Abstract. It is well known that transforming growth factor β (TGFβ), which is able to stimulate multiple intracellular signaling pathways, exerts an important role in Marfan syndrome, although the effects of TGFβ on congenital ectopia lentis (CEL) have yet to be fully elucidated. In the present study, the expression levels of TGFβ and matrix metalloproteinases (MMPs) were investigated in the aqueous humor of patients with CEL who differed in terms of the severity of the disease. A total of 17 CEL patients with 21 eyes (aged 12.76±9.37 years) and 12 congenital cataract (CC) patients with 17 eyes (aged 6.82±9.18 years) were randomized in the present study. The levels of active TGFβ and MMPs in the aqueous humor were analyzed with LumineX xMAP® technology by using commercially available Bio-Plex Pro™ Human MMP and TGFβ assays. The distance from the lens edge to the pupil edge and the white to white corneal diameter (i.e. the horizontal distance between the borders of the corneal limbus) were measured, and the ratio was calculated as the degree of lens dislocation. The association between TGFβ and MMP levels and the degree of lens dislocation was analyzed using Spearman’s correlation test. Compared with the patients with CC, the level of TGFβ2 in the patients with CEL was increased significantly. Specifically, the level of TGFβ2 in the CEL patients was 855.19 pg/ml (744.33, 1,009.24), whereas it was 557.08 (438.24, 692.71) pg/ml in the CC patients (P<0.001).

In addition, it was noted that the levels of MMP-2 and -10 in the aqueous humor of the patients with CEL were higher compared with those in the CC patients, although this increase did not reach the level of statistical significance. Notably, the levels of MMP-8 and -9 in the aqueous humor of patients with CEL were significantly lower compared with those in the CC patients (P=0.014 and P=0.002, respectively). Furthermore, a marginal correlation was identified between the severity of ectopic lentis and the levels of TGFβ2 in the aqueous humor (r=0.379; P=0.003) of the patients with CEL. Taken together, these results demonstrated that a significant correlation existed between high levels of aqueous humor TGFβ2 and the severity of ectopia lentis in patients with CEL. In addition, aqueous humor TGFβ2 levels in the CEL patients were significantly higher compared with those in CC patients.

Introduction
Congenital ectopia lentis (CEL) occurs in a variety of genetic diseases, including Marfan syndrome (MFS), acromicric dysplasia, geleophysic dysplasia 2 and Weill Marchesani syndrome. Fibrillin-containing microfibrils are the major components of zonular fibers, and disruption of zonular fibers causes ectopia lentis. The fibrillin 1 (FBN1) gene, encoding fibrillin 1 protein, is generally identified as being mutated in the majority of the cases of ectopia lentis. Microfibrils in the extracellular matrix are predominantly composed of FBN1 protein, and serve an important role in incorporating elastin into elastic fibers. As a result, FBN1 mutations are associated with impairments in protein synthesis and secretion, leading subsequently to their improper incorporation into the connective tissue (1).

It has been well established that transforming growth factor β (TGFβ) is a potent stimulator of inflammation, fibrosis and the activation of certain matrix metalloproteinases (MMPs), which serve multiple essential roles, including in fibrosis, proliferation and wound repair (2,3). TGFβ1, β2 and β3 are crucial isoforms expressed in mammals that are able to bind to the same receptor to elicit their biological effects. TGFβ2, in particular, is essential in ocular physiological and pathological processes, and is predominantly localized in
limbal epithelial cells and the ciliary body (4,5). In addition, activation of the TGFβ signaling pathways may accelerate the progression of aneurysms and encourage pneumothorax (collapsed lung) in MFS. Given what is currently known about TGFβ, treatments based upon restricting TGFβ signaling are currently undergoing clinical trials (6,7).

Isogai et al (8) reported that TGFβ produced by cells acts as a large latent complex that is able to bind to extracellular matrix (ECM) proteins, such as fibrillin 1. Current models have demonstrated that blockade of TGFβ by ECM chelating ligand leads to an inhibition of its activation, thus limiting the stimulation of its cell surface receptor. On the other hand, an elevation in the levels of TGFβ may exert its detrimental effects (with respect to MFS) through two distinct intracellular signaling pathways: The canonical and non-canonical signal transduction pathways (9-11).

