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Integrative analytical and statistical framework for optimization of multiplex qPCR detection of *TGFBI* mutations in refractive surgery candidates

Key words: Multiplex real-time quantitative polymerase chain reaction (qPCR); Transforming growth factor-β-induced (*TGFBI*) mutation; Preoperative genetic screening; Probit regression analysis; Refractive surgery

Graphical Abstract: Multiplex qPCR assay

This study developed a statistically optimized multiplex qPCR assay for *TGFBI* hotspot mutations, enabling rapid, high-throughput preoperative screening of 10,055 refractive surgery candidates with analytical thresholds defined by ROC analysis and limit-of-detection determined by Probit modeling, ensuring reliable detection within a unified Ct=36 cutoff.

Methodology: Targeted Screening

Targets 5 high-frequency *TGFBI* hotspot mutations (R124C/L/H, R555W/Q) for efficient preoperative screening

Validation: High Accuracy

100% concordance (κ=1.0) with Sanger sequencing; High precision (CV=0.34-1.21%) and robustness

Application: Large-Scale Cohort

Screened 10,055 refractive-surgery candidates; Identified 6 carriers (0.06%), preventing high-risk surgeries

Conclusion: Clinical Ready

A rapid, low-cost, and high-throughput solution for standardized genetic risk assessment

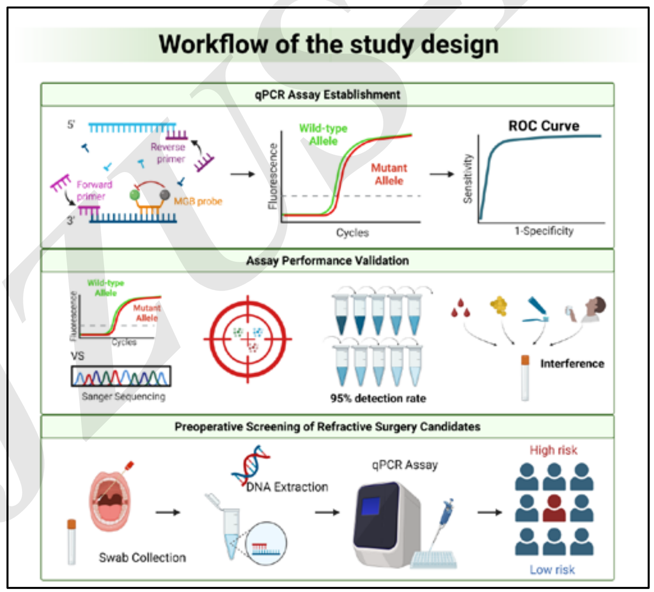

Fig. 1 Schematic overview of the workflow for multiplex allele-specific qPCR screening of *TGFBI* - hotspot mutations, including buccal swab collection, genomic DNA extraction, qPCR targeted amplification, and result interpretation.

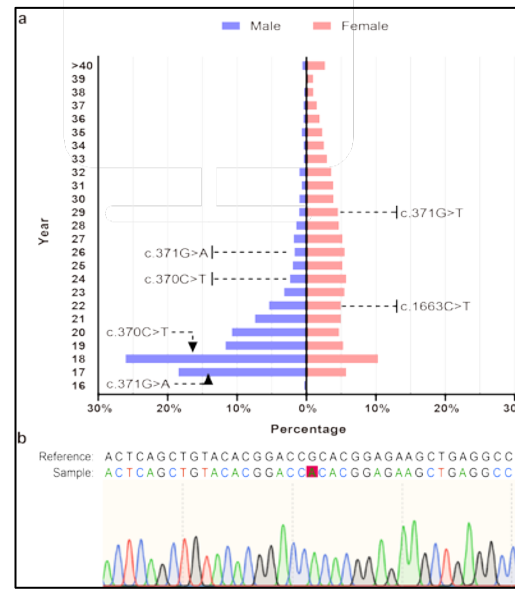

Fig. 4. Application of the validated multiplex qPCR assay in a large preoperative screening cohort of 10,055 refractive surgery candidates.

