A novel mutation (p.Glu1389AspfsX16) of the phosphoinositide kinase, FYVE finger containing gene found in a Japanese patient with fleck corneal dystrophy

Satoshi Kawasaki,¹ Kenta Yamasaki,² Hiroko Nakagawa,¹ Katsuhiko Shinomiya,¹ Mina Nakatsukasa,¹ Yoshihide Nakai,¹ Shigeru Kinoshita¹

¹Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; ²Department of Biomedical Engineering, Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan

Purpose: The phosphoinositide kinase, FYVE finger containing (PIKFYVE) gene has been identified as a gene responsible for fleck corneal dystrophy (FCD). The purpose of this study is to report a novel mutation of the PIKFYVE gene in a Japanese patient with fleck corneal dystrophy.

Methods: Slit-lamp microscopy, corneal topography, and optical coherence tomography were performed for the clinical examination of the patient’s eye. For genetic analysis, peripheral blood was obtained from the patient and her sister. DNA was extracted from the blood and subjected to mutation analysis by sequencing of the PIKFYVE gene. The sequencing results were validated with a PCR-fragment length polymorphism analysis.

Results: A 63-year-old woman presented at our clinic with complaints of decreased vision and metamorphopsia in her right eye occurring 1 month before presentation. Both eyes exhibited small, dot-like, white flecks scattered throughout all layers of the corneal stroma, which corresponds to the typical FCD phenotype. The opacities were relatively dominant at the peripheral region of the cornea, yet were found throughout the entire cornea. Sequence analysis revealed that the patient has a heterozygous c.4166_4169delAAGT mutation located at exon 24 of the PIKFYVE gene that may cause p.Glu1389AspfsX16 flame-shift mutation, which has never before been reported for FCD.

Conclusions: To the best of our knowledge, this is the first study to show that a novel mutation (p.Glu1389AspfsX16) causing the truncation of the PIKFYVE protein causes fleck corneal dystrophy in the Japanese population.

The cornea is one of the most transparent and non-vascularized tissues in the human body, and several active genes [1,2] are thought to be involved in maintaining the homeostasis of the cornea. Recent advances in molecular biology techniques have allowed the genes responsible for most hereditary corneal dystrophies to be identified, including transforming growth factor, beta-induced (TGFBI)-related corneal dystrophies (i.e., granular corneal dystrophy, lattice corneal dystrophy type I, granular corneal dystrophy type 2 (Avellino corneal dystrophy), Reis-Bücklers corneal dystrophy, and Thiell-Behnke corneal dystrophy) [3], Meesmann corneal dystrophy [4,5], macular corneal dystrophy [6], gelatinous drop-like corneal dystrophy [7], and Fuchs’ endothelial dystrophy [8].

Fleck corneal dystrophy (FCD, Online Mendelian Inheritance in Man (OMIM) #121850) was first reported in 1957 by Francois and Neetens [9], and is one of the hereditary corneal dystrophies in which the causative genes have already been identified. This corneal dystrophy is a rare autosomal dominant disease characterized by numerous tiny, dot-like white flecks scattered in all layers of the corneal stroma. Typically, the stroma located in between the flecks is clear, and the endothelium, the epithelium, Bowman’s layer, and Descemet’s membrane are normal. Patients are usually asymptomatic with normal vision, yet a small number of patients report the sensation of a minor photophobia. The flecks in FCD can appear as early as at 2 years of age, or sometimes even at birth, and appear not to progress significantly throughout life [10,11]. Histologically, the corneal flecks appear to correspond to abnormal keratocytes swollen with membrane-limited intracytoplasmic vesicles containing complex lipids and glycosaminoglycans [12]. It has been reported that there are no extracellular abnormalities [12].

In this study, we report a case of FCD bearing a heterozygous flame-shift mutation within the phosphoinositide kinase, FYVE finger containing (PIKFYVE) gene. The patient had no obvious vision loss or any complaints related to this corneal dystrophy, and the appropriateness of our identified mutation as a causative one for FCD is theoretically discussed.
METHODS

Measurement of corneal irregularity and higher-order aberration: Corneal irregularity and higher-order aberration in the patient were investigated using a commercially available corneal topography device (KR-1W; Topcon Corp., Tokyo, Japan).

