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May TGFBR1 act also as low penetrance allele in Marfan syndrome?

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Abstract

Marfan syndrome, a human disease involving cardiovascular and skeletal apparatuses and ocular and central nervous systems, is associated to mutations in *FBN1* gene; heterozygous mutations in *TGFBR2* and *TGFBR1* genes were found associated to MFS type 2, characterized by the presence of skeletal and cardiovascular major criteria and absence of eye major criterion.

We screened the *TGFBR1* gene in 46 Marfan patients in whom mutations in FBN1 and *TGFBR2* genes were excluded and the analysis of Ex1 was extended to additional 114 Marfan patients and 237 controls.

We detected two potentially pathological sequence variants: the TGFBR1 6Ala allele whose frequency was higher in the group of Marfan patients (0.13) than in the controls (0.08) ($p=0.013$; OR = 1.69) and an insertion of 20 nucleotides in the 5′UTR that turned out to be a familial silent rare polymorphism. We hypothesize that TGFBR1 sequence variants may act not only as major, but also as low penetrance alleles of the clinical phenotype in Marfan syndrome.
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Keywords: *TGFBR1*; Marfan syndrome; MFS type 2; TGFBR1 6Ala allele

Marfan syndrome (MFS; MIM#154700) is a multisystemic dominantly inherited connective tissue disorder with a prevalence of 1 in 5000–10000 [1]; MFS is mostly caused by mutations in fibrillin-1 (*FBN1*;MIM#134797;chr.15q21.1) [2]. MFS type 2 (MFS2;MIM#154705) is characterized by absence of major ocular involvement; it has been recently associated to heterozygous dominant mutations in both *TGFBR2* (MIM#190182;chr.3p22) [3–5], and *TGFBR1* (MIM#190181;chr.9q33-34) [4,5], the two genes coding for the receptors of transforming growth factor beta (TGFβ). TGFβ induces extracellular matrix (ECM) formation by binding to its two receptors and by activating the intracellular signalling through SMAD proteins [6]. Mutations in *FBN1*, *TGFBR1* and *TGFBR2* lead to altered TGFβ signalling resulting in degenerative changes of the ECM and causing aneurysms and dissections of the vessel wall in MFS2 [3,7], Thoracic Aortic Aneurysms and Dissections (TAAD; MIM#608967) [4,7], and Loeys–Dietz syndrome (LDS; MIM#609192) [5,8,9]. Noteworthy, in a mouse model of MFS, excessive TGFβ signalling was demonstrated to contribute to the formation of aortic aneurysms; furthermore, treatment with TGFβ antagonists was shown to block and reverse the demolition of ECM and consequentially the dilatation and dissection of aorta [10].

We investigated 46 MF patients (34 men and 12 women, median age 32.5 years, range 7–54 years) diagnosed according to Ghent criteria, referred to the Center for Marfan Syndrome and Related Disorders of the Azienda Ospedaliero-Universitaria, Careggi, University of Florence.

DNA mutation analysis of *FBN1* [11], *TGFBR2* [3] and *TGFBR1* genes (Table 1) was performed [11,12]. Clinical characteristics and molecular data of these patients (8 of them with MFS2) are shown in Table 2. No mutations in both *FBN1* and *TGFBR2* were found. We detected two potentially pathological mutations both in exon 1 of *TGFBR1*. In order to verify their pathogenicity, we extended the analysis to 114 Marfan patients (69 men and 45 women, median age 35.5 years, range 7–70 years; 18 MFS2, 103 carrying a mutation in FBN1) and 237 healthy unrelated controls (111 men and 126 women, median age 51.2 years, range 15–91 years) with primers Ex1ins and Ex1-9* (Table 1). The different alleles were separated on a 4% agarose gel. Patients and controls, all Italian, gave their

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Table 1
Primers of TGFBR1 gene

