Exclusion of known corneal dystrophy genes in an autosomal dominant pedigree of a unique anterior membrane corneal dystrophy

Andrea L. Vincent,1,2 David M. Markie,3 Betina De Karolyi,1 Catherine E. Wheeldon,1,2 Dipika V. Patel,1,2 Christina N. Grupcheva,1,2 Charles N.J. McGhee1,2

1Department of Ophthalmology, New Zealand National Eye Centre, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand; 2Eye Department, Greenlane Clinical Centre, Auckland District Health Board, Auckland, New Zealand; 3Department of Pathology, Otago University, Dunedin, New Zealand

Purpose: With advances in phenotyping tools and availability of molecular characterization, an increasing number of phenotypically and genotypically diverse inherited corneal dystrophies are described. We aimed to determine the underlying causative genetic mechanism in a three-generation pedigree affected with a unique anterior membrane corneal dystrophy characterized by early onset recurrent corneal erosions, small discrete focal opacities at the level of Bowman layer and anterior stroma, anterior stromal flecks, and prominent corneal nerves.

Methods: Twenty affected and unaffected members of a three-generation family were examined and extensively clinically characterized including corneal topography and in vivo confocal microscopy, and biological specimens were collected for DNA extraction. Mutational analysis of two corneal genes (TGFBI [Transforming Growth factor-beta induced] and ZEB1 [zinc finger E box-binding homeobox 1]) was undertaken, in addition to testing with the Asper Corneal Dystrophy gene chip (Asper Ophthalmics, Tartu, Estonia). Subsequent Genotyping To 11 Known Corneal Gene Loci (COL8A2 [Collagen, Type VIII, Alpha-2], TACSTD2 [Tumor-Associated Calcium Signal Transducer 2], PIP5K3 [Phosphatidylinositol-3-Phosphate 5-Kinase, Type III], GSN [Gelsolin], KERA [Keratocan], VSX1 [Visual System Homeobox Gene 1], COL6A1 [Collagen, Type VI, Alpha-1], MMP9 [Matrix Metalloproteinase 9], KRT3 [Keratin 3]), and two putative loci, 3p14-q13 and 15q22.33–24) was undertaken using polymorphic markers, and haplotypes constructed. Multipoint linkage analysis was performed to generate LOD scores and produce LOD plots across the candidate intervals.

Results: No pathogenic sequence variations were detected in TGFBI or ZEB1 of the proband nor on the Asper Corneal Dystrophy gene chip (302 mutations in 12 genes). Multipoint linkage analysis of 11 known corneal genes and loci generated negative LOD plots and was able to exclude all genes tested including PIP5K3.

Conclusions: Exclusion of linkage to candidate corneal loci combined with an absence of pathogenic mutations in known corneal genes in this pedigree suggest a different genetic causative mechanism in this dystrophy than the previously documented corneal genes. This unique phenotype of an anterior membrane dystrophy may therefore provide an opportunity to identify a new gene responsible for corneal disease.

The inherited corneal dystrophies represent a group of clinically and genetically heterogeneous disorders of the cornea, with many genes identified to date. With the advances in phenotyping tools and the availability of molecular characterization, an increasing amount of phenotypic and genotypic diversity is described in the corneal dystrophies. The autosomal dominantly inherited bilateral corneal dystrophies involving the anterior corneal layers of the epithelial basement membrane, Bowman layer, and/or anterior stroma are typically described as occurring in association with TGFBI (Transforming Growth factor-beta induced) [1] or PIP5K3 (Phosphatidylinositol-3-Phosphate 5-Kinase, Type III) [2] mutations. The TGFBI dystrophies (granular, lattice, granular dystrophy type 2, and Bowman layer [Reis-Bückler, Thiel-Behnke]) are well characterized and frequently demonstrate typical and reproducible phenotype-genotype correlations, yet there are an increasing number of new phenotypic and genotypic descriptions that are not compatible with the current forms of corneal dystrophies [1].

