

Prevalence of granular corneal dystrophy type 2-related *TGFBI* p.R124H variant in a South Korean population

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Purpose: Granular corneal dystrophy type 2 (GCD2) is an autosomal dominant disorder and is associated with the arginine to histidine substitution at codon 124 (p.R124H) of the *TGFBI* gene. Although *TGFBI* p.R124H is known to be the most common corneal dystrophy-related pathogenic variant, there are few data on the frequency of this variant in the South Korean population.

Methods: In total, 2,060 anonymous DNA samples from a public umbilical cord blood bank were tested for the *TGFBI* p.R124H variant using real-time PCR.

Results: Six of the 2,060 samples [0.29%; 95% confidence interval (CI), 0.12–0.67%] were heterozygous for the *TGFBI* p.R124H variant. The prevalence of the GCD2-related *TGFBI* p.R124H variant in this population was estimated to be 291.3 per 100,000 [95% confidence interval (CI), 118.5–667.0].

Conclusions: To our knowledge, this is the largest study that has estimated the prevalence of the GCD2-related *TGFBI* p.R124H variant in South Korea.

Corneal dystrophy is defined as a rare hereditary noninflammatory disorder in which substances accumulate abnormally in the cornea and is usually slowly progressive, symmetric, and not related to environmental or systemic factors [1]. Granular corneal dystrophy, type 2 (GCD2), is an autosomal dominant disorder that has clinical and histologic features of granular and lattice dystrophies and has granular and amyloid deposits [2]. GCD2 was first described in individuals from Avellino, Italy, in 1988 [2]. According to the International Classification of Corneal Dystrophies (IC3D) classification of corneal dystrophies, GCD2 along with granular corneal dystrophy, type 1 (GCD1) and lattice corneal dystrophy, type 1 (LCD1) are epithelial–stromal *TGFBI* (Gene ID: 7045; OMIM 601692) dystrophies [1].

TGFBI encodes a protein called TGFBI, which is involved in cell adhesion, migration, proliferation, and differentiation [3]. To date, 69 different *TGFBI* pathogenic or likely pathogenic variants that cause epithelial–stromal *TGFBI* dystrophies have been described. The most common pathogenic variants are located at two major codons (Arg124 or Arg555) in fasciclin 1 domains of TGFBI (Figure 1) [4]. GCD2 is associated with the arginine to histidine substitution at codon 124 (p.R124H) of the *TGFBI* gene [5].

Patients with GCD2 have different ages of onset and progression rates depending on whether the p.R124H variant is heterozygous or homozygous. In patients with the homozygous p.R124H variant, corneal deposits appear as early as 3 years of age, and visual loss begins in childhood; whereas patients with the heterozygous p.R124H variant are diagnosed as teenagers or young adults and progress slowly [6,7].

Laser in situ keratomileusis (LASIK) is a popular laser refractive surgery for the treatment of refractive errors worldwide, along with laser epithelial keratomileusis (LASEK) and photorefractive keratectomy (PRK) [8]. However, cases of exacerbated GCD2 after LASIK, LASEK, and PRK have been reported [9–12]. The reason for the worsening after laser refractive surgery in patients with GCD2 is that corneal trauma activates and overproduces TGFBI, the main component of corneal opacity [13,14]. For these reasons, these laser refractive surgeries were contraindicated in patients with GCD2 [1]. Among patients with the heterozygous *TGFBI* p.R124H variant who underwent LASIK surgery, 8.9% did not have corneal deposits before LASIK surgery [15]. Therefore, it is difficult to diagnose GCD2, which is a contraindication to laser refractive surgery only with clinical symptoms at the time of refractive surgery. It is important to know the exact prevalence rate of GCD2 and exclude these high-risk patients before refractive surgery.

Among South Korean patients with corneal dystrophy, the distribution of GCD2 has been reported in several studies;

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thus, the frequency of GCD2 among patients with CD is well-known [16,17]. There is only one study predicting the frequency of GCD2 in the South Korean population, but this study predicted the frequency of GCD2 using patients with the homozygous p.R124H variant based on the Hardy–Weinberg principle [18]. In this study, we estimated the prevalence of the GCD2-related *TGFBI* p.R124H variant using more than 2,000 umbilical cord blood samples in a South Korean population.

