

Characterization of Bietti Crystalline Dystrophy Patients with *CYP4V2* Mutations

Kelvin Y. C. Lee,^{1,2} Adrian H. C. Koh,¹ Tin Aung,^{1,2,3} Victor H. K. Yong,² Kit Yeung,^{2,3} Chong-Lye Ang,^{1,2} and Eranga N. Vitthana²

PURPOSE. Mutations of the *CYP4V2* gene, a novel family member of the cytochrome P450 genes on chromosome 4q35, have recently been identified in patients with Bietti crystalline dystrophy (BCD). The aim of this study was to investigate the spectrum of mutations in this gene in BCD patients from Singapore, and to characterize their phenotype.

METHODS. Nine patients with BCD from six families were recruited into the study. The 11 exons of the *CYP4V2* gene were amplified from genomic DNA of patients by polymerase chain reaction and then sequenced. Detailed characterization of the patients' phenotype was performed with fundal photography, visual field testing, fundal fluorescein angiography, and electroretinography (ERG).

RESULTS. Three pathogenic mutations were identified; two mutations, S482X and K386T, were novel and found in three patients. The third mutation, a previously identified 15-bp deletion that included the 3' splice site for exon 7, was found in all nine patients, with six patients carrying the deletion in the homozygous state. Haplotype analysis in patients and controls indicated a founder effect for this deletion mutation in exon 7. Clinical heterogeneity was present in the patients. Compound heterozygotes for the deletion in exon 7 seemed to have more severe disease compared to patients homozygous for the deletion. There was good correlation between clinical stage of disease and ERG changes, but age did not correlate with disease severity.

CONCLUSIONS. This study identified novel mutations in the *CYP4V2* gene as a cause of BCD. A high carrier frequency for the 15-bp deletion in exon 7 may exist in the Singapore population. Phenotype characterization showed clinical heterogeneity, and age did not correlate with disease severity. (*Invest Ophthalmol Vis Sci.* 2005;46:3812–3816) DOI:10.1167/iavs.05-0378

Bietti crystalline dystrophy (BCD) is a rare retinal dystrophy that is autosomal recessive in inheritance. This systemic condition was first described by Bietti in 1937, and is characterized by the presence of crystals in the corneal limbus, glistening intraretinal crystals associated with atrophy of retinal pigment epithelium (RPE), pigment clumping, and choroidal

sclerosis.^{1–2} Patients are usually affected in the prime of their life, presenting in the third to fourth decade of life with nyctalopia, decreased vision, and paracentral scotomas. There is progressive visual loss and constriction of the visual fields leading to legal blindness in the fifth to sixth decade.^{3–5}

Biochemical studies show that abnormal lipid metabolism is present in patients with BCD, with lower than normal conversion of fatty acid precursors into n-3 polyunsaturated fatty acids, and two fatty acid-binding proteins were found to be absent or nonfunctional in cells of patients with BCD.^{6,7} The gene for BCD has been localized on chromosome 4q35, and mutations of the *CYP4V2* gene, a novel family member of the cytochrome P450 genes, have recently been identified in patients with BCD.⁸ This gene codes for a 525-amino acid protein, the structure of which suggests that it may be active in fatty acid metabolism, and has been found to be expressed in various tissues, including the retina and RPE.⁸ However, the specific enzymatic activity of this cytochrome P450 family member remains to be elucidated.

The aim of this study was to expand the spectrum of mutations in this novel gene by screening patients with BCD, and to characterize their phenotype.

MATERIALS AND METHODS

Patients with BCD were recruited from the Singapore National Eye Centre, Singapore. Written informed consent was obtained from all patients, and the study had the approval of the Ethics Committee of the hospital and was performed according to the tenets of the Declaration of Helsinki. Patients who had been on medications that would cause crystalline deposits were excluded from the study.

All subjects underwent a complete eye examination including visual acuity testing, slit-lamp biomicroscopy, applanation tonometry, funduscopy, fluorescein and indocyanine green angiography, automated perimetry, and fundus photo-documentation. The clinical fundus findings were staged into stages 1 to 3 using the classification of Yuzawa et al.,⁹ which is summarized as follows:

Stage 1: RPE atrophy with uniform, fine, white, crystalline deposits is observed at the macular area.

Stage 2: RPE atrophy extends beyond the posterior pole. Choriocapillaris atrophy in addition to the RPE atrophy appears markedly at the posterior pole.

