ORIGI NAL RESEARCH

Mutations of the TGFBR2 gene in Chinese patients with Marfan-related syndrome

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Abstract

Purpose: Transforming growth factor beta receptors II gene (TGFBR2) mutations associated with Marfan syndrome and Marfan-associated disorders have been investigated. However, such studies are limited in China. To obtain more information about TGFBR2 mutations, we analyzed 6 unrelated Chinese patients with Marfan-associated disorders and without ocular manifestation.

Methods: The genomic DNA from blood leukocytes of these 6 patients and their relatives was isolated, and the entire coding region of TGFBR2 was amplified using PCR. We determined the sequence of TGFBR2 with the ABI 3100 Genetic Analyzer.

Results: Three mutations were identified in TGFBR2. Two mutations were associated with Loeys-Dietz syndrome (LDS), which were distributed as following: one missense mutation R528C (caused by a 1582C>T substitution) and one polymorphism T315M (a rare SNP). The third mutation was a novel silent mutation associated with MFS2, which was K291K caused by an 873 C>T substitution.

Conclusions: The TGFBR2 gene missense mutations are possibly causative mutations of Loeys-Dietz syndrome. This result suggests an increase in the mutation spectrum of Marfan-related disorders in China and possibly worldwide.

Marfan syndrome (MFS) (OMIM #154700) is a connective tissue disorder with autosomal dominant inheritance, characterized by a broad range of clinical manifestations involving skeletal, ocular, cardiovascular, skin and integument, pulmonary and central nervous systems with great phenotypic variability. More than 90% of MFS are caused by mutations in the fibrillin-1 gene (FBN1).1 The existence of a second locus for non-FBN1-related MFS (MFS2) was hypothesized. In 2004, patients with MFS2 were shown to have mutations in the transforming growth factor beta receptors II gene (TGFBR2).2 Recently TGFBR2 and TGFBR1 mutations were identified in a subset of patients with Loeys–Dietz syndrome (LDS), familial thoracic aortic aneurysms and dissections (TAAD2) and Shprintzen–Goldberg craniosynostosis syndrome (SGS), all of which were called TGFBR mutation-related disorders.3–5 LDS patients have cardiovascular and skeletal manifestations consistent with those seen in MFS, along with other features not present in MFS, which include hypertelorism, bifid uvula, cleft palate, and generalized arterial tortuosity with widespread vascular aneurysm and dissection.3
Recently we identified 6 Chinese patients who presented with features of MFS but without ocular manifestation. We analyzed the coding sequences of TGFBR2 in these patients.

Materials and Methods
A total of 6 unrelated individuals from Hunan province of China were suspected of having Marfan-related syndrome. These patients did not fulfill the Ghent diagnostic criteria of Marfan syndrome. The clinical presentations of the 6 probands studied were the following: two LDS, two MFS2, and two who had involvement of one or two organ systems associated with MFS. All patients have negative or unknown family histories. Blood samples were collected from the patients, 100 normal controls, and live relatives of case No.1. Controls were unrelated and lacked any features of MFS and MFS-related disorders. Genomic DNA was extracted from blood using standard protocols. This study followed the rules set by the Research Ethics Committee of the Second Xiangya Hospital of Central South University.

All exons covering the TGFBR2 coding region were amplified using PCR. Primers used for TGFBR2 (Table 1) have been reported previously (2). All primers were synthesized by the Shanghai Bioasia Company, China. The PCR conditions were as following: initial denaturation at 95°C for 5 minutes, which was followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and elongation at 72°C for 1 min, with a final elongation at 72°C for 5 min in a 20 uL reaction mixture containing 1 x buffer, 1 x Q solution, and 2.5 U Taq Polymerase (QIAGEN, Valencia, CA). PCR products were purified and sequenced with BigDye Terminator chemistry version 1.1 by standard protocol (ABI, Hilden, Germany). The analysis of the corresponding DNA fragment was performed using the ABI 3100 Genetic Analyzer. All the detected mutations have been independently sequenced 3 times. Controls were 200 chromosomes from 100 unrelated control individuals.

Results
Genetic mutations in TGFBR2 we identified in three patients of the six patients. In the other three patients with similar phenotypes, mutations in the region studied were not detected.