METHODS

Subjects: A total of 2,060 anonymous DNA samples were included in this study. DNA samples were obtained from a public umbilical cord blood bank (Seoul Metropolitan Public Cord Blood Bank, Korea). All samples were collected from unrelated South Koreans who provided written informed consent for donation and research, and the study protocol was reviewed and approved as a review exemption by the Institutional Review Boards of Seoul National University Boramae Hospital (07–2014–3). This study complied with the tenets of the Declaration of Helsinki and the Association for Research in Vision and Ophthalmology (ARVO) statement on human subjects. Genomic DNA was extracted from cord blood using a Puregene DNA purification kit (Gentra Systems, Minneapolis, MN) or QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions and stored at -70 °C until the experiment

***TGFBI* p.R124H analysis and statistical analysis:** DNA was extracted using standard protocols. To detect the p.R124H *TGFBI* variant, real-time PCR was performed using the 7500 Real-Time PCR system (Applied Biosystems Inc., Foster City, CA) using the GENEDIA® Avellino corneal dystrophy mutation Detection Kit (GENEDIA ACD kit; Green Cross Medical Science Co., Yongin, South Korea) according to the manufacturer's instructions. The amplification protocol for this reaction consisted of an initial denaturation at 95 °C for 15 min, followed by 45 cycles of 20 s at 95 °C and 40 s at 68 °C. Positive and negative control samples were tested in

each batch for quality control, and the positive results were confirmed using Sanger sequencing (primer sequences available upon request). The statistical analysis was performed using *VassarStats*, and 95% confidence intervals (CIs) were calculated for each value.

RESULTS

Six of the 2,060 samples [0.29%; 95% confidence interval (CI), 0.12–0.67%] were heterozygous for the *TGFBI* p.R124H variant, which was confirmed with Sanger sequencing. The estimated allele frequency of the *TGFBI* p.R124H variant was 0.15% (95% CI, 0.07–0.32%), and the prevalence of the GCD2-related *TGFBI* p.R124H variant was estimated to be 291.3 per 100,000 [95% confidence interval (CI), 118.5–667.0] in this population.

DISCUSSION

The exact prevalence of GCD2 remains unclear. There have been few studies on the prevalence of GCD2, especially prevalence studies of the genetically identified GCD2-related *TGFBI* p.R124H variant (Table 1) [18,19]. In this study, the prevalence of the *TGFBI* p.R124H variant was 291.3 per 100,000, which was two to three times higher than the previous South Korean study [18]. The previous prevalence study of the GCD2-related *TGFBI* p.R124H variant was performed in patients with the homozygous p.R124H variant based on the Hardy–Weinberg principle, whereas this study predicted prevalence using umbilical cord blood samples. This difference is thought to be a difference in the method for calculating the prevalence, and we believe that this study predicted a more accurate prevalence of the GCD2-related *TGFBI* p.R124H variant in South Koreans than the previous study. The prevalence in this study was similar to or higher than the results from a GCD2 prevalence study in a refractive surgical candidate population in China (241.8 per 100,000; 95% CI, 89.00–598.3 per 100,000) [19]. Although no homozygous p.R124H variants were identified in this study, the predicted prevalence of the homozygous p.R124H variant

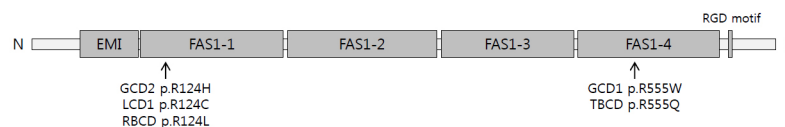


Figure 1. Schematic diagram of the *TGFBI* protein and the five most common pathogenic variants in the *TGFBI* gene. The *TGFBI* gene encodes the 64 kDa *TGFBI* protein composed of four fasciclin 1 (FAS1) domains, an N-terminal cysteine-rich (EMI) domain, and an RGD (Arg-Gly-Asp) motif. The most common pathogenic variants are in the two major codons (Arg124 or Arg555) in the FAS1 domains of *TGFBI*. C, C-terminus; GCD1, granular corneal dystrophy, type 1; GCD2, granular corneal dystrophy, type 2; LCD1, lattice corneal dystrophy, type 1; N, N-terminus; RBCD, Reis-Buckler corneal dystrophy; TBCD, Thiel-Behnke corneal dystrophy.

calculated in this study using the Hardy–Weinberg equation was 0.2 per 100,000 in South Koreans.

According to data from the [Korean Statistical Information Service](#) (accessed on 9 June 2020), in 2018, the total population of South Korea was 51.6 million with 326,882 births. Based on this population size, the number of patients with GCD2 who are heterozygous for the *TGFBI* p.R124H variant could be estimated to be approximately 150,000 in total and carried by 952 newborns per year. The number of estimated patients with GCD2 with the homozygous *TGFBI* p.R124H variant would be approximately 116 based on the Hardy–Weinberg equilibrium in the total population of South Korea. However, exact penetrance of the *TGFBI* p.R124H variant is not known, and reduced penetrance could not be excluded. Some unaffected cases that were heterozygous for the *TGFBI* p.R124H variant until the age of 55–56 years have been reported [20,21]. Therefore, there is a possibility that some individuals who are heterozygous for the *TGFBI* p.R124H variant will not develop GCD2; consequently, the actual prevalence of GCD2 may be lower than estimated in this study.