TGFBR1 gene	Forward	Reverse	TM	Size (bp)
EX1	5′-agc agt tac aaa ggg ccg g-3′	5′-gcg cca tgt ttg aga aag ag-3′	60 °C	313
EX1ins	5′-gga cgc gcg tcc tcc gag c-3′	5′-gac cgc cgc ctc cat ggt c-3′	62 °C	157
EX1-9*	5′-gac cat gga ggc ggc ggt c-3′	5′-gtc gcc ccc ggg agc ag-3′	62 °C	99
EX2	5′-gag caa caa aca gtg cat aga aa-3′	5′-tcc ctt cca gtt cta aaa tca ca-3′	58 °C	448
EX3	5′-ggc tct ttg gct aag tgg tg-3′	5′-ttc tag caa gtt ggc tta tta g-3′	58 °C	449
EX4a	5′-tca gtt ttc tgg gtc act cat t-3′	5′-gtt ctt ctc tag agg aga ata tc-3′	58 °C	237
EX4b	5′-gtt tgg aga gga aag tgg c-3′	5′-gtc tca tct act ttg atg atg g-3′	56 °C	228
EX5	5′-ttg cag tgt gtg act cag ga-3′	5′-agg ccc agc ctc cac ctt-3′	60 °C	351
EX6	5′-ttt ggg ttg gga gaa gag ac-3′	5′-ttt ggg ttg gga gaa gag ac-3′	58 °C	399
EX7	5′-tgt ctg aaa gga ggt tca tcc-3′	5′-aat tct tga aca act tct gct c-3′	60 °C	256
EX8	5′-gcc ttg gca tta gct gaa taa-3′	5′-cat tgg ttt gac tgc tat gaa aa-3′	58 °C	316
EX9a	5′-cgt aaa aat tct tat cca gac c-3′	5′-cct tct gtt ccc tct cag tg-3′	60 °C	300
EX9b	5′-ctt cag atc tgc tcc tgg g-3′	5′-cat tca aag tct gaa tca agg-3′	60 °C	429
EX9c	5′-gta cat tct cag agg att ctg-3′	5′-gaa tac caa atg aca tac cac-3′	60 °C	403

* = primers modified from [13].
Accession number NC_000009.10 for TGFBR1 gDNA.

informed consent and the study was approved by the local ethics committee of the Faculty of Medicine, University of Florence.

The 6Ala allele polymorphism in Ex1 (Fig. 1; Table 3a) [5,13] was a trinucleotide GCG, corresponding to alanine (Ala), repeated 6 or 9 times. The 6Ala allele was the rare variant (Fig. 2).

The heterozygous insertion of 20nts in Ex1-5′UTR (Fig. 1; Table 3a) was identified in the proband, his healthy son, and an affected niece of one Marfan family. It was a duplication of 20nts (nts 3–22), inserted between nts 22 and 23 of exon 1, 55nts before the ATG start of protein translation. It is located in a sequence that does not seem to contain any site for transcription binding proteins

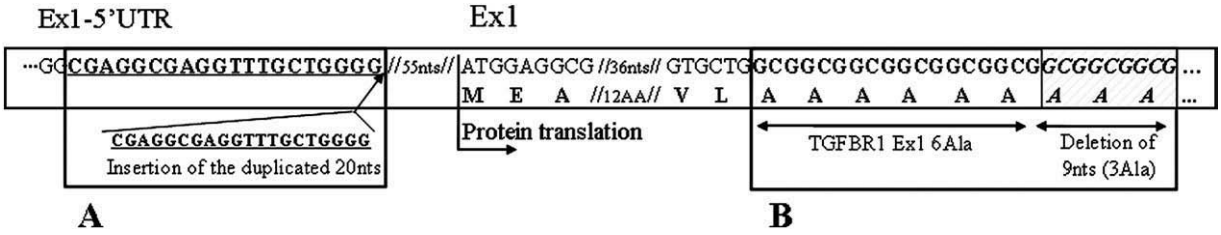

Fig. 1. Sequence variants found in Exon 1 of TGFBR1: A: insertion of the 20 duplicated nucleotides in the Ex1-5′UTR; B: the TGFBR1 6Ala allele is indicated and the 9nts (3Ala) deleted in the polymorphism are highlighted. Nts = nucleotides; AA = aminoacids; Ala = alanine.

Genotype distribution and allele frequency were in Hardy–Weinberg equilibrium for the 6Ala allele polymorphism both in patients and controls. A 9Ala/7Ala genotype was detected in one control.