Stage 3: RPE-choriocapillaris atrophy is observed throughout the fundus.

Patients also had full-field and multifocal electroretinography (ERG) in accordance with the guidelines of the International Society for Clinical Electrophysiology of Vision.^{10–13} The ERGs (full field) were classified as follows:

Group A: Rod dysfunction only; normal rod implicit times; cone function normal;

Group B: Rod and cone dysfunction but normal implicit times;

Group C: Severe rod and cone dysfunction with abnormal implicit times;

Group D: No observable response; extinguished ERG.

From the ¹Singapore National Eye Centre, Singapore; ²Singapore Eye Research Institute, Singapore; and ³National University of Singapore, Singapore.

Supported by the National University of Singapore and the Singapore Eye Research Institute.

Submitted for publication March 24, 2005; revised June 14, 2005; accepted August 25, 2005.

Disclosure: **K.Y.C. Lee**, None; **A.H.C. Koh**, None; **T. Aung**, None; **V.H.K. Yong**, None; **K. Yeung**, None; **C.-L. Ang**, None; **E.N. Vitthana**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Tin Aung, Glaucoma Service, Singapore National Eye Centre, 11 Third Hospital Avenue, Singapore 168751; tin11@pacific.net.sg.

TABLE 1. The Clinical Data of the 9 Patients with Bietti Crystalline Dystrophy and Their Corresponding Mutations

Patient	Pedigree	Age	Sex	Visual Acuity	Clinical Stage	ERG Group	Mutations
1		42	Female	RE 6/6 LE 6/6	3	B	IVS6-Ex7del + Ex11(S482X)
2	1	39	Female	RE 6/6 LE 6/6	2	NA	IVS6-Ex7del + Ex11(S482X)
3	2	49	Female	RE 6/7.5 LE 6/7.5	2	B	Homozygous IVS6-Ex7del
4	2	45	Female	RE 6/6 LE 6/9	2	B	Homozygous IVS6-Ex7del
5	2	40	Male	RE 6/12 LE 6/7.5	2	B	Homozygous IVS6-Ex7del
6	3	32	Female	RE HM LE 6/7.5	3	D	Homozygous IVS6-Ex7del
7	4	77	Female	RE 6/30 LE 6/60	2	NA	Homozygous IVS6-Ex7del
8	5	56	Female	RE 6/15 LE 6/60-6/18	3	D	IVS6-Ex7del + Ex9(K386T)
9	6	53	Female	RE 6/12 LE 6/9	3	NA	Homozygous IVS6-Ex7del

RE, right eye; LE, left eye; NA, Not recorded. Novel mutations are indicated in bold.

Mutation Analysis

Genomic DNA was extracted from leukocytes of the peripheral blood of the patients, and exons 1 to 11 of the *CYP4V2* gene were amplified by polymerase chain reaction (PCR; DNA Thermocycler 9700; Applied Biosystems, Foster City, CA). Primers were obtained according to previously published sequences.⁸ PCR reactions were carried out in 50- μ L reaction volumes containing 10 mM TrisHCl (pH 8.9), 50 mM KCl, 1.5 mM MgCl₂, 25 pmol each primer, 200 μ M each deoxyribonucleoside triphosphate, 50 to 100 ng patient genomic DNA, and 0.7 U *Taq* thermostable DNA polymerase (Promega, Madison, WI). Cycling parameters were 3 minutes at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at the melting temperature (T_m) of the primers (52°C–62°C), and 30 seconds at 72°C, with a final 5-minute extension at 72°C. PCR products were purified using PCR cleanup columns (GFX; Amersham, Piscataway, NJ). Sequence variations were identified by automated bidirectional sequencing (BigDye Terminator, v3.1 chemistry; Applied Biosystems). An automated DNA sequencer (Model ABI PRISM 3100; Applied Biosystems) was used. Primers for sequence reactions were the same as those for the PCR reaction.

The presence of a 15-bp deletion mutation in IVS6-exon 7 (see below) was investigated in 236 healthy Chinese control subjects using a restriction endonuclease assay, or by direct sequencing. The PCR products (10 μ L) were digested with 1 U of *Mwo*I enzyme in the corresponding buffer by incubating overnight at 60°C. DNA fragments were detected by electrophoresis on 2% agarose gels.