Optical coherence tomography of cornea: An optical section of the patient’s cornea was obtained using a commercially available optical coherence tomography (OCT) device (Cirrus HD-OCT; Carl Zeiss Meditec Co. Ltd., Tokyo, Japan).

Mutation analysis: A 63-year-old woman presented at our clinic with complaints of decreased vision and metamorphopsia in her right eye occurring 1 month before presentation. Her best-corrected visual acuity was 0.7 in her right eye and 1.2 in her left eye. The decreased visual acuity and metamorphopsia seemed to be due to a transient focal retinal detachment caused by the traction of the posterior vitreous membrane.

All experimental procedures were approved by the Institutional Review Board for Human Studies of Kyoto Prefectural University of Medicine. This study was performed in accordance with the tenets of the Declaration of Helsinki for research involving human subjects.

Peripheral blood was obtained from the patient and her younger sister, the patient’s only remaining living relative using a plastic syringe attached with a 23G needle. Prior informed consent was obtained from both subjects after a detailed explanation of the study protocols. Genomic DNA was extracted from the blood using a commercially available kit (DNeasy Blood & Tissue Kit; Qiagen GmbH, Hilden, Germany). Genomic DNA samples from 96 normal Japanese volunteers (48 men and 48 women) were obtained from a research-resource bank (Human Research Resource Bank, Osaka, Japan). Using 10 ng of genomic DNA, all exons of the PIKFYVE gene were amplified with polymerase chain reaction (PCR) in a 50 μl reaction buffer containing 1 x ExTa buffer, 0.2 mM dNTP, 0.2 μM primer pair, and 1.25 U Taq polymerase (ExTa Hot Start version; Takara Bio Inc., Otsu, Japan). All primer pairs were designed according to a previous study [13]. The PCR products were treated with a mixture of exonuclease and alkaline phosphatase (ExoSAP-IT; GE Healthcare UK, Ltd., Buckinghamshire, UK), heat-inactivated, and sequenced using a commercially available kit (BigDye 3.1; Applied Biosystems Inc., Foster City, CA). The sequencing products were purified with a commercially available kit (BigDye Xterminater Purification Kit; Applied Biosystems), electrophoresed on an automated sequencer (3130xl Genetic Analyzer; Applied Biosystems), and analyzed with sequence alignment software (Variant Reporter Version 1.0; Applied Biosystems). Thermal cycle conditions for all primer pairs were 30 cycles of three-temperature thermal cycles at 94 °C for 30 s for heat denaturation, at 55 °C for 30 s for annealing, and 72 °C for 30 s for extension.

Polymerase chain reaction–fragment length polymorphism analysis: Sequencing data were validated with PCR–fragment length polymorphism (PCR–FLP). Briefly, a partial sequence of exon 24 of the PIKFYVE gene was amplified by PCR using a primer pair (PIKFYVE_FLP_F_Ex24; 5’-CTC AGT TAT TCT CCC ATT CGG CTT C-3’, PIKFYVE_FLP_R_Ex24; 5’-AAT GAA TAT TTT GGG GAG TGG AAC A-3’). The PCR product was electrophoresed on a 10% acrylamide gel. After the electrophoresis, the gel was stained with a DNA-staining fluorescent dye (SYBR® Green I; Takara Bio), observed on a UV transilluminator, and photographed in
a dark box equipped with a charge-coupled device (CCD) camera (LAS-3000 UV mini; GE Healthcare UK).

**RESULTS**

Both eyes exhibited small, dot-like, white-fleck opacities scattered in all layers of the corneal stroma. The opacities were relatively dominant at the peripheral region of the cornea, yet were found throughout the entire cornea. The opacities were almost invisible under diffuse illumination (Figure 1A), but became more apparent under slit-lamp illumination or iris retroillumination (Figure 1B). It seems difficult to recognize the opacities in ordinary care, especially for ophthalmologists unfamiliar with such faint corneal opacity. OCT analysis successfully identified some of the small stromal flecks (Figure 1C). Higher-order aberration was within the normal limit in both corneas. Specular microscopy examination demonstrated that the endothelial cell density was 2,000 cells/mm$^2$ in her right cornea and 2,200 cells/mm$^2$ in her left cornea, which is sufficient for endothelial function but appears slightly decreased compared to the average cell density in persons of her same age. She had previously undergone clinical examination by an ophthalmologist several times in her life; however, it was never pointed out to her that she had such corneal abnormalities. Her sister did not exhibit any corneal manifestations in either eye (Figure 1D).
No treatment was undergone by this patient, but her retinal problem spontaneously ameliorated as judged by OCT findings along with the improvement of visual acuity from 0.7 to 1.2 in 2 weeks, indicating that the decrease in the visual acuity of her right eye at her first visit was not due to the corneal opacities.