Less is published on Fleck corneal dystrophy (CFD; OMIM 121850), a term characteristically used in reference to the dystrophy initially described by François and Neetens as “heredodystrophie Mouchetee [2],” which is translated as “speckled.” Multiple flecks are predominantly located centrally and peripherally in the stroma with clear normal tissue intervening and no involvement of other layers of the cornea, including Bowman layer. The disease is usually non-progressive, and the flecks are typically seen by slit-lamp
biomicroscopy to be predominantly in the posterior corneal stroma. The majority of patients are asymptomatic. Recurrent erosions are not a typical feature, although photophobia is reported [3,4]. In vivo confocal microscopy of fleck dystrophies highlights small bright deposits in and around keratocyte nuclei that occur throughout the stroma despite the clinical appearance [5,6].

Four families with classic Fleck dystrophy demonstrated linkage to chromosome 2q35 between D2S117 and D2S116 with a maximum multipoint LOD score of 5 halfway between D2S2289 and D2S325 [7]. Subsequent sequencing of candidate genes within the interval between D2S2289 and D2S126 identified pathogenic mutations in PIP5K3 in 8 of 10 families with Fleck dystrophy [8], although incomplete penetrance was documented in some families. PIP5K3 encodes for a member of the phosphoinositide 3-kinase family and is involved in the regulation of endosomal transport. However, it must be noted that in addition to being dystrophic, corneal flecks may also be degenerative, a feature of certain systemic or topical drugs, or associated with contact lens wear [5,9].

We have identified a three-generation family with a unique corneal dystrophy demonstrating autosomal dominant inheritance and presenting with frequent, recurrent corneal erosions in the first decade but with a relatively mild clinical appearance. This phenotype is not that of the classic (posterior) stromal Fleck dystrophies, and while it clearly exhibits multiple anterior stromal flecks, the corneal localization of larger circular/oval opacities at the level of Bowman layer and anterior stroma suggests an anterior membrane type dystrophy. This pedigree is assumed dystrophic predominantly because of the inheritance, bilaterality, and exclusion of long-term and/or recent contact lens wear.

We aimed to determine the causative genetic mechanism for this disease by undertaking mutational analysis of candidate corneal genes and linkage analysis to known corneal genes and loci.

METHODS
Following informed consent, 20 members of the three-generation family (Figure 1) were recruited, and all members were characterized clinically with visual acuity, autorefraction, slit-lamp biomicroscopic examination, and Orbscan II slit-scanning elevation topography (Bausch & Lomb Surgical, Rochester, NY). Selected individuals also underwent in vivo confocal microscopy (Confoscan 2; Fortune Technologies, Greensboro, NC and/or Heidelberg Retina Tomograph II, Rostock Corneal Module [RCM]; Heidelberg Engineering GmbH, Heidelberg, Germany). This study adhered to the principles of the Declaration of Helsinki and received institutional ethics approval (AKX0200002 and NTX0612161).

Since the clinical manifestation of the disease is relatively mild, being restricted to corneal erosive episodes without visual impairment, none of the affected individuals have undergone corneal biopsy or penetrating keratoplasty. Therefore, histological analysis was unavailable.

Biological samples (peripheral venous blood or saliva specimen) were collected for DNA extraction, which was obtained using the salt extraction method from blood [10] and according to the manufacturer’s instructions for saliva specimens (Oragene™; DNA Genotek, Ottawa, ON, Canada). Mutational analysis of all 17 coding exons of TGFBI was undertaken using previously described primers and methods [11,12].

Recurrent corneal erosions in a patient with epithelial basement membrane dystrophy have been described as occurring concurrently with posterior polymorphous...
dystrophy (PPD) [13]. Localized bullous epithelial changes are also described occurring in association with PPD [14].