Haplotype Analysis, Estimation of Haplotype Frequencies, and Determination of Statistical Significance

To investigate the possibility of a founder event for the 15-bp deletion mutation within exon 7, 10 intragenic polymorphic markers flanking the mutation were analyzed in patients who were homozygous and heterozygous for this mutation. These polymorphisms were located in the 5' UTR, exon 1, IVS2, IVS3, IVS4, exon 6, IVS8, and IVS9 of the *CYP4V2* gene.

To calculate the frequencies of *CYP4V2* haplotypes present in the population, 50 healthy unrelated Chinese individuals were also genotyped for the aforementioned markers. Haplotypes were predicted by Bayesian algorithm using the PHASE program,¹⁴ which handles uncertainty in phase determination.

Due to the small sample size of the patient group, the statistical significance of the difference in haplotype distribution in the patient and control groups was tested by Fisher's exact test.

RESULTS

Patient Demographics

Nine subjects with BCD were recruited into the study. Of these, eight were Chinese and one was Indonesian. There were eight females and one male, and the age of the patients ranged from 32 to 77 years (Table 1 and Fig. 1). Among the patients were two groups of siblings; a pair of sisters (patients 1 and 2) and a family of three siblings (one male and two females; patients 3–5). All subjects were born of non-consanguineous marriages.

Clinical Phenotype

Five patients (1, 3, 6, 8, and 9) presented with symptoms of nyctalopia, with patients 1 and 6 presenting in the second decade. Patient 2 had photopsia with onset in the third decade;

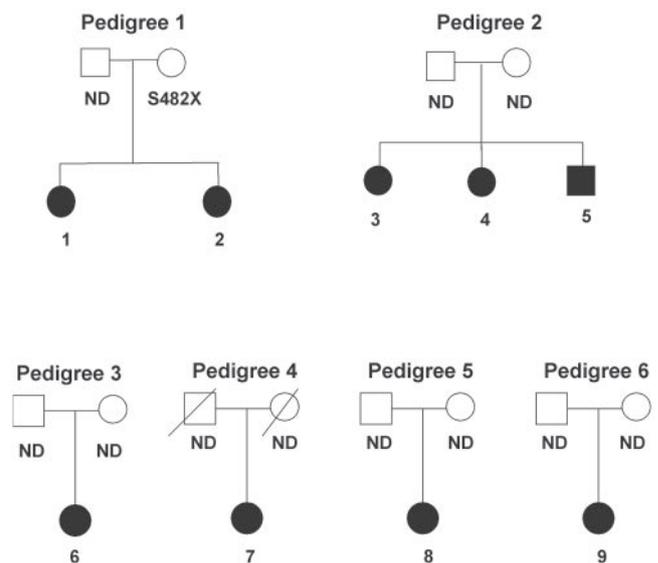


FIGURE 1. Nine Bietti crystalline dystrophy probands from five different Chinese families and one Indonesian family were analyzed. Filled symbols: BCD-affected patients; open symbols: unaffected subjects; ND denotes phenotype and genotype not determined.