**Mutation analysis:** The sequence data revealed that the patient had a heterozygous 4-base-pair deletion mutation (c.4166_4169delAAGT or c.4167_4170delAGTA) within the PIKFYVE gene (Figure 2A-C). This mutation may produce a new reading frame starting from amino acid number 1389, leading to a premature termination at the 16th codon counted from the first affected amino acid (p.Glu1389AspfsX16), which has never been reported in patients with FCD. PCR-FLP analysis confirmed these sequencing data (Figure 2D). In addition to this mutation, 20 nucleotide changes were found within the PIKFYVE gene (Table 1), yet all were of known single-nucleotide polymorphisms, of synonymous amino acid alteration, or located at the non-coding region, and hence are considered non-pathological. The c.4166_4169delAAGT mutation was not found in any of the examined 96 normal Japanese volunteers (data not shown). The sister of the patient did not have the c.4166_4169delAAGT mutation.

**DISCUSSION**

In 2003, Jiao et al. [14] performed linkage analysis of four families with FCD and found that the critical region for FCD mapped to a 27.9 cM region of chromosome 2q35 flanked by the genomic markers D2S117 and D2S126. Subsequently, in 2005, Li et al. [15] further narrowed the linked region to a 24 cM interval containing 18M bases. Li et al. subsequently sequenced genes included within the narrowed region and found mutations in the PIKFYVE gene in patients with FCD. The PIKFYVE gene encodes a widely expressed, 2,089-amino-acid-long, phosphoinositide 3-kinase family member that functions in post-Golgi vesicular sorting [15].

In the present study, we found a heterozygous c.4166_4169delAAGT mutation within the PIKFYVE gene in our patient with FCD. As this mutation is of 4-base-pair deletion, the mutation may cause a flame-shift amino acid

| # | Region      | Nucleotide change | Zygosity | Type of mutation | Effects on amino acid | SNP            |
|---|-------------|-------------------|----------|------------------|-----------------------|----------------|
| 1 | Intron 9    | g.32610C>T        | Homozygous| Substitution     | none (non-coding)     | none           |
| 2 | Intron 15   | g.48601A>G        | Homozygous| Substitution     | none (non-coding)     | none           |
| 3 | Exon 16     | c.2087G>A         | Homozygous| Substitution     | p.696S=N              | rs10932258     |
| 4 | Exon 16     | c.2106C>T         | Homozygous| Substitution     | p.702p>P              | rs10932259     |
| 5 | Exon 19     | c.2795T>C         | Homozygous| Substitution     | p.932L>S              | rs2363468      |
| 6 | Exon 19     | c.2984A>T         | Homozygous| Substitution     | p.995Q>L              | rs893254       |
| 7 | Exon 19     | c.2993C>G         | Homozygous| Substitution     | p.998T>S              | rs893253       |
| 8 | Exon 19     | c.2984A>T         | Homozygous| Substitution     | p.995Q>L              | rs893254       |
| 9 | Exon 19     | c.2993C>G         | Homozygous| Substitution     | p.998T>S              | rs893253       |
| 10| Exon 19     | c.3547C>A         | Homozygous| Substitution     | p.1183Q>K             | rs1529979      |
| 11| Exon 19     | c.3564T>C         | Homozygous| Substitution     | p.1188N>A             | rs1529978      |
| 12| Exon 24     | c.4166_4169delAAGT| Heterozygous| Insertion    | p.Glu1389AspfsX16     | none           |
| 13| Intron 27   | g.65496T>C        | Homozygous| Substitution     | none (non-coding)     | none           |
| 14| Intron 31   | g.73584G>A        | Homozygous| Substitution     | none (non-coding)     | none           |
| 15| Intron 32   | g.73754C>T        | Homozygous| Substitution     | none (non-coding)     | none           |
| 16| Exon 34     | c.5334G>A         | Homozygous| Substitution     | p.1778T>T             | rs2304545      |
| 17| Exon 35     | c.5397A>G         | Homozygous| Substitution     | p.1799T>T             | rs2118297      |
| 18| Intron 35   | g.79205A>G        | Homozygous| Substitution     | none (non-coding)     | none           |
| 19| Exon 36     | c.5526A>G         | Homozygous| Substitution     | p.1842E>E             | rs994697       |
| 20| Exon 38     | c.5727G>T         | Heterozygous| Substitution | p.1909A>A             | none           |
| 21| Intron 39   | g.82947A>G        | Homozygous| Substitution     | none (non-coding)     | none           |