Mutational analysis of nine coding exons of ZEB1 (zinc finger E box-binding homeobox 1), formerly TCF8 (transcription factor 8) a corneal gene implicated in PPD, was also undertaken using previously described primers and methods. For exon 1; primers were from Aldave et al. [15], for exons 2-9 primers were from Krafchak et al. [16]. Polymerase chain reaction (PCR) amplification was undertaken using Roche Faststart polymerase (F. Hoffman-La Roche Ltd., Basel, Switzerland). The amplification cycles were 95 °C for 5 min, then 30 cycles of 95 °C for 30 s, annealing temperature (varied by primer pair) for 1 min, 72 °C for 1 min. A final extension step of 72 °C for 10 min then completed the PCR reaction. Product was visualised on a 1.5% agarose gel. For sequencing, the products were purified with Roche HighPure PCR product purification kit (F. Hoffman-La Roche Ltd.), and then sequenced using the same primer pair. Sequences were examined manually and with CLC DNA workbench (CLCbio, Aarhus, Denmark) and aligned with genomic DNA reference: NC_000010.10 and mRNA reference sequence NM_030751.4.

A DNA sample of the proband was sent to Asper Ophthalmics (Tartu, Estonia) and tested against the Corneal Dystrophy chip, Version 2.0 (Asper Ophthalmics). A review of the literature identified known corneal genes and loci implicated in the pathogenesis of corneal disease, and bioinformatic databases were used to identify flanking and intragenic polymorphic microsatellite markers for each known gene. For these candidate loci, polymorphic markers were selected to cover the interval. If one marker failed to adequately amplify or demonstrated low informativeness in the family, a further marker was identified. The loci and markers used are shown in Table 1.

Primer pairs were synthesized and PCR amplification undertaken using Roche Faststart polymerase (F. Hoffman-La Roche Ltd.). The amplification cycles were as described for TCF8/ZEB1 above, with annealing temperature varying for each primer pair in order to optimise the amount of product. Samples were then run on ABI3700 Genotyper, (Applied Biosystems Inc., Foster City, CA), and ABI software was used to assign the peaks. Haplotypes were constructed, and multipoint linkage analysis using GeneHunter v2.1 [17] was undertaken to generate LOD plots across the candidate regions.

**RESULTS**

The clinical features of members of this pedigree are summarized in Table 2.

Affected individuals typically presented in childhood at an average age of six years with recurrent corneal erosions (RCEs), occurring approximately every two to three months. These RCEs became much less frequent in the third and fourth decade, indeed, with increasing age, the recurrent epithelial erosions became less frequent, and the episodes appeared to largely “burn out” by early to mid 20s. A few of the older adults reported photophobia and foreign body sensation and continued to use topical ocular lubricants frequently for ocular comfort. Due to their very young age, four individuals in the youngest generation could not yet be assigned affection status.

Three slit-lamp biomicroscopic corneal features were common to the six affected individuals: (1) accumulation of small, variable size (0.2–1.5 mm diameter), discrete, grayish-

| Table 1. Candidate corneal genes and loci. |
|-------------------------------------------|
| **Gene or locus** | **Chromosomal location** | **Number of markers** |
| COLVIIA2 | 1p34.3-p32 | 3 |
| TACSTD2 | 1p32–31 | 3 |
| PIP5K3 | 2q33.3 | 10 |
| KTCN3 locus | 3p14-q13 | 6 |
| GSN | 9q34 | 3 |
| KERA | 12q22 | 3 |
| KRT3 | 12q12-q13 | 3 |
| Hughes Keratoconus [25,26] | 15q22.33–24.2 | 8 |
| VSX1 | 20p11-q11 | 3 |
| MMP9 | 20q12 | 4 |
| COLVIA1 | 1q22.3 | 3 |

