lines of evidence indicate augmented TGF- β signalling in aortic lesions *in vivo*.^{6,9,10} This is intriguing as many pathogenic mutations associated with TAAD have a loss-of-function effect.^{2,11,12} Recently, two studies have shown that haploinsufficiency for *TGFB2* causes a familial syndrome of TAAD with additional clinical manifestations overlapping MFS and LDS.^{13,14} *TGFB2* encodes TGF- β 2, one of three ligand isoforms that exert their cellular effects, eg, the regulation of organ-specific morphogenesis and adult tissue homeostasis by binding to heterotetrameric complexes of the TGF- β serine/threonine kinase receptors.¹⁵

MATERIALS AND METHODS

Patients

We obtained clinical data and blood samples from 88 individuals (both familial and sporadic cases) with a Marfan-like phenotype and/or TAAD, who were assessed by SS, MR and YvK. All individuals were negative for mutations in *FBN1*, *TGFBR1* and *TGFBR2*. Sixteen patients out of these eighty-eight individuals showed clinical features overlapping with syndromic/familial forms of TAAD including vascular type of the Ehlers–Danlos syndrome (MIM 130050), aortic valve disease (MIM 109730), arterial tortuosity syndrome (MIM 208050), aneurysms-osteoarthritis syndrome (MIM 613795), familial thoracic aortic aneurysm 6 (MIM 611788) and/or familial thoracic aortic aneurysm 4 (MIM 132900). In these cases, we excluded mutations in clinically relevant, aortic disease-associated genes including *COL3A1* (10 probands), *ACTA2* (9 probands), *SMAD3* (8 probands), *SLC2A10* (3 probands), *NOTCH1* (8 probands) and/or *MYH11* (5 probands).

The clinical data and samples were obtained with informed consent, including consent to use the photographs in this report, under protocols approved by the Institutional Review Board.

Sequencing of *TGFB2*

We isolated genomic DNA from blood by standard procedures and amplified the coding region including the flanking intronic sequences of *TGFB2* (exons 1–8; GenBank accession no. NM_001135599.2) from genomic DNA.

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Primer sequences are available on request. We directly sequenced amplicons using the ABI BigDye Terminator Sequencing Kit (Applied Biosystems, Weiterstadt, Germany) and an automated capillary sequencer (ABI 3500; Applied Biosystems, Weiterstadt, Germany). We analyzed the sequence data by the Sequence Pilot software (JSI medical systems, Kippenheim, Germany). Exon numbering of *TGFB2* is according to Refseq accession number NM_001135599.2 and ensemble transcript ID ENST00000366929.

TGFB2 transcript analysis

RNA was isolated from fresh whole blood from the index patient (II-2) using the PAXgene Blood RNA System (PreAnalytics, Hombrechtikon, Switzerland) according to the manufacturer's recommendations. First-strand cDNA was synthesized from 1 µg of RNA using the Omniscript Reverse Transcriptase Kit (Qiagen, Hilden, Germany) and gene-specific oligonucleotides. Of a total of 20 µl of volume from the reaction, 1 µl was used as template to amplify transcripts by using primer pairs spanning different exon-exon junctions in nested PCR reactions. Amplicons were directly sequenced or cloned into pCR2.1 TOPO TA Cloning Vector (Invitrogen, Darmstadt, Germany). For semi-quantitative analysis, 34 *Escherichia coli* clones were subjected to colony PCR, and PCR products from individual clones were sequenced.

Database

Mutation data (*TGFB2* c.1165dupA) were submitted to Leiden Open Variation Database (LOVD) for *TGFB2* at <http://www.LOVD.nl/TGFB2>.