The notation convention of the nucleotide and protein changes follows the nomenclature guidelines of human genome variation society (HGVS).
alteration and have a significant impact on the function of the PIKFYVE protein. A homology search of its amino acid sequence identified six putative functional domains in the PIKFYVE protein \cite{15,16}, including a zinc-finger-containing phosphoinositide kinase (FYVE) located at the 150–219 amino acid region, Disheveled, EGL-10, plextrin homology domain (DEP) of unknown function located at the 365–440 amino acid region, a common kinase core motif found in the type IIβ phosphatidylinositol-4-phosphate 5-kinase (PIP kinase) located at the 1791–2085 amino acid region, a small β-sheet “winged helix” DNA/RNA-binding motif (Winged) located at the 348–489 amino acid region, and two spectrin repeats (SPEC) located at the 1490–1538 and 1679–1729 amino acid regions (Figure 3). Our mutation may lead to a truncation of the PIKFYVE protein lacking the two spectrin repeats and phosphatidylinositol-4-phosphate 5-kinase core domains. Thus, the mutation might imply the necessity of at least each of the two domains for completing the function of the PIKFYVE protein, at least in corneal keratocytes. The fact that the mutation was not found in any of the examined 96 normal Japanese volunteers, along with the fact that the phenotype well cosegregated with genotype in our pedigree, supports the pathogenicity of the mutation.

There are several genetically modified model organisms for the PIKFYVE gene. In \textit{C. elegans} \cite{17} and \textit{Drosophila} \cite{18}, several mutant lines were generated for the PIKFYVE gene with the ethyl methanesulfonate mutagenesis technique. In both organisms, mutant lines harboring loss-of-function mutations of the gene displayed numerous vacuoles in their cells and died at the embryonic or pupal stages. However, partial loss-of-function situation in the worm model displayed growth retardation. In mice, although no knockout models have been established for the gene, knockout lines were generated for the \textit{Sac3} \cite{19} and \textit{ArPIKfyve} \cite{20} genes, both of which are functionally associated with the PIKFYVE gene.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
Region & Nucleotide change & Amino acid change & Original description & Report \\
\hline
Exon 16 & c.2098delA & p.Asn701ThrfsX7 & 2256delA & Li et al. \cite{15} \\
Exon 16 & c.2116_2117delCT & p.Leu706ValfsX6 & 2274delCT & Li et al. \cite{15} \\
Intron 19 & c.3619−1G>C & p.Val1207AlafsX11 & IVS19−1G→C,intron 19 & Li et al. \cite{15} \\
Exon 19 & c.2551C>T & p.Arg851X & R851X & Li et al. \cite{15} \\
Exon 19 & c.2962C>T & p.Gln988X & Q988X & Li et al. \cite{15} \\
Exon 19 & c.3088G>T & p.Glu1030X & E1030X & Li et al. \cite{15} \\
Exon 19 & c.3112C>T & p.Arg1038X & R1038X & Li et al. \cite{15} \\
Exon 19 & c.3308A>G & p.Lys1033Arg & K1033R & Li et al. \cite{15} \\
Exon 19 & c.2902_2905delCCCTT & p.Asp1021ThrfsX28 & c.3060−3063delCCCTT & Kotoulas et al. \cite{13} \\
Exon 24 & c.4166_4169delAGT & p.Glu1389AsfsX16 & p.Glu1389AsfsX16 & This report \\
\hline
\end{tabular}
\caption{List of mutations thus far reported within the PIKFYVE gene in FCD patients.}
\end{table}

The notation convention of the nucleotide and amino acid changes follows the nomenclature guidelines of human genome variation society (HGVS). Note that there is a confusing situation in that the numbers of the exons and the intron of the PIKFYVE mutations listed in IC3D \cite{21} appear to be incorrect, while those originally described by Li et al. \cite{15} are correct. There is also another confusing situation in that the nucleotide number of the mutation reported by Li et al. and Kotoulas et al. \cite{13} appears to be of mRNA, not of the coding sequence. We corrected this nucleotide number so that it is now in accordance with the nomenclature convention of the HGVS.
In both knockout lines, neurologic defects and juvenile or perinatal death was seen. Thus, complete loss of function of the PIKFYVE gene may lead to death in many organisms, possibly also in humans, which may account for the fact that all of the mutations thus far reported within the PIKFYVE gene, including the mutation shown in this report, are heterozygous.