For each candidate gene or locus of interest (column 1) the chromosomal location is given (column 2). Also for each locus the number of polymorphic microsatellite markers used for linkage analysis across each interval is stated in column 3. Abbreviations in the table are: COLVIIA2 = Collagen, Type VIII, Alpha-2, TACSTD2 = Tumor-Associated Calcium Signal Transducer 2, PIP5K3 = Phosphatidylinositol-3-Phosphate 5-Kinase, Type III, KTCN3 = keratoconus 3, GSN = Gelsolin, KERA = Keratocan, KRT3 = Keratin 3, VSX1 = Visual System Homeobox Gene 1, MMP9 = Matrix Metalloproteinase 9, COLVIA1 = Collagen, Type VI, Alpha-1.
Table 2 lists the demographic features for each individual in the pedigree including sex (F = female, M = male), age at last examination in years, affectation status, and age in years at onset and offset of the recurrent corneal erosions for those affected. For those unaffected this information is not applicable (N/A). Clinical examination findings listed include visual acuity measured with the Snellen chart, pachymetry indicating central corneal thickness measured in microns, and anesthesiometry, units are in millibars. When the information was not available, it is listed in the table as Not Done (ND).

| Patient ID | Sex | Affected status | Age at last exam | Age at onset | Age at offset | Visual acuity | Pachymetry | Anesthesiometry |
|------------|-----|-----------------|------------------|--------------|---------------|---------------|-------------|-----------------|
| I-1        | F   | Unaffected      | 65               | N/A          | N/A           | 6/6           | 568         | 569             | ND              |
| I-2        | M   | Affected        | 64               | ‘childhood’   | unsure        | 6/7.5-1       | 559         | 558             | 0.76            |
| II-2       | M   | Affected        | 41               | 10 years     | 21            | 6/12          | 550         | 552             | 0.38            |
| II-3       | M   | Affected        | 39               | 6 years      | 13            | 6/6+          | 550         | 552             | 0.38            |
| II-5       | M   | Affected        | 38               | 5 years      | 22            | 6/6           | 513         | 491             | 0.78            |
| II-7       | M   | Unaffected      | 37               | N/A          | N/A           | 6/6           | 554         | 549             | 0.42            |
| III-1      | F   | Unaffected      | 15               | N/A          | N/A           | 6/9           | 566         | 558             | 0.29            |
| III-2      | F   | Affected        | 14               | 5 years      | ongoing       | 6/5           | 542         | 540             | 0.41            |
| III-3      | F   | Affected        | 12               | 5 years      | ongoing       | 6/5           | 558         | 549             | 0.26            |
| III-4      | F   | Unaffected      | 11               | N/A          | N/A           | 6/5-          | 566         | 558             | 0.28            |
| III-5      | M   | Unknown         | 5                | Nil to date  | N/A           | 6/5-          | ND          | ND              | ND              |
| III-6      | M   | Unknown         | 4                | Nil to date  | N/A           | 6/6           | ND          | ND              | ND              |
| III-7      | F   | Unknown         | 10               | Nil to date  | N/A           | 6/5-2         | ND          | ND              | ND              |
| III-8      | M   | Unknown         | 5                | Nil to date  | N/A           | 6/6-2         | ND          | ND              | ND              |
white oval-round or annular opacities at the level of Bowman layer and the superficial anterior stroma (typically less than six lesions per cornea; Figure 2); (2) numerous, prominent small gray flecks in the anterior 20%–25% of the stroma extending from the central cornea to the limbus; and (3) five of the six affected subjects also had prominent corneal nerves (with reduced corneal sensation more prevalent in the older members of the pedigree). The lesions identified appeared on a background of a generally clear translucent cornea with no involvement of the deeper stroma, Descemet membrane, or the endothelium, and there was an absence of neovascularization.

Orbscan II slit-scanning elevation topography (Bausch & Lomb Surgical) of affected individuals demonstrated normal keratometric patterns and pachymetry. Subsequent in vivo confocal microscopy confirmed that the corneal opacities were restricted to the area of Bowman layer and anterior stroma (Figure 3 and Figure 4), and there appeared to be no abnormalities of the anterior keratocytes or stromal deposits consistent with the clinical appearance of multiple diffuse anterior stromal flecks.

Three clinically unaffected individuals demonstrated faint stromal flecks of size consistent with keratocytes (I:1, II:7, III:7). None of the clinically unaffected individuals demonstrated the Bowman layer circular/oval opacities observed in the affected subjects.

