Alterations in Serum Fatty Acid Concentrations and Desaturase Activities in Bietti Crystalline Dystrophy Unaffected by *CYP4V2* Genotypes

Timothy Y. Y. Lai, Kai-On Chu, Kwok-Ping Chan, Tsz-Kin Ng, Gary H. F. Yam, Dennis S. C. Lam, and Chi-Pui Pang

Purpose. To evaluate the serum fatty acid changes in Chinese patients with Bietti crystalline dystrophy (BCD) in association with *CYP4V2* mutation.

Methods. Sixteen Chinese patients with BCD confirmed with *CYP4V2* mutation were recruited. Peripheral venous blood was obtained after fasting and serum fatty acid concentrations were measured and compared with those in 13 control subjects. Δ-9-desaturase and Δ-5-desaturase activities were estimated based on serum fatty acid compositions. Serum insulin and glucagon concentrations and their correlations with fatty acid and desaturase activities were also evaluated. Fatty acid concentrations were compared among patients with BCD with different genotypes or phenotypes.

Results. Patients with BCD were found to have a significantly higher concentration of octadecanoic acid (18:0) than that in control subjects (18.28% versus 13.52%, $P = 0.007$), as well as a lower concentration of octadecadienoic acid (18:1n-9) than that in control subjects (10.97% vs. 14.88%, $P = 0.007$). The total monounsaturated fatty acid concentration was significantly lower in BCD than in the control (11.82% vs. 15.85%, $P = 0.012$). The activity of Δ-9-desaturase was also significantly lower in BCD (0.71 vs. 1.14, $P = 0.004$). Serum glucagon was significantly associated with increased total unsaturated fatty acid and decreased polyunsaturated fatty acid in control subjects but not in patients with BCD. No significant difference in the fatty acid concentration and desaturase activities was found in patients with different genotypes or phenotypes.

Conclusions. Abnormal serum fatty acid composition with reduced Δ-9-desaturase activity was detected in patients with BCD, and the metabolic derangement was unaffected by *CYP4V2* mutations. The findings suggest that systemic abnormality in fatty acid metabolism occurs in patients with BCD independent of *CYP4V2* genotype. (*Invest Ophthalmol Vis Sci.* 2010;51:1092–1097) DOI:10.1167/iovs.09-3665

Bietti crystalline dystrophy (BCD) is a progressive retinal degenerative disease first described by Bietti in 1937.[1] The disease is characterized by progressive atrophy of the retinal pigment epithelium (RPE) and choriocapillaris, with or without limbal corneal and retinal crystals.[1,2] The pure retinal form of the disease is more common in Asians particularly in Chinese and Japanese when compared with Caucasians.[3–5] BCD typically manifests between the second and fourth decades of life, and patients present with progressive night blindness, reduced vision, and visual field constriction.[3]

CYP4V2 has been identified as the causative gene for BCD, which confirms that BCD is a genetic disorder with an autosomal recessive inheritance.[6–12] *CYP4V2* is a member of the cytochrome P450 hemethiolate protein superfamily and is responsible for oxidation of various substrates in the metabolic pathway. Metabolic studies of cells cultured from patients with BCD have demonstrated abnormally high triglycerides and cholesterol storage, with reduced conversion of fatty acid precursors into n-3 polyunsaturated fatty acids (PUFAs).[13] These findings suggest that BCD may result in systemic lipid metabolism abnormalities. Altered fatty acid compositions have also been demonstrated in tissue phospholipids of rats with retinal dystrophy as well as of sperm in patients with retinitis pigmentosa.[14,15] Moreover, by evaluating the ratios of different fatty acids, it has been found that the miniature poodle with progressive rod–cone degeneration has impaired desaturase activity, which catalyzes the introduction of double bond into fatty acids.[16] Since BCD is also a form of hereditary retinal dystrophy, we hypothesize that changes in systemic fatty acid metabolism can also occur. Such metabolic derangement may be associated with *CYP4V2* or with fatty acid–metabolizing enzymes. We therefore performed a study to evaluate the serum fatty acid changes and desaturase activities in patients with BCD with *CYP4V2* mutations. In addition, we also assessed the serum insulin and glucagon concentrations, to investigate the potential alteration in fatty acid metabolism during fasting in patients with BCD.

Methods

Recruitment of Subjects

This was a cross-sectional study and patients with BCD were recruited at the Department of Ophthalmology and Visual Sciences, Chinese University of Hong Kong. All index patients had a diagnosis of BCD based on the typical ophthalmic findings and confirmed by the presence of pathogenic *CYP4V2* mutations. A genetic mutation analysis of the *CYP4V2* gene has been published.[12] Control subjects were non–blood-related family members with normal ophthalmic examination results (e.g., spouse of affected subjects). Written informed consent was obtained from all patients and their family members. The research protocol was approved by an institutional review board, and the study was performed in accordance with the tenets of the Declaration of Helsinki.