Regarding the canonical signaling pathway, TGFβ and its receptor binding complex are able to significantly enhance Smad2 and/or Smad3 phosphorylation, and, once phosphorylated, these proteins cause the recruitment of Smad4, its translocation to the nucleus, and the transcription of Smad-dependent genes (12). On the other hand, TGFβ activates non-canonical pathways, including the MAPK and JNK signaling pathways, p38 and RhoA cascades, the first of which includes ERK (13-15).

In mouse models, blocking JNK leads to a reduction in the activity of MMPs, which serve an important role in MFS pathology, indicating that JNK might be a therapeutic target (16). In addition, inhibiting the p38 MAPK pathway leads to a suppression in the levels of the MMPs. Furthermore, ERK1/2 blockade was revealed to elevate the levels of MMP inhibitors on cell membranes (17). TGFβ increases the expression levels of elastase and numerous MMPs (18). As an elevated level of elastase is able to promote elastin degradation, and MMPs can invoke a major collapse in the elastic fibers, overexpression of these proteases is able to reduce connective tissue elasticity, thereby contributing to instability of the aortic wall. The association between TGFβ and MMP expression has been well elucidated in the MFS mouse model (19).

High concentrations of active TGFβ are able to activate multiple intracellular signaling pathways that are involved in the progression of MFS. However, the underlying mechanisms have yet to be properly elucidated. The aim of the present study was to investigate the expression levels of TGFβ and MMPs in the aqueous humor of patients with CEL and congenital cataracts (CC), and their potential correlation with the severity of ectopic lentis.

**Patients and methods**

**Patients and samples.** An observational study was conducted on 17 patients with CEL and 12 patients with CC. It was necessary that the patients who applied to Zhongshan Ophthalmic Center, Sun Yat-sen University of Medicine between January 2015 and December 2016 had been diagnosed with either CEL or CC. Inclusion criteria included the following: i) Diagnosed with CEL or CC, respectively; ii) patient aged between 1 and 32 years; and iii) patients in the CEL group had no other ocular diseases, such as CC or other congenital anterior segment malformations, with the exception of the abnormal lens position. Exclusion criteria included the following: i) Previous intraocular surgery; ii) lens dislocation or cataract caused by head trauma, ocular trauma or surgery; and iii) the presence of inflammatory or infectious eye diseases, glaucoma, or use of topical or systemic anti-inflammatory or anti-infectious agents prior to screening. Aqueous humor samples were collected during clear lens extraction or cataract surgery. A total of 17 CEL patients with 21 eyes and 12 CC patients with 17 eyes were selected for the study, in order to deduce whether the TGFβ2 and MMP levels were related to the severity of the lens dislocation in patients with CEL.

**Ethics.** This research was performed in accordance with The Declaration of Helsinki and was approved by the Institutional Review Board at the Zhongshan Ophthalmic Center, Sun Yat-sen University of Medicine. Written informed consent was obtained from each of the 6 adult subjects, and from the legal guardians of the 23 children, in accordance with The Declaration of Helsinki. All participants provided informed consent for the publication of their data, including images and examination results.

**Analysis of the degree of lens dislocation.** The degree of lens dislocation was defined by the ratio of the distance from the lens edge to the pupil edge (a) and the length of the white to white corneal diameter (i.e. the horizontal distance between the borders of the corneal limbus) (b) (Fig. 1). This was assessed on anterior segment images from the hospital database of patients with CEL. All the pupils of the patients were pharmacologically dilated with 0.1 ml 0.5% tropicamide drops 30 min before examination. Subsequently, two ophthalmologists and one non-ophthalmic physician assessed the images.