Case No.1 ID 645365
A 6 years old girl, with congenital heart disease including atrial septal defect (ASD) and patent ductus arteriosus (PDA), showed typical characteristics of Loeys–Dietz syndrome: hypertelorism, bifid uvula, pectus carinatum, crowding of the teeth, dolichostenomelia, arachnodactyly, aortic sinus aneurysm, aneurysm of sinus arteriae pulmonalis, aortic root aneurysm, innominate arterial tortuosity and right coronary arterial aneurysm (Fig.1). A corrective operation for

### TABLE 1. TGFBR2 gene primers

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer sequence (5’→3’)</th>
<th>Annealing Temperature ºC</th>
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<tr>
<td>1</td>
<td>F GGA ACT CCG TGC AGCT TCC</td>
<td>R CACA ATCC CGT CAG CTACG</td>
</tr>
<tr>
<td>2</td>
<td>F TGAA ATG CAT AAC ATCT TGAG</td>
<td>R GGAA AGGG AAA AT TG GAAC AGG</td>
</tr>
<tr>
<td>3</td>
<td>F CAG ATT GCCT TTT TGCT GG</td>
<td>R CCAC GGA GGA AT GTG CTCT</td>
</tr>
<tr>
<td>4a</td>
<td>F GCA TTCG AAT CCG TGA</td>
<td>R ACCT CGG AAA ACG GAC TT</td>
</tr>
<tr>
<td>4b</td>
<td>F GGA AGAT GAC CGC CGT GCA</td>
<td>R ACT GTG GAG GTG GCA ATCC</td>
</tr>
<tr>
<td>4c</td>
<td>F GGGA ACAA ATAC T GG C TGA</td>
<td>R TTC CTA GA ACC AGT TG CCT AGA</td>
</tr>
<tr>
<td>5</td>
<td>F AGGG GGC ACC ATC AGCT</td>
<td>R CCCT GGA ATAT GCT CGA AG</td>
</tr>
<tr>
<td>6</td>
<td>F AGCC AGG CAT CT ACC AT</td>
<td>R CAGGG CCA TAG AAC ACAA TG</td>
</tr>
<tr>
<td>7</td>
<td>F GAC CTC TT GGC ACT CA</td>
<td>R TCT GCT AT TCCC CAC AG CT</td>
</tr>
</tbody>
</table>
ASD and PDA was performed and histologic examination of pulmonary arterial tissue revealed pulmonary artery wall thickening and intima fibroplasia. The R528C mutation in TGFBR2 was identified in this patient. This mutation affects a highly conserved residue of the serine/threonine kinase domain of the TGFBR2 and is absent in 100 healthy controls. Live relatives of the proband were phenotypically and genetically normal. This patient was diagnosed to be

LDS based on the clinical findings and the presence of a TGFBR2 mutation.

Case No.2 ID 636579

The second proband was a 34 year old man, who had a history of hypertension. The patient presented to the hospital because of sudden onset of severe chest pain. Physical findings in this patient included a small sub-maxilla, crowding of the lower teeth, broad implant uvula, easy bruising, joint laxity but not cleft palate, hypertelorism, or craniosynostosis. Echocardiographic evaluation and Computed Tomography (CT) scan with contrast and 3D reconstruction of the major arteries showed the following: aortic root dilation with a type I dissection extending from the ascending aortic to the iliac artery, descending aortic mild tortuosity and abdominal arterial branches with severe tortuosity (Fig. 2). Emergency surgery with aortic arch replacement and stent implantation into the descending aorta was performed because of acute dissection. A polymorphism, T315M, was detected in this patient.

Case No.3 ID 633488

The third proband was a 42-year-old man (height: 180 cm, weight: 62kg) who was referred for chest pain. Physical findings include joint laxity without ocular manifestation. Echocardiography showed aortic valve regurgitation without ascending aortic dissection. CT scan demonstrated aortic dissection from the aortic arch to the bilateral common iliac arteries, and a Bentall procedure was urgently performed. A novel silent mutation in TGFBR2 exon 4, K291K, was detected.

DNA sequences of the TGFBR2 mutations from the three patients are listed in Fig.3.

Discussion

Loeys-Dietz syndrome is a newly recognized genetic condition, characterized by hypertelorism, cleft palate or bifid uvula, cardiac abnormalities, aortic root aneurysm, arterial tortuosity and aneurysms of other vessels. Mutations in the serine-threonine kinase domain of TGFBR1 or TGFBR2 are likely the cause of this syndrome. In 2006 Loeys et al. categorized the LDS to type I and type II. Patients who had typical craniofacial manifestations, including cleft palate, craniosynostosis or hypertelorism, were assigned to LDS type I. LDS type II lacks these typical manifestations, but has a presentation suggestive of vascular Ehlers–Danlos syndrome without a deficiency of type III collagen. In our study, case No. 1 had typical craniofacial and vascular features of type I. Case No. 2 belongs to LDS type II because this patient had no typical craniofacial involvement, but had easy bruising, joint laxity, arterial tortuosity, and aortic root dilation with a type I dissection. Type III collagen was evaluated and no deficiency was found. This study is the first report of LDS in the Chinese population.