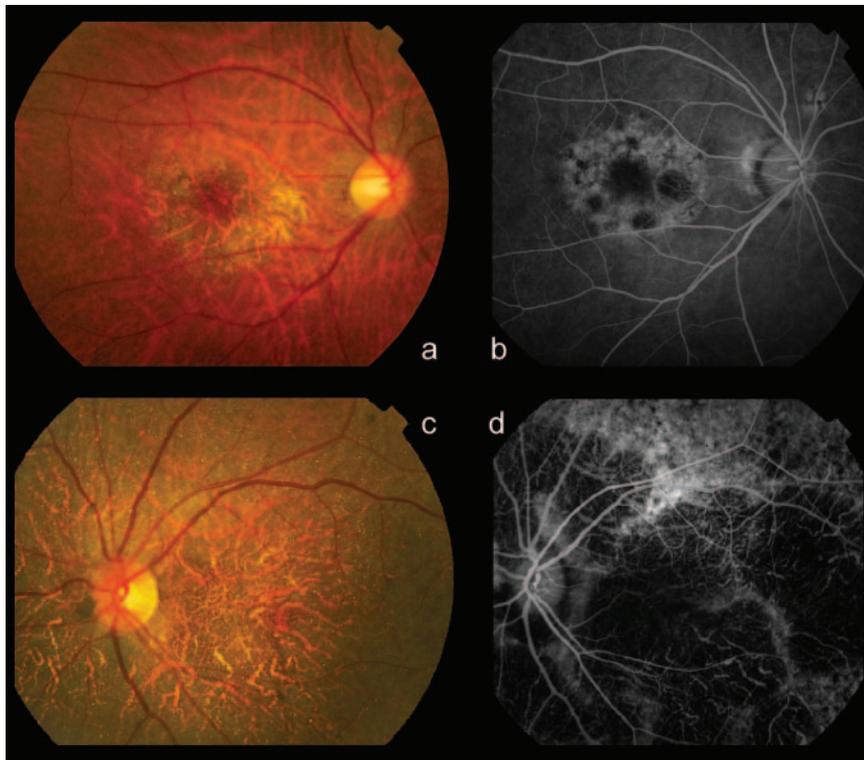


FIGURE 2. Fundal photographs with corresponding fluorescein angiography of patient 5 with stage 2 disease (**a, b**); and patient 9 with stage 3 disease (**c, d**). Both patients were homozygous for the exon 7 deletion mutation in the *CYP4V2* gene.

and seven patients (3–9) presented with decreased vision. Within the family of three siblings, patient 5 had onset of symptoms (nyctalopia and decreased vision) in the third decade, and the two sisters (patients 3 and 4) presented with decreased vision in the fourth decade. The Snellen visual acuity of the study subjects is listed in Table 1. All the patients were myopic, with refraction ranging from -1.0 to -10.75 D (spherical equivalent). Corneal limbal crystals were absent in all nine patients. Patient 4 had bilateral polar cataracts, and patient 7 had bilateral nuclear sclerotic cataracts.

Fundus examination showed a range of severity, with glistening intraretinal crystals distributed in the posterior pole and also midperiphery, RPE and choriocapillaris atrophy, pigment clumping, and retinal scarring (Fig. 2 and Table 1). Visual field testing was performed in eight patients. Unilateral paracentral and central scotomas were documented in patients 3 and 5. Patients 1, 2, 4, 6, 8, and 9 had bilateral central scotomas. In the sibling pair, patient 2 had smaller scotomas than patient 1. Patient 6 had a tiny central island of vision with surrounding scotomas in the left eye.

Six patients (1, 3, 4, 5, 6 and 8) underwent ERG (Table 1). Full-field ERG in patient 5 showed decreased scotopic b-wave amplitudes with normal implicit times. The maximal responses were within normal limits but demonstrated an electronegative waveform (left eye worse than right eye). The photopic a- and b-wave amplitudes were both decreased with normal implicit times. Multifocal ERG in the right eye showed widespread attenuation of P1 responses with significant delay; the left eye had paracentral attenuation of P1 responses with significant delay in implicit time (ring 2–3) corresponding to the area of maximal crystal deposits around the fovea. These findings have not been previously described in patients with BCD.

Mutation and Haplotype Analysis

Three different mutations were identified in the patients (Table 1). The previously reported deletion within exon 7,⁸ a 15-bp deletion that also included the 3' splice site for exon 7, was the

most common mutation identified. Six patients were homozygous for this mutation, and three patients were compound heterozygotes with this mutation. The frequency of this mutation in the healthy population was estimated by the analysis of 236 healthy Chinese individuals, and the carrier frequency was determined to be 0.004 (1 of 236).

We also identified two novel pathogenic mutations in the *CYP4V2* gene: a C to A transversion mutation in exon 11 that resulted in the premature termination of the protein at codon 482 (S482X) in patients 1 and 2 (sibling pair) and an A to C transversion mutation in exon 9 that resulted in the substitution of the amino acid lysine for threonine at codon 386 (K386T) in patient 8. Neither of these mutations was identified in a panel of 100 healthy Chinese control subjects.

The haplotype analysis in patients who were homozygous for the deletion mutation in exon 7 revealed that polymorphic markers within exons 3 to 8, which flanked the deletion mutation, were all homozygous (Table 2). Heterozygous single-nucleotide polymorphisms (SNPs) were observed in the 5'UTR, exon 1, intron 2, and intron 9 of these patients. The haplotype CT₆-T-A-C₅ was associated with the deletion mutation in all the patients carrying this mutation, including the heterozygotes.