RESULTS

Mutation analysis of *TGFB2*

We sequenced the *TGFB2* gene in a cohort of 88 individuals with a Marfan-like phenotype and/or TAAD and detected a single sequence alteration: the heterozygous mutation c.1165dupA in the penultimate codon of exon 7 (Figure 1a) in a 51-year-old male with TAAD and a phenotype resembling MFS (Figure 2a). The 1-bp duplication likely leads to a frameshift and introduction of a premature stop codon after seven TGF-β2-unrelated amino acids (p.Ser389Lysfs*8; Figure 1b). By RNA analysis, we demonstrated expression of both the wild-type and the mutated *TGFB2* allele in blood cells of the index patient (Figure 1c); semi-quantitative evaluation suggested 59% and 41% of wild-type and mutant *TGFB2* transcripts, respectively (data not shown). We sequenced exon 7 of *TGFB2* in four additional family members of the index patient and identified the c.1165dupA mutation in one of his two affected brothers (II-4 in Figure 2b; II-6 was not available for testing) and his nephew (Figure 2b, III-2), who showed aortic aneurysm and/or a Marfan-like phenotype. We could exclude the mutation in the healthy daughter (III-1 in Figure 2b) and the healthy brother (II-5 in Figure 2b) of the index patient. In summary, the *TGFB2* mutation co-segregates with the disease phenotype in the family.

Clinical data of family members with the *TGFB2* c.1165dupA mutation

The index patient II-2 (Figure 2b) was clinically evaluated at age 51 years. His height was 191 cm and weight 85 kg. He had mild scoliosis, pes planus and a positive ratio of arm span and body height. Craniofacial dysmorphism included malar hypoplasia, prominent uvula, elongated face and retrognathia (Figure 2a). He already had undergone several surgical operations including testicular torsion repair, inguinal hernia surgery, appendectomy and sigma resection after acute diverticulitis. Magnetic resonance angiography revealed profound cerebral and cervical arterial tortuosity (Figure 2a). After we diagnosed aortic insufficiency due to an aneurysm with a diameter of 51 mm, patient II-2 underwent aortic aneurysm surgery. Based on these clinical manifestations, we made the diagnosis of a syndromic

Figure 1 Molecular aspects and consequences of the *TGFB2* c.1165dupA mutation. (a) Part of the sequence electropherogram of *TGFB2* exon 7–intron 7 (gDNA, genomic DNA). The index patient is heterozygous for the 1-bp duplication c.1165dupA in *TGFB2* (indicated by an arrow, ↓). The sequence of the wild-type and mutated allele is given above the electropherogram; the exon–intron boundary and encoded amino acids (one-letter-code) are indicated. (b) Schematic presentation of *TGFB2* messenger RNA aligned with protein domains of the TGF-β2 proprotein. The c.1165dupA mutation is predicted to cause a frameshift leading to a premature stop codon (PTC) in *TGFB2* exon 8 and a hypothetically COOH-terminal truncated protein (p.Ser389Lysfs*8). Protein domains are indicated according to Simple Modular Architecture Research Tool (<http://smart.embl-heidelberg.de/>). ATG, start codon; TAA, stop codon; UTR, untranslated region; aa, amino acid. (c) Transcript analysis: partial sequence electropherogram of *TGFB2* exon 7–exon 8 junction obtained from cDNA (complementary DNA). The index patient is heterozygous for the *TGFB2* c.1165dupA mutation (indicated by an arrow, ↓). The sequence of the wild-type (WT) and mutated (MUT) allele is given under the electropherogram; the exon 7–exon 8 junction and the PTC (STOP) is shown.

TAAD disorder. The brother of patient II-2, II-4 in Figure 2b, whose height was 186 cm and weight 104 kg, presented with arterial tortuosity, mild scoliosis, joint hyperflexibility, retrognathia and a high arched palate at age 47 years. He had undergone aortic aneurysm surgery because of an aneurysm with a diameter of 58 mm and inguinal hernia surgery. Another brother of the index patient, II-6 in Figure 2b, also had undergone aortic aneurysm surgery, and he showed clinical features strongly suggestive of a MFS-related disorder. He denied genetic testing. The index patient's nephew, III-2 in Figure 2b, was clinically evaluated at the age of 13 years. He had scoliosis, pectus deformity, joint hyperflexibility and club foot, which were indicative for a syndromic TAAD disorder. The father I-1 (Figure 2b) of the index patient and his brothers died at the age of 52 years after acute aortic dissection.