The phenotype of LDS involves multiple systems. Aortic aneurysms and dissections are the common
marker of all known phenotypes. About 20 percent of patients have arterial aneurysms. Arterial tortuosity, a cardinal feature of LDS, is commonly found in the head and neck vessels, sometime even throughout the body (6). Togashi Y et al. reported a Japanese family with LDS who suffered hemifacial spasms because tortuous arteries might have been compressing the facial nerve.\(^7\) In LDS, the risk of thoracic aortic rupture and dissection exceeds that of most other MFS-related disorders caused by TGFBR mutations. The affected patients are predisposed to severe vascular consequences at a young age.\(^8,9\) Therefore, the management of vascular disease is a focus in the care of patients with LDS. If the patient is asymptomatic, treatment includes the use of beta-blockade and losartan, clinical assessment with periodic physical examination and cardiovascular imaging procedures. Losartan, an angiotensin II type 1 receptor blocker, can prevent aortic aneurysm by reducing increased TGF-beta signaling.\(^10\) For case No.1, the corrective operation for ASD and PDA was performed, and beta-blockers were prescribed for management of the aneurysm of sinus arteriae pulmonalis and the aortic root aneurysm. This patient was followed every 6 months with echocardiography. If aortic root exceeded 5.0 cm in diameter in adults and older children, or the growth rate of the aorta is high in younger children, aggressive surgical repair of arterial aneurysms may be considered because the very low intraoperative mortality rate.\(^8\) For case No. 2, replacement of the aortic arch and stent implantation into the descending aorta were performed. Both patients now remain healthy.

To date, 35 mutations associated LDS have been found in the TGFBR2 gene. All the mutations are listed in Fig. 4, including missense mutations, nonsense mutations, splice-site alterations and deletions. Mizuguchi summarized 28 TGFBR2 mutations.\(^9\) Among the 28 amino acid mutations, V258G and R356P were reported by Matyas et al. and Ki et al. respectively.\(^11,12\) Other mutations were all described by Loeys et al.\(^3,6\) Stheneur C et al. reported 5 novel mutations associated with LDS.\(^13\) E440K and T289-K291del were found in pediatric patients by Yetman AT et al.\(^14\) In our patients two amino acid mutations associated LDS were identified, which were R528C and T315M. With the exception of one splice-site mutation (IVS1-2A→G), all the mutations are located in the serine/threonine kinase domain of the TGFBR2.
R528C, a recurrent missense mutation identified in Case No. 1, has been previously described in 4 patients with LDS, but not in MFS patients. This patient showed typical characteristics of LDS. Her parents, uncles and cousins are clinically and genetically unaffected. The proband 2, possibly a LDS type II, was found to carry the mutation T315M. The germinal TGFBR2 mutation T315M was found in familial non-polyposis colorectal cancer. This mutation was observed in one out of 100 controls, with an estimated allele frequency of 0.005. Therefore it is proposed to be a rare polymorphism. This is in agreement with previous reports.

The height of case No. 3 was 180 cm, who was considered to be very tall in south China. Joint laxity, aortic valve regurgitation, aortic dissection but no ocular manifestation was found in this patient. The patient was diagnosed with MFS2 because his phenotypes did not fulfill the Ghent criteria. He carried the de novo K291K silent mutation of TGFBR2. Silent mutations of genes do not produce altered coding sequences, therefore they are not expected to change the function of the protein encoded. However Kimchi-Sarfaty C et al. reported that a synonymous SNP could alter the structure of substrate and inhibitor interaction sites, and proposed that silent mutation maybe not be really “silent”. Whether the K291K variation, which was not found in 100 normal controls and not listed in the single nucleotide polymorphism database, was a causative mutation requires more studies.

According to previous reports and our study, LDS is a distinct disease different from classic MFS, and the causal gene could be TGFBR2, TGFBR1 or key components of TGF-beta signaling pathway. Mutations in TGFBR2 occur at a higher rate than mutations in TGFBR1. We propose that for patients presenting some features of LDS, or being non-FBN1-related MFS, sequence determination of exons and flanking intronic regions of TGFBR2 and TGFBR1 should be performed.

In this study we identified genetic mutations in TGFBR2 from three patients (see Results) and failed to identify mutations in TGFBR2 from the other three patients despite the similar phenotypes. It is possible that genes interacting with TGFBR2 or acting in the same pathway as TGFBR2 are affected in these...
patients, which are beyond the scope of current study. It is also possible that genetic mutations located at a distance from the TGFBR2 loci in these patients affected the expression of TGFBR2. As we only determined the coding sequences and the 5' UTR and 3' UTR of TGFBR2, this type of mutations would not be identified in our study. Future research may identify such additional mutations.

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References


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