No mutations have been previously reported for this disease in the Japanese population. In addition, even on a global scale, only two studies have been conducted to report nine mutations within the PIKFYVE gene in patients with FCD [13,15] (Table 2). This may be mostly due to the quite faint corneal phenotype with almost no disturbance in visual function in patients with this disease, even when those patients are older. We theorize that most ophthalmologists may fail to notice the subtle opacities associated with this corneal dystrophy. Moreover, there is a good chance that most patients with this disease might never visit an eye clinic complaining of symptoms associated with this disease. We imagine that the prevalence of patients with FCD might be much more common than has been recognized in previous reports, and potentially may exist in many countries.

In summary, we show here that a novel mutation (p.Glu1389AspfsX16) causing the truncation of the PIKFYVE protein causes fleck corneal dystrophy in the Japanese population. We hope that this study will contribute to future investigations focusing on understanding the biochemical properties and physiologic significance of the PIKFYVE gene as well as elucidating the molecular pathogenesis of FCD.

ACKNOWLEDGMENTS

This work was supported in part by grant-in-aids (#23390404, #24592675) from the Japanese Ministry of Education, Science, Culture and Sports and by a grant (H23-Nanchi-Ippan-084) from Japanese Ministry of Health, Labour and Welfare. The authors wish to thank John Bush for his assistance in reviewing this manuscript. The authors also wish to thank the staff members at Tokai Eye Clinic for their excellent clinical support, as well as the case report patient and her family members for their participation in this study. There is no commercial interest with regard to this manuscript. There is no prior presentation regarding this manuscript.

REFERENCES

1. Nishida K, Adachi W, Shimizu-Matsumoto A, Kinoshita S, Mizuno K, Matsubara K, Okubo K. A gene expression profile of human corneal epithelium and the isolation of human keratin 12 cDNA. Invest Ophthalmol Vis Sci 1996; 37:1800-9. [PMID: 8759347].

2. Sakai R, Kinouchi T, Kawamoto S, Dana MR, Hamamoto T, Tsuru T, Okubo K, Yamagami S. Construction of human corneal endothelial cDNA library and identification of novel active genes. Invest Ophthalmol Vis Sci 2002; 43:1749-56. [PMID: 12036975].

3. Munier FL, Frueh BE, Othenin-Girard P, Uffer S, Cousin P, Wang MX, Heon E, Black GC, Blasi MA, Balestazzi E, Lorenz B, Escoto R, Barraquer R, Hoeltztenbein M, Gloor B, Fossarello M, Singh AD, Arsenijevic Y, Zografas L, Schorderet DF. BIGH3 mutation spectrum in corneal dystrophies. Invest Ophthalmol Vis Sci 2002; 43:949-54. [PMID: 11923233].

4. Irvine AD, Corden LD, Swensson O, Swensson B, Moore JE, Frazer DG, Smith FJ, Knowlton RG, Christophers E, Rochels R, Uitto J, McLean WH. Mutations in cornea-specific keratin K3 or K12 genes cause Meesmann's corneal dystrophy. Nat Genet 1997; 16:184-7. [PMID: 9171831].

5. Nishida K, Honna Y, Dota A, Kawasaki S, Adachi W, Nakamura T, Quantock AJ, Hosotani H, Yamamoto S, Okada M, Shimomura Y, Kinoshita S. Isolation and chromosomal localization of a cornea-specific human keratin 12 gene and detection of four mutations in Meesmann corneal epithelial dystrophy. Am J Hum Genet 1997; 61:1268-75. [PMID: 9399908].

