Supplementary files

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Table S1 Primers and probe sequences used in qPCR and sanger sequencing

Assay type	Locus	Oligo name	Sequence (5′ to 3′)	Product length (bp)
qPCR	c.370	370-qF1	TTACGAGACCCTGGGAGTC	89
		370-qR1	CGGGCCCCTCCATCTC	
		370C-qP1	VIC-TACACGGACCGCACG-MGB	
		370T-qP2	FAM-TACACGGACTGCACG-MGB	
	c.371	371-qF1	TTACGAGACCCTGGGAGTC	89
		371-qR1	CGGGCCCCTCCATCTC	
		371G-qP1	VIC-TACACGGACCGCACG-MGB	
		371A-qP2	FAM-CACGGACCACACG-MGB	
		371T-qP3	ROX-CACGGACCTCACGG-MGB	
	c.1663	1663-qF1	ACACAGTCTTTGCTCCCACAAA	96
		1663-qR1	GAGACGTGTACTTAAGTTGGTCTTTACC	
		1663C-qP1	VIC-CCAAGAGAACGGAGC-MGB	
		1663T-qP2	FAM-CAAGAGAATGGAGC-MGB	
	c.1664	1664-qF1	ACACAGTCTTTGCTCCCACAAA	96
		1664-qR1	GAGACGTGTACTTAAGTTGGTCTTTACC	
		1664G-qP1	VIC-CAAGAGAACGGAGCA-MGB	
		1664A-qP2	FAM-CAAGAGAACAGAGCA-MGB	
Sanger sequencing	c.370/c.371	370/371-SF1	CCTCGTCCTCTCCACCTGTA	325
		370/371-SR1	GGGAAGTAAGGCAGTTCCCC	
	c.1663/c.1664	1663/1664-SF1	TTTGTGCATACGGAGGGCAT	611
		1663/1664-SR1	ATGGTTCTGACAGTGGCTGG	

Table S2 Concordance analysis between qPCR and sanger sequencing

		Sanger sequencing	
		Positive	**Negative**
qPCR	**Positive**	38	0
	Negative	0	120

Table S3 Validation of the lower limit of detection (LLOD)

DNA concentration (ng/μL)	**Positive number/total number**				
	370CT genotype	**371GA genotype**	**371GT genotype**	**1663CT genotype**	**1664GA genotype**
0.4	20/20	20/20	20/20	20/20	20/20
0.2	20/20	20/20	19/20	20/20	20/20
0.1	18/20	17/20	15/20	20/20	19/20

Table S4 Linear regression analysis parameters for each locus

Locus	**Slope**	**Efficiency (%)**	**R^2 Value**	**LoQ**
370C	−3.413	96	0.9995	
370T	−3.370	98	0.9977	
371G	−3.224	104	0.9996	
371A	−3.194	106	0.9986	
371T	−3.235	104	0.9997	0.2 ng/μL*
1663C	−3.234	100	0.9992	
1663T	−3.159	107	0.9986	
1664G	−3.212	105	0.9997	
1664A	−3.165	107	0.9988	

* The LoQ was determined to be 0.2 ng/μL of genomic DNA, corresponding to 300 copies/reaction.

Table S5 Specificity for qPCR assay in samples harboring *TGFBI* mutations

Gene	Mutation	Detected genotype			
		370C>T	**371G>A/T**	**1663C>T**	**1664G>A**
TGFBI (NM_000358.3)	c.346A>G	CC	GG	CC	GG
	c. 367G>C	CC	GG	CC	GG
	c. 370C>T	CT	GG	CC	GG
	c. 371G>A	CC	GA	CC	GG
	c. 371G>T	CC	GT	CC	GG
	c. 374C>T	CC	GG	CC	GG
	c. 1652C>A	CC	GG	CC	GG
	c. 1663C>T	CC	GG	CT	GG
	c. 1664G>A	CC	GG	CC	GA
	c. 1673T>C	CC	GG	CC	GG

Table S6 Specificity for the qPCR assay in samples with other ocular disease-related genes

Gene	Mutation	Detected genotype			
		370C>T	371G>A/T	1663C>T	1664G>A
NMNAT1 (NM_001297778.1)	c. 500A>G	CC	GG	CC	GG
EYS (NM_001142800.2)	c. 8107G>T	CC	GG	CC	GG
CRX (NM_000554.6)	c. 238G>A	CC	GG	CC	GG
RHO (NM_000539.3)	c. 792_794del	CC	GG	CC	GG
MERTK (NM_006343.3)	c. 296_297del	CC	GG	CC	GG
MT-ND4 (GRCh38.p14)	m. 11778G>A	CC	GG	CC	GG
USH2A (NM_007123.6)	c. 8559-2A>G	CC	GG	CC	GG
RPGR (NM_001034853.2)	c. 3178_3179del	CC	GG	CC	GG

Table S7 Distribution of *TGFBI* hotspot mutations in positive samples from preoperative refractive surgery screening

Mutation type	Sample count	Disease subtype
c. 370C>T heterozygous	1	Lattice corneal dystrophy type I
c. 371G>A heterozygous	1	Avellino corneal dystrophy
c. 371G>A homozygous	1	Avellino corneal dystrophy
c. 371G>T heterozygous	2	Reis-Bücklers corneal dystrophy
c. 1663C>T heterozygous	1	Granular corneal dystrophy type I
c. 1664G>A heterozygous	0	Thiel-Behnke corneal dystrophy
Total	6	/