No pathogenic mutations were identified in any of the 17 coding exons and flanking intronic regions of TGFBI or in the nine coding exons and flanking intronic regions of ZEB1. Testing of an affected individual’s DNA against the Asper Corneal Dystrophy microarray Version 2.0 (302 mutations in 12 genes: COL8A2 (Collagen, Type VIII, Alpha-2), TGFBI, VSX1 (Visual System Homeobox Gene 1), CHST6 (Carbohydrate Sulfotransferase 6), KRT3 (Keratin 3), KRT12 (Keratin 12), GSN (Gelsolin), TACSTD2 (Tumor-Associated Calcium Signal Transducer 2), CYP4V2, (Cytochrome P450, Family 4, Subfamily V, Polypeptide 2) SOD1 (Superoxide Dismutase 1), ZEB1 and SLC4A11 (Solute Carrier Family 4 [Sodium Borate Cotransporter]), Member 11) revealed a non-pathogenic heterozygous sequence change in CHST6 (c. 484C>G), resulting in p.R126G but no pathogenic mutations.
Multipoint linkage analysis of nine genes and loci shown in Table 1 was predominantly negative across the intervals. Although the maximum LOD score achievable in this family with the current information is 2.1, the LOD plots generated by multipoint linkage analysis (Figure 5) suggest that all tested candidate loci are excluded or highly unlikely to be responsible.

**DISCUSSION**

This pedigree consisted of three generations with six affected individuals that clearly demonstrate autosomal dominant inheritance. The clinical phenotype in this pedigree is of early onset recurrent erosions associated with an unusual anterior membrane/fleck dystrophy. Some features are similar to the original description in 1966 of an eponymous entity, Grayson Wilbrandt dystrophy (GWD) [18]. GWD varies from the classic fleck description—GWD was described with infrequent erosions, and a less severe effect on vision than occurred in the Bowman layer dystrophies. The original clinical description of GWD is of gray-white amorphous opacities of various sizes over the central cornea, consisting of mounds extending into the epithelium from a thickened Bowman layer with a clear intervening stroma. Corneal nerves were described as prominent.

Further characterization of corneal dystrophies, particularly fleck dystrophy, can be achieved using in vivo confocal microscopy (IVCM) with typical findings consisting of hyper-reflective spots within pleomorphic keratocyte nuclei as previously described [5,6,19]. The phenotypic presentation in the pedigree here particularly with frequent erosions is more in keeping with a TGFBI dystrophy. Given the spectrum of phenotypic variability demonstrated in the TGFBI-associated dystrophies to date with the presence of photophobia and recurrent corneal erosions in many of the family members, mutational analysis of TGFBI was believed to be a valid first approach. Interestingly, TGFBI analysis failed to demonstrate any pathogenic mutation.

Mutational analysis using the corneal dystrophy disease microarray (Asper Ophthalmics) tested for all known mutations in addition to single nucleotide polymorphisms (SNPs) in 12 corneal genes, but none of the known mutations were identified in the proband. As no TGFBI mutation was identified in this pedigree and the phenotype is a “unique” dystrophy, genotyping, haplotype construction, and multipoint linkage to candidate loci and genes including the Fleck locus on 2q35 (where subsequently PIP5K3 was identified as the causative gene) were undertaken. This method is useful to determine if any candidate genes warrant sequencing including PIP5K3, a very large gene of 41 exons encompassing more than 89 kb.

**PIP5K3** is a member of the phosphoinositide 3-kinase family and regulates the sorting and traffic of peripheral endosomes that contain lysosomally directed fluid phase cargo by controlling the morphogenesis and function of multivesicular bodies.

Limitations of the methods used for gene or loci exclusion include the Asper Corneal Dystrophy microarray only excluding known mutations in known genes. As the genes not
excluded by microsatellite analysis and linkage (e.g., *CHST6*, *KRT12*, *SLC4A11*, and *SOD1*) were not fully sequenced, it is feasible that a unique mutation may reside on one of the candidate genes. Similarly, sequencing of *TGFBI* did include all coding exons and flanking intronic regions but, theoretically, would not detect copy number variants within the *TGFBI* open reading frame (ORF).