Haplotype analysis in 50 Chinese control individuals showed that three common haplotypes accounted for approximately 85% of alleles at the *CYP4V2* locus, as defined by the markers within intron 3, intron 4, exon 6, and intron 8 of *CYP4V2*. These were CT₇-G-C-C₅, CT₆-T-A-C₅, and CT₇-G-C-C₄, of which CT₆-T-A-C₅ may be the ancestral haplotype associated with the deletion mutation. The frequency of normal alleles bearing this haplotype was 29%. There was a significant difference in the distribution of haplotype T-CT₆-T-A-C₅ in patients and controls ($P < 0.0001$, Fisher's exact test).

DISCUSSION

Bietti first reported cases of tapetoretinal degeneration characterized by yellow glistening retinal crystals, choroidal sclerosis,

TABLE 2. Haplotype Data from Individuals with Bietti Crystalline Dystrophy Who Were Homozygous and Heterozygous for the Deletion Mutation in Exon 7

Nucleotide Position* and Location of SNP/Marker	Type of Polymorphism	Haplotypes						
		Homozygotes†					Heterozygotes‡	
		Patients 3, 4, and 5	Patient 6	Patient 7	Patient 9	Patients 1 and 2‡	Patient 8	
-349 (Promoter)	G/C	C.G						
-296 (5'UTR)	C/A	C.C	C.A	C.A	C.C	C.C	C.C	C.C
-152 (5'UTR)	G/A	G.A	G.A	G.A	G.A	G.G	G.A	G.A
-146 (5'UTR)	C/T	C.T	C.C	C.C	C.T	C.C	C.C	C.T
64 (Ex 1)	C/G	C.G	C.C	C.C	C.G	C.C	C.C	C.G
2864 (IVS 2)	C/T	T.T	T.T	T.C	T.T	T.T	T.T	T.C
5079 (IVS 3)	(CT) _n	(CT)₆ , (CT)₆	(CT)₆ , (CT)₇					
5685 (IVS 4)	G/T	T.T	T.T	T.T	T.T	T.T	T.T	T.G
7234 (Ex 6)	A/C	A.A	A.A	A.A	A.A	A.A	A.A	A.C
	IVS6-Ex7							
9334 (IVS6-Ex7)	15 bp del	Ex7del. Ex7del	Ex7del. Ex7	Ex7del. Ex7				
13579 (IVS8)	C/-	C₅.C₅	C₅.C₅	C₅.C₅	C₅.C₅	C₅.C₅	C₅.C₅	C₅.C₄
17221 (IVS 9)	A/G	A.A	G.G	A.G	A.G	A.G	A.G	A.G

* The A of the initiator codon of *CYP4V2* is denoted nucleotide +1. The genomic sequence of *CYP4V2* is available at GenBank accession no. NT_022792. SNP, single-nucleotide polymorphism.

† Haplotype phases were determined through the PHASE program; founder haplotype appears in bold.

‡ Haplotypes were determined from parental haplotypes.

and marginal crystalline dystrophy of the cornea. Interestingly, none of the patients in the present study had corneal limbal crystals. Similar cases in which there were no limbal crystalline deposits have been reported and have been called Bietti crystalline fundus dystrophy or crystalline retinopathy.¹⁵⁻¹⁸

The patients presented with visual symptoms from the third decade onward. The most common presenting symptom was a decrease in visual acuity, often accompanied by nyctalopia. Visual acuity was good even in patients with severe disease, as central vision had not been affected. This sparing of the central vision until late in the disease was also recently described in European patients with BCD.¹⁹ The clinical grading used in this study correlated well with the ERG classification we proposed. Patients with RPE atrophy and intraretinal crystals confined to the posterior pole tended to show lesser disturbances in the full-field ERG. This is in contrast to Stargardt's disease, in which the distribution of flecks is not a good indicator of the extent of retinal dysfunction, as demonstrable on full-field ERG.²⁰ Visual field testing in patient 6 showed a tiny island of preserved central vision, surrounded by large paracentral scotomas in the left eye. Despite having a difference in visual acuity, the ERG of patient 6 (classified as group D) showed that the patient did have severe bilateral disease.

As BCD has been described as a progressive disease, one would expect older patients to have more severe disease. In the sibling pair (patients 1 and 2), the older sibling (patient 1) had more severe disease (stage 3) than her younger sister (patient 2). However, the rate of progression may be variable, as illustrated by patients 6 and 7. At the age of 32, patient 6 was the youngest patient in the series, and ironically she had the most severe clinical phenotype; whereas patient 7, in her 80s, had stage 2 disease. Thus, age does not always correlate with disease severity and progression. This suggests the possible involvement of environmental factors such as diet or modifier genes in the pathogenesis of BCD. Cells cultured from patients with BCD have been reported to have abnormally high levels of triglycerides and cholesterol storage, and an abnormal lipid metabolism associated with BCD could be the result of defects in fatty acid metabolism in binding, elongation, or desaturation.^{6,8} It is possible that environmental factors such as diet

may affect lipid metabolism and hence the phenotypic expression of the disease.