**Surgical technique.** A single experienced surgeon (DYZ) performed the CEL or CC operations in all cases. Topical 0.3% ofloxacin eye drop was applied four times each day at 0.1 ml each time, for 3 days prior to surgery. According to standard procedures, none of the patients was in receipt of steroids prior to surgery.
**Aqueous humor sampling.** In the CEL and CC groups, the primary incision was made under sterile conditions, and undiluted aqueous humor samples (0.1-0.2 ml) were collected into a 30-gauge needle on a tuberculin microsyringe. The samples were immediately frozen, and stored at -80˚C until measurements were made. All samples were collected during the year 2016, and stored for 2-3 months under identical conditions.

**Multiplex analysis of cytokines in the aqueous humor.** All samples were assessed for the total protein levels of TGFβ1, TGFβ2, TGFβ3 and MMP-1, -2, -3, -7, -8, -9, -10, -12 and -13 using the Luminex system (Luminex xMAP® technology; Bio-Rad Laboratories, Inc.) and commercially available Bio-Plex Pro™ Human MMP (catalog number: 171aM001M, Bio-rad laboratories, inc.) assays. Each sample present was quantified from the standard curve of each measurement. The same aqueous humor sample was measured in each plate as an internal control.

**Statistical analysis.** Every experiment was repeated twice. Statistical analysis was performed using SPSS 13.0 software for Windows (SPSS, Inc.). Some of the data values, which were not normally distributed, were analyzed using the Kolmogorov-Smirnov test, and the results are shown as the median and range (25th and 75th percentiles) for continuous variables under these conditions, including the expression levels of TGFβ/MMPs. The mean ± SD was evaluated for normally distributed continuous variables, such as the age of the patients. A nonparametric test (Mann-Whitney U test) was used to compare abnormally distributed values of TGFβ/MMPs among the different groups between males and females. The proportions (percentages) of sex were analyzed using Pearson's χ² test. The Spearman correlation coefficient method was used to analyze the correlation between TGFβ/MMPs and the degree of lens dislocation, or between TGFβ/MMPs and the age of the patients. P<0.05 was considered to indicate a statistically significant difference.

**Table I. Demographic characteristics of 29 patients (comprising 38 eyes in total) in the CEL and CC groups.**

| Characteristic                                      | CEL group | CC group |
|-----------------------------------------------------|-----------|----------|
| Total no. of subjects (no. of eyes)                 | 17 (21)   | 12 (17)  |
| Age, years; mean ± SD (range)                       | 12.76±9.37 (5-32) | 6.82±9.18 (1-32) |
| Sex, M/F                                            | 11/6      | 6/6      |
| Tricuspid prolapse, n                               | 1         | -        |
| Tricuspid incompetence                              | 4         | -        |
| Aorto-sinus dilatation                              | 6         | -        |
| Ascending aorta extension                           | 1         | -        |
| Patent oval foramen                                 | 1         | -        |
| Ventriculus sinister diastolic function decrease     | 1         | -        |
| Without heart disease                               | 3         | -        |

CEL, congenital cataract; CC, congenital cataract; M, male; F, female.

**Results**

**Demographic characteristics of 29 patients (38 eyes in total).** Aqueous humor samples were obtained from 38 eyes from 29 subjects. A total of 17 CEL patients with 21 eyes (aged 12.76±9.37 years) and 12 CC patients with 17 eyes (aged 6.82±9.18 years) were randomized in the present study (Table I). No statistically significant differences in either sex distribution or age were observed between these two groups. Furthermore, the 17 CEL patients were analyzed via standard echocardiography, and the presence of tricuspid incompetence and aorto-sinus dilatation was identified in 23% (4/17) and 35% (6/17) of the enrolled subjects, respectively; tricuspid prolapse, ascending aorta extension, patent oval foramen and ventriculus sinister diastolic function decrease were identified in 5% (1/17) respectively. Only for 3 of the patients were no apparent abnormalities identified in terms of cardiac architecture and function. These patients in the CEL group had no other ocular diseases, such as CC or other congenital anterior segment malformations, except for the abnormal lens position.

**Levels of TGFβ1,-2 and -3.** The difference between the TGFβ2 levels in the CEL and CC groups was identified to be statistically significant (P<0.001). The level of TGFβ2 in the CEL patients was 855.19 (744.33, 1,009.24) pg/ml, whereas it was 557.08 (438.24, 692.71) pg/ml in the CC patients (P<0.001). However, the differences in the levels of TGFβ1 and TGFβ3 in the two groups were not revealed to be statistically significant (Table II and Fig. 2).