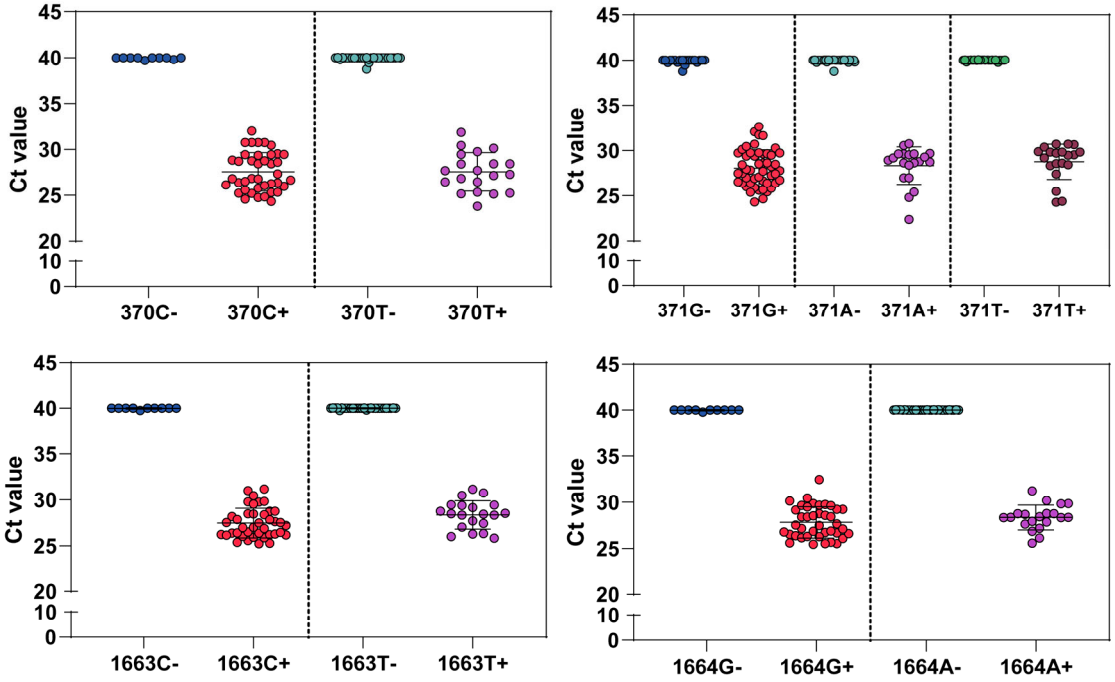

Fig. S1 Determination of the diagnostic Ct cutoff value for the qPCR assay. Distribution of Ct values from genomic DNA from 30 wild-type (WT), 10 heterozygous (HET), and 10-point serial dilutions of homozygous (HOMO) samples. These data were used to generate ROC curves to establish the diagnostic threshold.

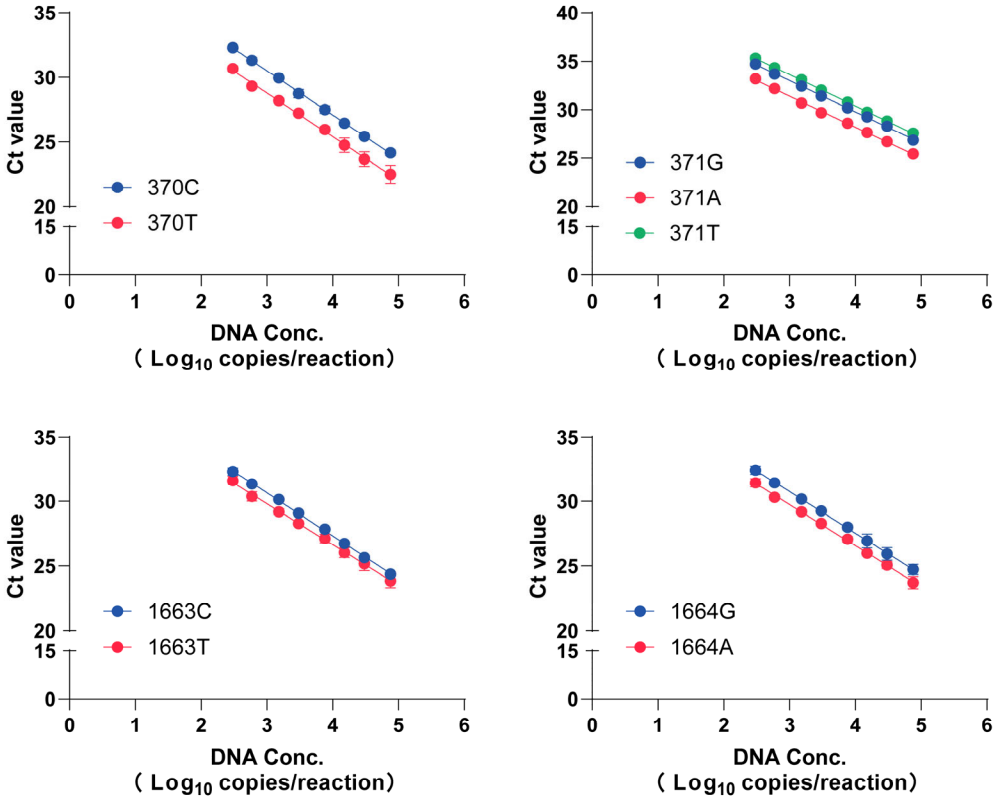

Fig. S2 The limit of quantification (LoQ) was determined. A linear regression analysis was performed with the logarithm (base 10) of DNA concentration on the x-axis and Ct values on the y-axis. Conc.: concentration.