DISCUSSION

We report a familial syndromic form of thoracic aortic aneurysm because of a heterozygous 1-bp duplication (c.1165dupA) in

Figure 2 Photographs and family pedigree. (a) Photographs of the index patient. Note the cerebral arterial tortuosity, the skeletal abnormalities including pes planus and retrognathia, but negative wrist and thumb signs and no restriction of elbow extension. The patient shows postoperative scars derived from aortic aneurysm surgery and former surgical operations. (b) The three-generation pedigree shows individuals with clinical manifestations suggestive of a MFS-LDS spectrum disorder (■). The index patient (II-2) is indicated by an arrow. Three affected individuals (II-2, II-4 and III-2) share the mutated *TGFB2* allele. Material from the deceased father (I-1) of the brothers for *TGFB2* sequencing was not available; one affected brother (II-6) denied genetic testing. Plus sign (+) in symbols, mutation present; minus sign (–) in symbols, mutation absent; gray bars above symbols, DNA available.

proximity of the last exon–intron boundary in *TGFB2* leading to a frameshift and a PTC (p.Ser389Lysfs*8). Because the PTC is located in the last *TGFB2* exon (Figure 1b), the mutant transcript is not expected to be degraded by nonsense-mediated mRNA decay.¹⁶ Indeed, we detected expression of both wild-type and mutant *TGFB2* transcripts. However, we propose that the mutated TGF- β 2 p.Ser389Lysfs*8 protein is not functional, either because of a severely compromised reactivity of the growth-factor domain or because of misfolding and degradation before secretion. In line with this, normal levels of mutant *TGFB2* transcripts but reduced proprotein levels have been detected in smooth muscle cells and fibroblasts from patients with the frameshift mutation c.1106_1110delACAAT (p.Tyr369Cysfs*26) in *TGFB2* (RefSeq NM_001135599.2, NP_001129071.1); this alteration was originally described as c.1021_1025delTACAA (p.Tyr341Cysfs*25) (*TGFB2* RefSeq NM_003238.3, NP_003229.1).¹³ Considering these

consequences, our finding of the c.1165dupA mutation in a three-generation family supports the idea that functional haploinsufficiency of TGF- β 2 causes a cellular compensatory overshoot leading to augmented TGF- β signalling in MFS-LDS spectrum disorders.¹⁷ On the other hand, we cannot exclude that, due to structural changes, the p.Tyr341Cysfs*25 mutation enhances TGF- β receptor affinity resulting in increased TGF- β signaling.

Auxiliary receptor TGFBR3/betaglycan is required for cellular responsiveness to TGF- β 2.^{18,19} Non-synonymous variants [c.334T>A (p.(W112R)), c.2368A>T (p.(I790F))] have been identified in *TGFBR3* in patients with familial intracranial aneurysms,²⁰ which strongly suggests a role of TGF- β 2-induced signaling in the molecular pathogenesis of aneurysms.

Interestingly, a mutation in *TGFB3* encoding the TGF- β 3 ligand is associated with a syndrome with few features of MFS and LDS, but

Table 1 Summary of clinical features of individuals with *TGFB2* mutations

Clinical feature	Index patient (II-2)	Brother (II-4)	Nephew (III-2)	Frequency ^a		
				Boileau <i>et al</i> ¹³	Lindsay <i>et al</i> ¹⁴	In total
Aortic or main pulmonary artery aneurysm	+	+	NA	14/19	1/4 (11)	17/25 (12) 68.0%
Aortic dissection	–	–	–	2/19	1/15	3/37 8.1%
Aortic repair	+	+	–	4/19	3/15	9/37 24.3%
Arterial tortuosity	+	+	NA	3/5 (14)	1/4 (11)	6/11 (26) 54.5%
Mitral valve impaired	–	–	NA	3/19	7/10 (5)	10/31 (6) 32.3%
Scoliosis	+	+	+	4/15 (4)	5/15	12/33 (4) 36.4%
Pectus deformity	–	–	+	7/16 (3)	9/15	17/34 (3) 50.0%
Joint hyperflexibility	–	+	+	10/15 (4)	7/15	19/33 (4) 57.6%
Arachnodactyly	–	–	NA	8/13 (6)	8/15	16/30 (7) 53.3%
Foot deformity	+	NA	+	11/15 (4)	8/15	21/32 (5) 65.6%
Retrognathia	+	+	NA	2/13 (6)	11/15	15/30 (7) 50.0%
High arched palate	–	+	NA	9/15 (4)	10/15	20/32 (5) 62.5%
Downslant palpebral fissures	NA	NA	NA	0/13 (6)	10/15	10/28 (9) 35.7%
Ectopia lentis	–	–	NA	1/13 (6)	0/15	1/30 (7) 3.3%
Striae atrophicae	–	–	NA	8/15 (4)	3/15	11/32 (5) 34.4%
Hernia	+	+	NA	6/17 (2)	10/15	18/34 (3) 52.9%
Easy bruising	–	–	NA	(19)	5/11 (4)	5/13 (24) 38.5%
Dural ectasia	–	–	NA	3/5 (14)	1/6 (9)	4/13 (24) 30.8%