**Levels of MMP1,-2,-3,-7,-9,-10,-12 and -13.** MMP-1 could not be detected in the majority of the aqueous humor samples in the two groups. The differences identified for MMP-2, -3, -7, -10, -12 and -13 in the two groups did not reach the level of statistical significance (Table II and Fig. 3). The Mann-Whitney U test demonstrated that the levels of MMP-2 and MMP-10 in the aqueous humor from the patients with CEL were higher compared with those from the patients with CC, although, again, this did not reach the level of statistical significance (P=0.325 and P=0.386, respectively). However, the levels of
Table II. Aqueous humor TGFβ/MMP levels in patients with CEL and CC.

| TGFβ/MMP, pg/ml | CEL group | Degree of lens dislocation | CC group | P-value |
|-----------------|-----------|-----------------------------|----------|---------|
|                 | All       | <0.4                        | >0.4     |         |
| TGFβ1, median (P25, P75) | 71.15 (68.62, 73.68) | 71.15 (68.87, 73.29) | 70.39 (68.62, 71.78) | 69.64 (67.36, 71.15) | 0.475 |
| TGFβ2, median (P25, P75) | 855.19 (744.33, 1,009.24) | 749.94 (730.39, 796.44) | 954.09 (883.86, 1,121.05) | 557.08 (438.2, 692.71) | <0.001a |
| TGFβ3, median (P25, P75) | 69.57 (69.07, 69.98) | 69.67 (69.07, 69.92) | 69.57 (69.21, 69.98) | 69.07 (68.79, 69.57) | 0.49 |
| MMP-2, median (P25, P75) | 552,803.83 | 561,063.74 | 541652.94 | 535,458.01 | 0.325 |
| (474,334.63, 676,702.56) | (549,706.36, 783,874.96) | (476399.61, 736999.94) | (394,6245, 652,955.31) | |
| MMP-3, median (P25, P75) | 24,874.10 (12,956.62, 36,791.58) | 24,874.09 (12,956.62, 36,791.57) | 28,449.34 (14,820.54, 39,770.94) | 24,874.1 (12,956.62, 36,791.58) | 0.552 |
| MMP-7, median (P25, P75) | 5,014.63 (4,122.69, 6,613.84) | 4,627.83 (3,731.67, 5,526.60) | 5,832.34 (4,628.28, 8,418.63) | 5,654.43 (3,953.61, 7,215.48) | 0.977 |
| MMP-8, median (P25, P75) | 13,207.60 (8,327.02, 19,019.98) | 16,005.08 (8,437.79, 19,547.61) | 11,872.91 (9,209.54, 15,019.31) | 18,608.34 (13,065.12, 30,151.73) | 0.014a |
| MMP-9, median (P25, P75) | 4,418.19 (4,018.74, 5,350.28) | 4,862.04 (4,174.08, 5,283.69) | 4,196.27 (4,085.31, 4,817.65) | 5,971.66 (4,773.27, 12,984.45) | 0.002a |
| MMP-10, median (P25, P75) | 36,248.38 (29,234.96, 47,501.42) | 38,152.74 (32,483.39, 47,485.99) | 33,956.23 (29,234.96, 44,845.86) | 27,846.82 (21,557.86, 50,116.39) | 0.386 |
| MMP-12, median (P25, P75) | 3,962.81 (2,975.89, 4,490.70) | 3,733.29 (3,159.50, 4,214.84) | 3,962.81 (3,320.17, 4,490.70) | 4,238.23 (3,733.29, 5,110.39) | 0.221 |
| MMP-13, median (P25, P75) | 2,682.62 (2,243.78, 3,034.13) | 2,682.62 (2,316.88, 3,004.82) | 2,594.83 (2,375.38, 2,814.41) | 2,624.07 (2,448.49, 2,887.65) | 0.929 |

TGFβ/MMPs protein levels in the aqueous humor from 29 patients (38 eyes) with CEL and CC were measured with Luminex xMAP Technology by using Milliplex xMAP kits. The Mann-Whitney U test was used to compare abnormally distributed values of TGFβ/MMPs among CEL and CC groups. *P<0.05. P25, 25th percentile; P75, 75th percentile; TGFβ, transforming growth factor β; MMP, matrix metalloproteinase; CEL, congenital ectopia lentis; CC, congenital cataract.
MMP-8 and MMP-9 in the aqueous humor of patients with CEL were significantly lower compared with those in the CC patients (P=0.014 and P=0.002; Table II and Fig. 3).