The 6Ala allele frequency significantly differed between the group of Marfan patients and controls ($p = 0.013$; 0.13*vs*.0.08). The genotype distribution of the 6Ala allele polymorphism was higher in the group of Marfan patients (9Ala/6Ala 21.9% and 6Ala/6Ala 2.5%) than in the controls (9Ala/6Ala 16%). Furthermore, the homozygous genotype 6Ala/6Ala was detected only in 4 MF patients (Table 3b). The odds ratio was 1.69 (95%CI:1.023–2.785), suggesting that the 6Ala allele may contribute to Marfan syndrome phenotype.

The 6Ala allele frequency was 0.13 in both classic MFS and MFS2, although the second group is still too small. No significant different distribution of the 6Ala allele was found between genres and between single systems or organs' involvement.

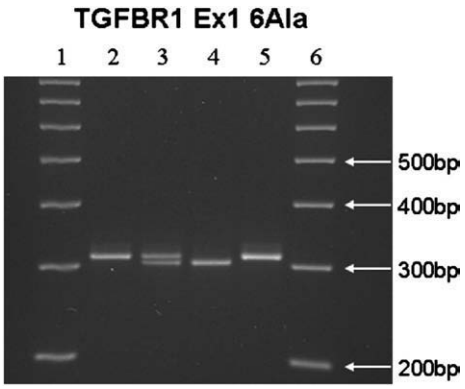

Fig. 2. PCR fragments of homozygous and heterozygous genotypes of TGFBR1 6Ala allele polymorphism and homozygous genotype of normal TGFBR1 9Ala allele on agarose gel (4%). Lanes 1 and 6 = molecular markers; lanes 2 and 5 = 9Ala/9Ala genotype; lane 3 = 9Ala/6Ala genotype; lane 4 = 6Ala/6Ala genotype. 9Ala = 319 bp; 6Ala = 310 bp. Ala = alanine; bp = base pairs.

Table 2
Clinical manifestations and mutations found in 46 Marfan patients

	Age (yrs)	Sex	Major criteria				Minor criteria			
			Familial history		CV	Ocular	Skel	Skin	Pneum	TGFBR1 6Ala genotype
1	36	F	+		−	+	+	+	−	9Ala/9Ala
2	35	M	+		+	−	−	+	−	9Ala/9Ala
3	38	M	−	§	+	−	+	−	−	9Ala/9Ala
4	42	M	−	*	+	−	−	+	−	9Ala/9Ala
5	46	M	−	*	+	−	−	+	−	9Ala/9Ala
6	46	F	−		+	+	+	+	−	9Ala/9Ala
7	40	M	−	§	+	−	+	+	−	9Ala/9Ala
8	11	M	−		+	+	−	−	−	9Ala/9Ala
9	34	F	+	§	+	−	+	+	−	**9Ala/6Ala**
10	24	M	−	§	+	−	+	+	+	9Ala/9Ala
11	8	M	−		+	+	−	−	−	9Ala/9Ala
12	23	F	+	§	+	−	+	+	−	9Ala/9Ala
13	44	M	+		+	−	−	+	−	9Ala/9Ala
14	13	M	+		+	+	+	−	−	9Ala/9Ala
15	30	M	+		+	−	−	+	−	9Ala/9Ala
16	42	M	+		+	−	−	+	−	9Ala/9Ala
17	40	M	+		+	+	−	+	−	9Ala/9Ala
18	33	F	+		+	+	−	−	−	9Ala/9Ala
19	36	M	+		+	−	−	+	−	**9Ala/6Ala**
20	42	M	−	§	+	−	+	+	−	**9Ala/6Ala**
21	25	F	+		−	−	+	+	−	9Ala/9Ala
22	22	M	−		+	+	−	+	−	9Ala/9Ala
23	34	F	−		+	+	−	−	−	9Ala/9Ala
24	30	M	−	*	+	−	−	+	−	9Ala/9Ala
25	34	F	−		+	+	−	−	−	9Ala/9Ala
26	42	M	−		+	+	−	−	−	9Ala/9Ala
27	30	M	−	*	+	−	−	+	−	9Ala/9Ala
28	44	M	+		+	−	−	+	−	9Ala/9Ala ins 20nts hetero
29	45	M	+		+	−	−	+	−	9Ala/9Ala
30	32	M	−	*	+	−	−	+	−	9Ala/9Ala
31	33	F	−	*	+	−	−	+	−	**9Ala/6Ala**
32	40	M	−	*	+	−	−	+	−	9Ala/9Ala
33	45	M	−	*	+	−	−	+	−	9Ala/9Ala
34	39	F	−	*	+	−	−	+	−	**9Ala/6Ala**
35	20	F	−		+	+	−	+	−	9Ala/9Ala
36	15	M	−	*	+	−	−	+	−	**9Ala/6Ala**
37	40	M	−	*	+	−	−	+	−	9Ala/9Ala
38	9	M	−		+	+	−	−	−	9Ala/9Ala
39	28	M	−	*	+	−	−	+	−	9Ala/9Ala
40	25	M	+		+	−	−	+	−	9Ala/9Ala
41	7	M	−	§	+	−	+	−	−	9Ala/9Ala
42	32	M	+		+	−	−	+	−	9Ala/9Ala
43	54	F	−	§	+	−	+	+	−	9Ala/9Ala
44	44	M	+		+	+	−	+	−	9Ala/9Ala
45	22	M	+		+	−	−	+	−	**6Ala/6Ala**
46	43	M	+		+	+	+	+	−	9Ala/6Ala