Research Summary: *TGFBI* preoperative genetic screening

Background & Clinical Need

- Risk: Surgery may unmask/accelerate *TGFBI* dystrophies
- Need: Rapid, standardized genetic screening is essential

Methodology

- Assay: Multiplex qPCR panel targeting 5 *TGFBI* hotspots
- Optimization: ROC & Probit analysis framework

Key Performance

- Accuracy: κ=1.0 (Perfect agreement with Sanger)
- Impact: Identified 6 carriers (0.06%) in 10k+ candidates

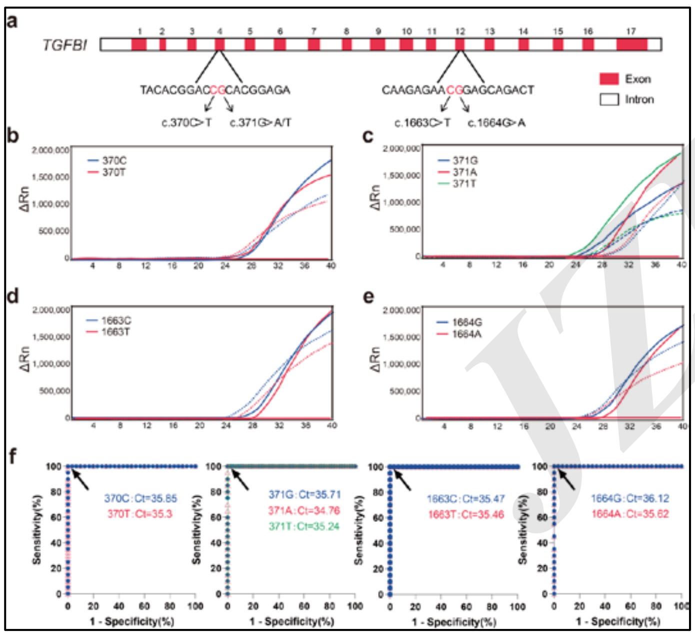

Fig. 2 Allele-specific qPCR performance for the four *TGFBI* loci, showing distinct amplification patterns for wild-type and mutant alleles and ROC-derived Ct thresholds supporting accurate discrimination across all targeted hotspot mutations.

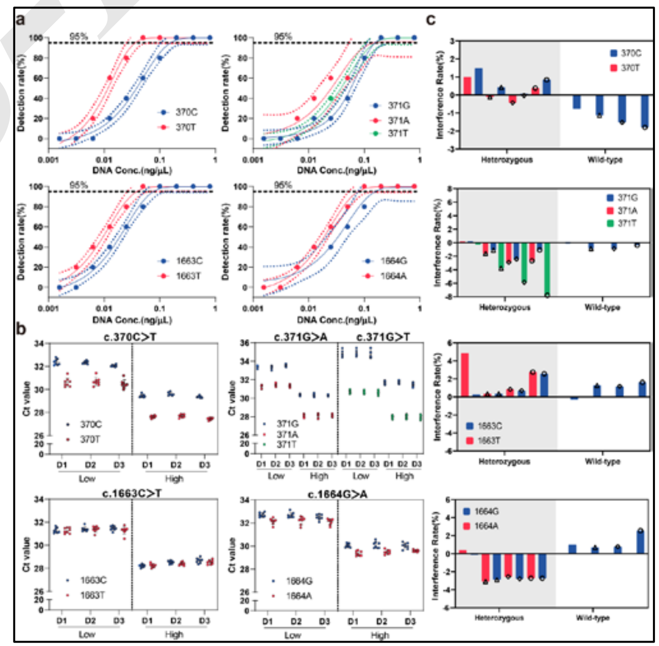

Fig. 3. Analytical sensitivity, precision, and robustness of the multiplex allele-specific qPCR assay across all *TGFBI* hotspot loci.

Innovation Points: Key advances in assay development

Statistical-Optimization

- **ROC-guided Cutoffs:** Unified threshold (Ct=36) for simple deployment.
- **Probit-defined LoD:** Robust analytical sensitivity.
- **Transparent:** Clear path to standardize screening.

Analytical Validation

- **Rigorous Testing:** Verified precision, specificity & inhibitor robustness.
- **Linear Calibration:** Stable Ct-log(copy) relation for QC.
- **Reliability:** Consistent across operators & days.

Clinical Implementation

- **High-Throughput:** Proven on 10,055 refractive candidates.
- **Cost-Effective:** First-tier assessment tool.
- **Actionable:** Informs standardized post-screening workflow.

Core Insight: Empowering Preoperative Screen  **en Precision**