We found three mutations in the series of BCD patients. Two of the mutations, K386T in exon 9 and S482X in exon 11, are novel and not found in a panel of 100 healthy control subjects, suggesting that these are pathogenic mutations and not common polymorphisms. The S482X mutation in exon 11 results in the loss of 44 amino acids at the C terminus of the protein, and therefore the removal of a beta sheet that lies adjacent to the central I helix,⁸ which is likely to perturb the protein structure in a major way. The K386T mutation lies adjacent to the first member of the ERR triad that coordinates the heme ring.⁸ Conservation of the K386 residue between human *CYP4V2* and *Mus musculus* *CYP4V3* (data not shown) further indicates it to be a functionally important residue. Therefore, these mutations are very likely to interfere with the protein's enzymatic function.

The 15-bp deletion that included the 3' (acceptor) splice site for exon 7 has been reported previously,⁸ and results in the skipping of exon 7, with the loss of amino acids 268 to 329. This was predicted to cause the uncoiling and displacement of the central helix, leading to protein dysfunction.⁸ This change was the most common mutation found in the study patients. Six of the patients were homozygous for this mutation, and the remaining three patients were compound heterozygotes. This finding implies high carrier prevalence for this deletion mutation in the study population and possibly the Chinese population. In our study the heterozygote carrier frequency was calculated to be 0.004 from the analysis of 236 healthy controls of Chinese ethnicity. This also gave an estimated population frequency of 0.002 for the deleted allele. This correlates well, as BCD has been reported to be more common in East Asia, especially in the Chinese and Japanese populations. An estimated gene frequency of 0.005 was reported in an epidemiologic survey of genetic disease in a general population in China.²¹

The haplotype analysis carried out to see whether the deletion mutation in exon 7 was a recurrent or an ancient founder mutation, like the F508del mutation in *CFTR* gene for cystic fibrosis,²² was limited by the small number of homozy-

gous individuals available. Yet the linkage disequilibrium between the deletion mutation and polymorphic markers were complete for four of the analyzed markers (Table 2). All the mutant exon 7 alleles derived from the homozygotes and heterozygotes bore the CT₆-T-A-C₅ core founder haplotype; in contrast, only 29% of the normal exon 7 alleles derived from 50 healthy controls bore this haplotype. Statistical analysis (two-tailed Fisher's exact test) confirmed that the difference in founder haplotype frequency between normal alleles and alleles bearing the exon 7 deletion mutation was significant ($P < 0.0001$, Fisher's exact test). This strongly supports the conclusion that the deletion mutation in exon 7 arose from a single founder. Therefore, the heterozygous SNPs observed in the 5'UTR, exon 1, exon 2, and within intron 9 could be due to intragenic recombination events and new mutations that occurred subsequent to the deletion mutation. Moreover the length of the chromosomal segment (at least 8.5 kb) that appears to be in linkage disequilibrium with the mutation indicates this deletion to be an ancient mutation, likely to have arisen in an ancestor common to the Chinese and the Japanese and predating the Chinese migration to Singapore. Further clues to the age and origin of this mutation may be provided by the analysis of Japanese individuals with the deletion mutation in exon 7.

Due to the small number of patients in this study, it was difficult to draw phenotypic and genotypic correlations. Of the patients who were homozygous for deletion in exon 7, four of six were in the earlier stages of the disease. The three siblings who were found to be homozygous for the deletion in exon 7 (patients 3, 4, and 5) seemed to express a similar stage of the disease. Patients who were compound heterozygotes, carrying the deletion in exon 7 in only one of the alleles, seemed to have a more severe form of BCD (patients 1 and 8, in stage 3) compared to patients homozygous for the deletion, who as mentioned earlier had a better prognosis in terms of severity and blindness.

There is a need for more research to investigate the function of the CYP4V2 protein and its interactions. Through such studies, it may be possible to see if dietary or other environmental modifications could result in a slower progression of the disease. Longitudinal studies on patients with BCD may reveal further differences in the clinical phenotype and severity of the disease according to their genotype.