Relationship between TGFβ/MMPs and the degree of lens dislocation in the CEL group. The Spearman correlation test revealed that there was a significant positive correlation between the TGFβ2 level and the degree of lens dislocation (r²=0.379, P=0.003; Fig. 4). By contrast, no significant correlation was identified between the degree of lens dislocation and the levels of TGFβ1 and TGFβ3, and MMP-2, -3, -7, -8, -9, -10, -12 and -13 (Table III; TGFβ1, P=0.263; TGFβ3, P=0.571; MMP-2, P=0.238; MMP-3, P=0.98; MMP-7, P=0.989; MMP-8, P=0.552; MMP-9, P=0.638; MMP-10, P=0.726; MMP-12, P=0.812; and MMP-13, P=0.853).

Relationship between TGFβ/MMPs and age in the CEL and CC groups. The Spearman correlation test revealed that TGFβ2 was positively correlated with age (r²=0.298, P<0.001; Fig. 5).

However, the correlation identified between age and the levels of TGFβ1 and β3, and MMP-2, -3, -7, -8, -9, -10, -12 and -13 did not reach the level of statistical significance (Table III; TGFβ1, P=0.592; TGFβ3, P=0.547; MMP-2, P=0.56; MMP-3, P=0.597; MMP-7, P=0.736; MMP-8, P=0.079; MMP-9, P=0.178; MMP-10, P=0.453; MMP-12, P=0.232; and MMP-13, P=0.085).

Discussion

To the best of the authors' knowledge, the present study is the first to confirm that the expression levels of TGFβ2 in the aqueous humor of patients with CEL is associated with the severity of ectopia lentis, suggesting the important role of TGFβ and MMPs in the pathological process of CEL.

MFS is characterized as a hereditary disorder with various symptoms, including skeletal, cardiovascular and ocular systems (20). CEL is one of the most commonly identified ocular disorders identified in people with MFS. It is well-recognized that reduced or mutant forms of fibrillin 1, which induces increased levels of TGFβ activation, is the precise factor that results in the different clinical features (13). Furthermore, the abnormal homeostasis resulting from overexpression of MMP-2 and MMP-9 leads to a slow deterioration of both the elastin fibers and other components of the ECM (21). The aforementioned reports established that TGFβ signaling is able to accelerate the progression of aneurysms and pneumothorax in patients diagnosed with MFS. Therefore, the present hypothesis was that TGFβ signaling may also drive ectopia lentis.

To validate this hypothesis, multiplex bead immunoassays were performed to confirm the levels of TGFβ and MMPs in...
It has been previously shown that TGFβ is secreted as a large latent complex that consists of TGFβ, its binding protein and latency-associated peptide (22). Fibrillin 1, which is characterized as an isomer of latent TGFβ binding proteins, is able to specifically bind to the TGFβ latent complex. TGFβ signaling is initiated by binding of TGFβ to its receptors, activating its downstream signaling cascades by releasing the mature TGFβ that is regulated by proteases or MMPs (23).

Previously, researchers have focused on the canonical and non-canonical TGFβ signaling pathways, due to the important roles they serve in aneurysms (16). On the basis of their results, the present hypothesis was that a deficiency of fibrillin 1 in the patients with cel may inhibit matrix sequestration of the large latent complex of TGFβ, subsequently rendering this cytokine more prone to activation. Furthermore, TGFβ signaling may be involved in the regulation of MMPs, causing cel.

TGFβ, transforming growth factor β; MMP, matrix metalloproteinase; cel, congenital ectopia lentis; cc, congenital cataract.