6. Akama TO, Nishida K, Nakayama J, Watanabe H, Ozaki K, Nakamura T, Dota A, Kawasaki S, Inoue Y, Maeda N, Yamamoto S, Fujiwara T, Thonar EJ, Shimomura Y, Kinoshita S, Tanigami A, Fukuda MN. Macular corneal dystrophy type I and type II are caused by distinct mutations in a new sulphotransferase gene. Nat Genet 2000; 26:237-41. [PMID: 11017086].

7. Tsujikawa M, Kurahashi H, Tanaka T, Nishida K, Shimomura Y, Tano Y, Nakamura Y. Identification of the gene responsible for gelatinous drop-like corneal dystrophy. Nat Genet 1999; 21:420-3. [PMID: 10192395].

8. Schmedt T, Silva MM, Ziaei A, Jurkunas U. Molecular bases of corneal endothelial dystrophies. Exp Eye Res 2012; 95:24-34. [PMID: 21855542].

9. Francois J, Neetens A. New hereditary-familial dystrophy of the corneal parenchyma (spotted hereditary dystrophy). Bull Soc Belge Ophtalmol 1957; 114:641-6. [PMID: 1082286].

10. 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].

11. Patten JT, Hyndiuk RA, Donaldson DD, Herman SJ, Ostler HB. Fleck (Mouchette) dystrophy of the cornea. Ann Ophthalmol 1976; 8:25-32. [PMID: 1082286].

12. Nicholson DH, Green WR, Cross HE, Kenyon KR, Massof D. A clinical and histopathological study of Francois-Neetens speckled corneal dystrophy. Am J Ophthalmol 1977; 83:554-60. [PMID: 141212].

13. Kotoulas A, Kokotas H, Kopsidas K, Droutsas K, Grigoriadou M, Bajrami H, Schorderet DF, Petersen MB. A novel PIKFYVE mutation in fleck corneal dystrophy. Mol Vis 2011; 17:2776-81. [PMID: 22065932].
14. Jiao X, Munier FL, Schorderet DF, Zografos L, Smith J, Rubin B, Hejtmancik JF. Genetic linkage of Francois-Neetens fleck (mouchetee) corneal dystrophy to chromosome 2q35. Hum Genet 2003; 112:593-9. [PMID: 12607114].
15. Li S, Tiab L, Jiao X, Munier FL, Zografos L, Frueh BE, Sergeev Y, Smith J, Rubin B, Meallet MA, Forster RK, Hejtmancik JF, Schorderet DF. Mutations in PIP5K3 are associated with Francois-Neetens mouchetee fleck corneal dystrophy. Am J Hum Genet 2005; 77:54-63. [PMID: 15902656].
16. Shisheva A. PIKfyve: Partners, significance, debates and paradoxes. Cell Biol Int 2008; 32:591-604. [PMID: 18304842].
17. Nicot AS, Fares H, Payrastre B, Chisholm AD, Labouesse M, Laporte J. The phosphoinositide kinase PIKfyve/Fab1p regulates terminal lysosome maturation in Caenorhabditis elegans. Mol Biol Cell 2006; 17:3062-74. [PMID: 16801682].
18. Rusten TE, Rodahl LM, Pattni K, Englund C, Samakovlis C, Dove S, Brech A, Stenmark H. Fabl phosphatidylinositol 3-phosphate 5-kinase controls trafficking but not silencing of endocytosed receptors. Mol Biol Cell 2006; 17:3989-4001. [PMID: 16837550].
19. Chow CY, Zhang Y, Dowling JJ, Jin N, Adamska M, Shiga K, Szigeti K, Shy ME, Li J, Zhang X, Lupski JR, Weisman LS, Meisler MH. Mutation of FIG4 causes neurodegeneration in the pale tremor mouse and patients with CMT4J. Nature 2007; 448:68-72. [PMID: 17572665].
20. Zhang Y, Zolov SN, Chow CY, Slutsky SG, Richardson SC, Piper RC, Yang B, Nau JJ, Westrick RJ, Morrison SJ, Meisler MH, Weisman LS. Loss of Vac14, a regulator of the signaling lipid phosphatidylinositol 3,5-bisphosphate, results in neurodegeneration in mice. Proc Natl Acad Sci USA 2007; 104:17518-23. [PMID: 17956977].
21. Weiss JS, Moller HU, Lisch W, Kinoshita S, Aldave AJ, Belin MW, Kivela 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:Suppl 2S1-83. [PMID: 19337156].

Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 12 December 2012. 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.