Acknowledgments

The authors especially thank the patients and their families for taking part in this study. They also thank Jianjun Liu at the Genome Institute of Singapore, for helping with the PHASE program, and Chi Luu of the Singapore Eye Research Institute, for help with electrophysiology.

References

1. Bietti G. Ueber familiars Vorkommen von "Retinitis punctata albescens" (verbunden mit "Dystrophia marginalis cristallinea cor-

- neae"). Glitzen des Glaskorpers and Andersen degenerativen Augen veränderungen. *Klin Monatsbl Augenbeilkd.* 1937;99:737-756.
2. Mataftsi A, Zografos L, Milla E, et al. Bietti's crystalline corneoretinal dystrophy: a cross-sectional study. *Retina.* 2004;24:416-426.
3. Bagolini B, Ioli-Spada G. Bietti's tapetoretinal degeneration with marginal corneal dystrophy. *Am J Ophthalmol.* 1968;65:53-60.
4. Bernauer W, Daicker B. Bietti's corneal-retinal dystrophy—a 16 year progression. *Retina.* 1992;12:18-20.
5. Wilson DJ, Weleber RG, Klein ML, et al. Bietti's crystalline dystrophy. A clinicopathologic correlative study. *Arch Ophthalmol.* 1989;107:213-221.
6. Lee J, Jiao XD, Hejtmancik JF, et al. The metabolism of fatty acids in human Bietti crystalline dystrophy. *Invest Ophthalmol Vis Sci.* 2001;42:1707-1714.
7. Lee J, Jiao XD, Hejtmancik JF. Identification, isolation, and characterization of a 32-kDa fatty acid-binding protein missing from lymphocytes in humans with Bietti crystalline dystrophy (BCD). *Mol Genet Metab.* 1998;65:143-154.
8. Li A, Jiao XD, Munier FL, et al. Bietti crystalline corneoretinal dystrophy is caused by mutations in the novel gene *CYP4V2*. *Am J Hum Genet.* 2004;74:817-826.
9. Yuzawa M, Mae Y, Matsui M. Bietti's crystalline retinopathy. *Ophthalmic Paediatr Genet.* 1986;7:9-20.
10. Marmor MF, Holder GE, Seeliger MW, Yamamoto S. Standard for clinical electroretinography (2004 update). *Doc Ophthalmol.* 2004;108:107-114.
11. Marmor MF, Zrenner E. Standard for clinical electroretinography (1999 update). *Doc Ophthalmol.* 1998;97:143-156.
12. Marmor MF, Hood DC, Keating D, Kondo M, Seeliger MW, Miyake Y. Guidelines for basic multifocal electroretinography (mfERG). *Doc Ophthalmol.* 2003;106:105-115.
13. Brigell M, Bach M, Barber C, Moskowitz A, Robson J. Guidelines for calibration of stimulus and recording parameters used in clinical electrophysiology of vision. *Doc Ophthalmol.* 2003;107:185-193.
14. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet.* 2001;68:978-989.
15. Hayasaka S, Okuyama S. Crystalline retinopathy. *Retina.* 1984;4:177-181.
16. Grizzard WS, Deutman AF, Nijhuis F, de Kerk AA. Crystalline retinopathy. *Am J Ophthalmol.* 1978;86:81-88.
17. Francois J, De Laey JJ. Bietti's crystalline fundus dystrophy. *Ann Ophthalmol.* 1978;10:709-716.
18. Kaiser-Kupfer MI, Chan CC, Markello TC, et al. Clinical biochemical and pathologic correlations in Bietti's crystalline dystrophy. *Am J Ophthalmol.* 1994;118:569-582.
19. Mataftsi A, Zografos L, Milla E, Secretan M, Munier FL. Bietti's crystalline corneoretinal dystrophy: a cross-sectional study. *Retina.* 2004;24:416-426.
20. Kretschmann U, Seeliger MW, Ruether K, Usui T, Apfelstedt-Sylla E, Zrenner E. Multifocal electroretinography in patients with Stargardt's macular dystrophy. *Br J Ophthalmol.* 1998;82:267-275.
21. Hu DN. Ophthalmic genetics in China. *Ophthalm Paed Genet.* 1983;2:39-45.
22. Dork T, Neumann T, Wulbrand U, et al. Intra- and extragenic marker haplotypes of CFTR mutations in cystic fibrosis families. *Hum Genet.* 1992;88:417-425.