Table III. Association between the levels of TGFβ/MMPs and degree of lens dislocation variable in CEL group and also association between the levels of TGFβ/MMPs and age in CEL and CC groups.

| Degree of lens dislocation variable | Age |
|-----------------------------------|-----|
| TGFβ1                             | R   | P-value |
| TGFβ2                             | 0.014 | 0.263 |
| TGFβ3                             | 0.379 | 0.003 |
| MMP2                              | 0.086 | 0.571 |
| MMP3                              | 0.000 | 0.98 |
| MMP7                              | 0.000 | 0.989 |
| MMP8                              | 0.019 | 0.552 |
| MMP9                              | 0.012 | 0.638 |
| MMP10                             | 0.007 | 0.726 |
| MMP11                             | 0.002 | 0.812 |
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TGFβ, transforming growth factor β; MMP, matrix metalloproteinase; cel, congenital ectopia lentis; cc, congenital cataract.
MMPs and their tissue inhibitors are all expressed in the aqueous humor with or without age-associated cataracts (25). Upon a review of the relevant literature, no studies reporting that MMPs in the aqueous humor are relevant to CC were identified. Some findings have indicated that lens cells may synthesize MMPs upon certain injuries, such as cataract surgery or oxidative stress (26-28).

It has been documented that TGFβ drives anterior subcapsular cataract formation due to the increasing levels of MMP-2 and MMP-9, thereby promoting the development of posterior capsule opacification (29-33). Researchers have previously observed that the level of MMP-9 activity varies in different types of cataracts (34). It has been demonstrated that the level of MMP-9 activity in lens epithelial cells increases with the age of patients with age-associated cataract (34). However, MMP proteins are not only secreted by ciliary non-pigment epithelial cells in the aqueous humor (35). It has been reported that scleral fibroblasts (36), uveal melanocytes (37), ciliary muscle cells (38-40), trabecular cells (41,42) and retinal pigment epithelial cells (43-45) are able to produce MMPs and secrete or exchange them to the aqueous humor. The changes in the MMP expression levels may result from the homeostatic mechanism of various cells in the aqueous humor. Therefore, the levels of MMPs in the aqueous humor of CC patients may or may not serve a key role in the disintegration of zonular fibers, and further studies are required to explore the role of MMPs in ectopia lentis.

A limitation of the present study was that these samples were not compared with age-matched control healthy persons, due to ethical reasons. On the other hand, active inflammatory factors in the aqueous humor in the eyes of cadavers are degraded slowly, because the protein in the aqueous humor of cadavers is easy to dissolve and inactivate. Due to the age factor, CC patients were included to provide a study contrast. Besides, the only accurate information about congenital ectopia lentis available was a general diagnosis, with no genetic information due to economic or technical reasons. Therefore, it was not possible to make the diagnosis of MFS. The small number of patients enrolled also limited the results, and further studies are required that include a larger number of subjects.

In conclusion, the findings in the present study indicated that TGFβ signaling activation may promote the progression of CEL. These findings may support targeting TGFβ signaling as a potential therapy to prevent the complications in patients with MFS that arise from CEL.

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**Availability of data and materials**

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

**Authors’ contributions**

QC, BX, GJ and DZ conceived and designed the experiments. BX performed the experiments. BZ, MC and KW analyzed the data. JI-L, YW, Ju-L and YZ contributed reagents/materials/analysis tools, and helped perform the experiments and acquired data. QC, BX, GJ and CAY wrote the paper. CAY analyzed and interpreted the patient data regarding the standard echocardiography and was a major contributor in writing the manuscript.

**Ethics approval and consent to participate**

This research was performed in accordance with The Declaration of Helsinki and was approved by the Institutional Review Board at the Zhongshan Ophthalmic Center, Sun Yat-sen University of Medicine. Written informed consent was obtained from each of the 6 adult subjects, and from the legal guardians of the 23 children, in accordance with The Declaration of Helsinki.

**Patient consent for publication**

All participants provided informed consent for the publication of their data, including images and examination results.

**Competing interests**

The authors declare that they have no competing interests.

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