GCD2 is frequently observed among corneal dystrophies, especially in South Korea and Japan. According to Song et al., 74 of 89 patients with corneal dystrophy were clinically diagnosed with GCD2, and 82.0% (73/89) of patients with corneal dystrophy had the p.R124H variant [17]. In Japan, GCD2 is the most common corneal dystrophy at 72%, followed by LCD1 [22]. Among Chinese patients with corneal dystrophy, GCD1, followed by GCD2 and LCD1, is the most common [23]. In the United States, endothelial and anterior corneal dystrophies are the most common among patients with corneal dystrophy, while GCD is the least common [24]. Among patients with corneal dystrophy, it can be inferred that the prevalence of GCD2 in South Koreans is higher than that of other ethnic

groups due to higher prevalence of the GCD2-related *TGFBI* p.R124H variant. According to Hashemi et al., the prevalence of myopia is highest in Southeast Asia (32.9%) but lowest in America (16.2%) [25]. In South Korea, the prevalence of myopia and the prevalence of high myopia in young adult men are 50.6–53.0% and 11.3–12.9%, respectively, and the prevalence increases every year [26]. As myopia is widespread in the Southeast Asian population including South Koreans, the use of refractive surgeries, such as LASIK, LASEK, or PRK, is steadily increasing and is expected to continue to increase. As shown in this study, especially in South Korea, the prevalence of patients with GCD2 with the heterozygous *TGFBI* p.R124H variant is higher than in other ethnicities; thus, the number of patients with contraindications to laser refractive surgery is also expected to be higher than in other countries. Recently, several studies have suggested that genetic testing for prescreening for the *TGFBI* mutation is recommended before LASIK or PRK [27–29]. Chao-Shern et al. reported that the *TGFBI* genetic test is used primarily as a screening tool before refractive surgery within South Korea, whereas in European clinics the test is mainly used for clinical confirmation [27]. The approach to genetic test subjects should differ according to the proportion of patients with myopia, an indication for laser refractive surgery, and the proportion of patients with GCD2, a contraindication for laser refractive surgery. South Korea has more patients requiring laser refractive surgery than other countries, and the proportion of patients with GCD2, a contraindication, is higher. Through this study, we believe that genetic screening before laser refractive surgery for at least the *TGFBI* p.R124H variant would be useful for South Koreans.

According to Chao-Shern et al., in South Korea, the genetic test is administered as a general screening for all refractive surgery candidates, whereas in Japan, patients are first subjected to a rigorous clinical examination, and

TABLE 1. PREVIOUSLY REPORTED PREVALENCE OF GCD2-RELATED *TGFBI* p.R124H.

Country	Study population	Total samples (N)	Positive samples	Estimated prevalence per 100,000 (95% confidence interval)	Year	Reference
Korea	p.R124H homozygous patients	Not applicable	21*	115	2010	[18]
China	refractive surgery candidates	2,068	5	241.8 (89.0 – 598.3)	2017	[19]
Korea	cord blood from public cord blood bank	2,060	6	291.3 (118.5 – 667.0)	2020	This study

* All of these patients have a homozygous p.R124H variant.

only patients who have no detected corneal abnormalities have samples submitted for the genetic test [8]. In the United States, some clinics and hospitals use the test for screening during the preoperative examination for vision corrective surgery, whereas others use the genetic test as confirmation of a clinical diagnosis or to exclude mutations in *TGFBI* if the surgeon has any doubt about imperfections noted in the patient's cornea. European clinics utilize the test mostly for this type of clinical confirmation.

The present study has several limitations. First, this study targeted the *TGFBI* p.R124H variant using real-time PCR, so it was not possible to identify atypical forms of GCD2 that were not associated with the *TGFBI* p.R124H variant [4]. Second, this study was performed using umbilical cord blood from newborns. GCD2 prevalence could be overestimated, because some individuals with the *TGFBI* p.R124H variant may not have a GCD2 phenotype due to reduced penetrance [20,21]. Nevertheless, this study has advantages. There have been no large-scale population studies on GCD2 prevalence in South Korea. The frequency of the *TGFBI* p.R124H allele can be predicted more accurately because this study used umbilical cord blood from newborns.

These data indicated that GCD2 with the heterozygous *TGFBI* p.R124H variant is not a rare disease in the general South Korean population, and its prevalence is similar to that in the East Asian population and higher than that of other ethnic groups. To our knowledge, this is the largest cohort study that has estimated the prevalence of the GCD2-related *TGFBI* p.R124H variant. We believe these data provide evidence to support the usefulness of genetic screening for the *TGFBI* p.R124H variant before laser refractive surgery in South Koreans.

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