This dystrophy shows some similarities to two recently described autosomal dominant dystrophies with recurrent corneal erosions, Dystrophia Helsinglandica [20] and the Dystrophia Smolandiensis variant [21]. However, Dystrophia Helsinglandica gradually manifests with subepithelial fibrosis, and the Dystrophia Smolandiensis variant results in keloid-like corneal nodules with penetrating keratoplasty.
often being required. There are also some similarities to subepithelial mucinous corneal dystrophy (SMCD), although more significant vision loss resulted in the one SMCD family described [22]. Franceschetti [23] described a pedigree with RCE in 1928. However, as this original description is limited to one boy, it is difficult to determine similarities. Using the IC3D (International Committee for Classification of Corneal Dystrophies) classification [1], this anterior membrane dystrophy falls in the descriptive category 4: “This category is reserved for a suspected new, or previously documented corneal dystrophy, although the evidence for it, being a distinct entity, is not yet convincing” [1].

By resolving the affection status of the four children in the youngest generation (III-5, III-6, III-7, and III-8), the statistical power increases so as to achieve a potential LOD score of around 3.3, suitable for providing positive gene localization in a genome-wide linkage study. This phenotype has an age-dependent penetrance, so until all of these younger children reach 10 years of age, one cannot confidently label them as unaffected. Non-penetrance may also be an issue as has been described occurring in fleck dystrophy associated with PIP5K3 mutations [8] and in some of the TGFBI - associated corneal dystrophies [24]. This issue may reduce somewhat the power of linkage based approaches in this family.

Thus, to genetically characterize this family further, ongoing clinical examination and observation of the younger and clinically unaffected members of the pedigree must be undertaken. Once affection status can be more tightly assigned, it may be possible to obtain a higher potential LOD score, which will permit a tighter search of the genome. This could be rapidly achievable using SNP microarrays such as the Affymetrix Gene chip systems (Affymetrix, Santa Clara, CA). The clinical findings, analysis of linkage, and mutational analysis suggest this family may provide a clue to a novel genetic mechanism and/or genes implicated in the pathogenesis of the dominantly inherited anterior corneal dystrophies.

ACKNOWLEDGMENTS
The authors would like to thank participating family members, and acknowledge financial support from the Save Sight Society, School of Medicine Foundation, University of Auckland Research fund and the Maurice and Phyllis Paykel Foundation.

REFERENCES
1. Weiss JS, Møller HU, Lisch W, Kinoshita S, Aldave AJ, Belin MW, Kivelä T, Busin M, Munier FL, Seitz B, Sutphin J, Bredrup C, Mannis MJ, Rapuano CJ, Van Rij G, Kim EK, Klintworth GK. The IC3D classification of the corneal dystrophies. Cornea 2008; 27:S1-83. [PMID: 19337156]
2. François J, Neetens A. New hereditary-familial dystrophy of the corneal parenchyma (spotted hereditary dystrophy). Bull Soc Belge Ophtalmol 1957; 114:641-6. [PMID: 13446668]
3. Akova YA, Unlu N, Duman S. Fleck dystrophy of the cornea; a report of cases from three generations of a family. Eur J Ophthalmol 1994; 4:123-5. [PMID: 7950337]
4. Patten JT, Hyndiuk RA, Donaldson DD, Herman SJ, Ostler HB. Fleck (Mouchetee) dystrophy of the cornea. Ann Ophthalmol 1976; 8:25-32. [PMID: 1082286]
5. Grupcheva CN, Malik TY, Craig JP, Sherwin T, McGhee CN. Microstructural assessment of rare corneal dystrophies using...
real-time in vivo confocal microscopy, Clin Experiment Ophthalmol 2001; 29:281-5. [PMID: 11720152]