Abbreviations: +, present; –, absent; NA, not analyzed.

^aFeature present/number of clinically evaluated patients (number of patients with no data).

no vascular findings suggesting a pathophysiology distinct from that caused by excess TGF- β signaling.²¹

Up to date, 12 independent *TGFB2* mutations in 34 individuals have been reported (Table 1).^{13,14} In our study, the three subjects with the c.1165dupA mutation in *TGFB2* share clinical features with other autosomal dominant aortic aneurysm syndromes. Among these features, aortic aneurysm, scoliosis, pectus deformity, joint hyperflexibility, pes planus, retrognathia, high arched palate and hernia occur both in MFS and LDS.²² Conversely, arterial tortuosity and club feet are manifestations, which are only seen in LDS. Moreover, the index patient did not present with reduced elbow extension or positive wrist/thumb signs, which are common manifestations of MFS. Most notably, the patients in this report did not exhibit ectopia lentis, which occurs in MFS but not in LDS. Similarly, Lindsay *et al*¹⁴ did not observe ectopia lentis in any of the 15 individuals with *TGFB2* mutations (Table 1),¹⁴ and Boileau *et al*¹³ described only a single patient among 13 individuals with *TGFB2* mutations who exhibited some sort of 'minor ectopia lentis', which they stated was 'not recognized as lens dislocation in the USA'.¹³ Thus, the clinical presentation of the three affected male relatives described here strengthens the notion that mutations in *TGFB2* are associated with an LDS rather than a MFS phenotype.¹⁴

We identified only one mutation carrier in a cohort of 88 individuals (1.1%) with a phenotype within the MFS-LDS spectrum. Similarly, *TGFB2* mutations were identified in 4 out of 409 probands (1%) including both familial and sporadic cases with thoracic aortic disease.¹³ In contrast, Lindsay *et al*¹⁴ found a relative high frequency of *TGFB2* mutations (in 6 out of 86 individuals, 7.0%), which may be the result of testing a cohort of probands with phenotypic features within the LDS spectrum.¹⁴ Notably, only two patients with MFS/LDS-related disorders in combination with developmental delay and heterozygous *de novo* microdeletions (6.5 Mb and 3.5 Mb) including the *TGFB2* gene have been described;¹⁴ however, more detailed copy number analysis may uncover further *TGFB2* mutations in patients with thoracic aortic disease. Taken together, sequence

analysis identified pathogenic *TGFB2* lesions in about 2% of patients with thoracic aortic disease, which is a rather low frequency compared with the three major TGF- β vasculopathy-associated genes *FBN1*, *TGFBR1* and *TGFBR2*. However, in view of the high risk for aortic aneurysm and dissection associated with *TGFB2*-mutations, molecular testing of this gene is crucial. Therefore, we suggest that a clinical presentation including thoracic aortic disease, arterial tortuosity, skeletal abnormalities and hernia but not ectopia lentis is indicative for mutation testing of *TGFB2*. Nevertheless, there are still many mutation-negative individuals with thoracic aortic disease indicating further genetic heterogeneity and additional genetic studies are necessary to recognize all details of the pathogenesis underlying this disorder.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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