Wt = wild type; yrs = years; M = male; F = female; CNS = central nervous system; CV = cardiovascular system; skel = skeletal system; Pneum = pneumothorax; * = presence of dural ectasia, required for diagnosis of Marfan; § = diagnosis of MFS2. Data in bold indicates patients with the polymorphism.

nor translational regulatory sequences; therefore, this may be considered a "silent" and private rare polymorphism.

The frequencies of three known polymorphisms appeared to be higher in patients than in controls (Table 4).

In vitro studies on TGFβ type I receptor showed that the molecular differences between 6Ala and 9Ala are due to altered 6Ala signal sequence; this mechanism may result either from direct transcriptional inactivation or from alterations of TGFβ signalling pathways [14]. The 6Ala signal sequence could directly interact with regulatory elements of the signalling pathway genes [14].

A highly significant association between the 6Ala allele of *TGFBR1* gene and patients with Marfan syndrome is here demonstrated for the first time; moreover, homozygosity for

Table 3a
Modifier mutations in TGFBR1 gene in Marfan patients and healthy controls

EX/IVS	Position (nt)		Status	Patients (n=160)	Genotype distribution	Controls (n=237)	Genotype distribution
Ex1-5′UTR	ins 20nts (22/23)	Novel	Hetero	1	ins=0.006	0	ins=0
Ex1	del 9nts (146-154)	Known [a]	Homo	4	6Ala/6Ala=0.025	0	9Ala/6Ala=0.16 [b]
			Hetero	35	9Ala/6A=0.219	38	

[a] [13].
[b] Frequency from literature (9Ala/6A=0.14).

Table 3b
TGFBR1 6Ala genotype distribution and allele frequency in MFS patients *versus* controls

Genotype distribution	MFS patients (n=160)	Controls (n=237)	p
TGFBR1 6Ala polymorphism			
9Ala/9Ala	121 (75.6%)	199 (84.0%)	
9Ala/6Ala	35 (21.9%)	38 (16.0%)	
6Ala/6Ala	4 (2.5%)	0	0.014 [a]
TGFBR1 6Ala allele frequency	0.13	0.08	0.013 [b]

[a] Genotype distribution.
[b] Allele frequency.

Table 4
Polymorphisms in TGFBR1 gene in Marfan patients and healthy controls

EX/IVS	Position (nt)		Status	Patients (n=46)	Genotype distribution	Controls (n=92)	Genotype distribution
IVS7	+24 G>A	Novel	Hetero	16	A=0.35	26	A=0.28
IVS8	del 5nts+(90–94)	Novel	Hetero	16	del=0.35	25	del=0.27
Ex9-3′UTR	+69 A>G	Known [a]	Hetero	13	G=0.28	25	G=0.27

nt = nucleotide; ins = insertion; del = deletion.
[a] [5].

the 6Ala allele was found only in patients. For this reason, at the moment it may be defined as a low penetrance allele. A larger number of patients is required to investigate the possibility that 6Ala allele may act as modifier mutation.

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