6. Holopainen JM, Moilanen JA, Tervo TM. In vivo confocal microscopy of Fleck dystrophy and pre-Descemet’s membrane corneal dystrophy. Cornea 2003; 22:160-3. [PMID: 12605053]

7. Jiao X, Munier FL, Schorderet DF, Zografos L, Smith J, Rubin B, Heijtmancik JF. Genetic linkage of Francois-Neetens fleck (mouchetee) corneal dystrophy to chromosome 2q35. Hum Genet 2003; 112:593-9. [PMID: 12607114]

8. Li S, Tiab L, Jiao X, Munier FL, Zografos L, Frueh BE, Sergeev Y, Smith J, Rubin B, Meallet MA, Forster RK, Heijtmancik JF, Schorderet DF. Mutations in PIP5K3 are associated with Francois-Neetens mouchetee fleck corneal dystrophy. Am J Ophthalmol 1996; 80:21-4. [PMID: 8664225]

9. Pimenides D, Steele CF, McGhee CN, Bryce IG. Deep corneal stromal opacities associated with long term contact lens wear. Br J Ophthalmol 1996; 80:21-4. [PMID: 8664225]

10. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16:1215. [PMID: 3344216]

11. Munier FL, Frueh BE, Othenin-Girard P, Uffer S, Cousin P, Wang MX, Héon E, Black GC, Blasi MA, Balestrazzi E, Lorenz B, Escoto R, Barraque R, Hoelztenbein M, Gloor B, Fossarelllo M, Singh AD, Arsenijevic Y, Zografos L, Schorderet DF. BIGH3 mutation spectrum in corneal dystrophies. Invest Ophthalmol Vis Sci 2002; 43:949-54. [PMID: 11923233]

12. Wheeldon CE, De Karolyi BH, Patel DV, Sherwin T, McGhee CN, Vincent AL. A novel phenotype-genotype correlation with a de novo TGFBI exon 14 mutation in a pedigree with a unique Corneal Dystrophy of Bowman’s layer. Mol Vis 2008; 14:1503-12. [PMID: 18728790]

13. Cremona FA, Ghosheh FR, Laibson PR, Rapuano CJ, Cohen EJ. Meesmann corneal dystrophy associated with epithelial basement membrane and posterior polymorphous corneal dystrophies. Cornea 2008; 27:374-7. [PMID: 18362674]

14. Srinivasan S, Skarmoutsos P, O’Donnell C, Kaye SB. Localized bullous keratopathy secondary to posterior polymorphous dystrophy. Clin Experiment Ophthalmol 2008; 36:800-1. [PMID: 19128391]

15. Aldave AJ, Yellore VS, Yu F, Bourla N, Sonmez B, Salem AK, Rayner SA, Sampat KM, Krafcak CM, Richards JE. Posterior polymorphous corneal dystrophy is associated with TCF8 gene mutations and abdominal hernia. Am J Med Genet A 2007; 143A:2549-56. [PMID: 17935237]

16. Krafcak CM, Pawar H, Moroi SE, Sugar A, Lichter PR, Mackey DA, Mian S, Nairus T, Elnor V, Schteingart MT, Downs CA, Kijek TG, Johnson JM, Trager EH, Rozsa FW, Mandal MN, Epstein MP, Vollrath D, Ayyagari R, Bohnhke M, Richards JE. Mutations in TCF8 cause posterior polymorphous corneal dystrophy and ectopic expression of COL4A3 by corneal endothelial cells. Am J Hum Genet 2005; 77:694-708. [PMID: 16252232]

17. Markanos K, Daly MJ, Kruglyak L. Efficient multipoint linkage analysis through reduction of inheritance space. Am J Hum Genet 2001; 68:963-77. [PMID: 11254453]

18. Grayson M, Wilbrandt H. Dystrophy of the anterior limiting membrane of the cornea. (Reis-Buckler type). Am J Ophthalmol 1996; 81:345-9. [PMID: 12605053]

The print version of this article was created on 22 August 2009. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.