From the Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong.

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Corresponding author: Timothy Y. Y. Lai, Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong Eye Hospital, 147K Argyle Street, Kowloon, Hong Kong; tyylai@cuhk.edu.hk.

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TABLE 1. Target Fatty Acids and Their Corresponding Retention Times in Gas Chromatography Mass Spectrometry Analysis

Fatty Acids	Retention Time (min)
15:1cis (internal standard)	24.72
16:0	25.25
16:1	26.21
18:0	29.27
18:1n-9	30.55
18:2n-6	33.01
18:3n-3	35.73
20:3n-6	40.09
20:4n-6	41.16
24:0	44.87
24:1	46.02
22:5n-3	48.65
22:6n-3	49.78

Samples and Standards Preparations

Peripheral venous blood was collected from all subjects after a minimum fasting period of at least 8 hours. Plasma were extracted from blood samples of the subjects and stored in a −83°C freezer until analysis. The fatty acids we targeted are shown in Table 1. Fatty acid standard mixture (Supelco Inc., Bellefonte, PA) was prepared by diluting the fatty acids in hexane, resulting a concentration of 50 μg/mL for the standards and an internal standard concentration of 15 μg/mL for *cis*-pentadecenoic acid (15:1 cis). A 300-μL sample of plasma was evaluated using 30 μL of internal standard solution mixed with 300 μL methanol/chloroform (1:2) solution. After extraction, the mixture was centrifuged and the supernatant was extracted with a pipette twice. After drying under nitrogen, the residue was dissolved in 80 μL hexane and mixed with 20 μL commercial esterification reagent (Meth-Prep II; Alltech, Deerfield, IL) and allowed to stand for 30 minutes. One microliter of the mixture was taken for gas chromatography mass spectrometry (GCMS) analysis.

GCMS Analysis of Fatty Acids

GCMS was performed on a commercial system (Agilent 6890 GC connected to a 5973 Agilent MS system; Agilent Technologies, Santa Clara, CA). Chromatography of methylated fatty acids were conducted by a 0.2 × 0.25 × 60-m column (HP-88; Agilent Technologies). Splitless mode was used with 2 mL/min helium carrier flow. Inlet temperature was set at 280°C and initial oven temperature was set at 100°C and kept for 10 minutes. The temperature was then raised to 175°C at 6°C/min ramping rate and kept for 10 minutes and further raised to 210°C for 5 minutes at a ramping rate of 3°C/min. Finally, it was increased to 230°C for 5 minutes at the rate of 3°C/min. Mass spectra were measured in a range of 40 to 550 m/z and the compositions of serum fatty acids expressed as mol% of total fatty acids after subtraction of unidentified peaks.

Estimation of Desaturase Activity

The activities of various desaturases were estimated from the product-to-precursor ratio of individual fatty acids. Therefore, the Δ-9 desaturase was estimated from the concentration of 18:1n-9 divided by 18:0 and Δ-5 desaturase from the concentration of 20:4n-6 divided by 20:3n-6.[17]

Measurement of Serum Insulin and Glucagon Concentrations

Serum insulin concentration was measured using the ACTIVE Insulin ELISA Kit (Diagnostic Systems Laboratories, Inc., Webster, TX) according to manufacturer's instruction. Briefly, 25 μL of known standards or serum samples and 100 μL insulin antibody-enzyme conjugate solution were added into each well. The wells were then incubated for 1 hour at room temperature, with 500 rpm shaking. After aspiration and washing with washing solution, 100 μL of TMB chromogen solution was added into the wells and incubated in darkness for 10 minutes at room temperature. The reaction was then stopped by adding 100 μL of stopping solution and the absorbances of the wells were read by a microplate spectrophotometer (PowerWave XS; Bio-Tek) at 450 nm, with background wavelength correction at 600 nm. The lower detection limit of the insulin assay was 0.26 μIU/mL.

Glucagon concentration in the plasma samples were quantified by the rat, mouse, and human EIA kit (GLP-1; Kamiya Biomedical Company, Seattle, WA) according to the manufacturer's instruction. Briefly, the wells were washed with 350 μL diluted saline. After aspiration, 40 μL of biotinylated pancreatic glucagon was added, followed by addition of 30 μL of standards or serum samples to the individual wells. After addition of 40 μL GLP-1 antibody, the wells were incubated at 4°C for 16 hours. After aspiration and washing, 100 μL of diluted HRP-labeled streptavidin solution was added, and the wells were incubated for 1 hour at room temperature. After aspiration and washing, 100 μL of 0.015% hydrogen peroxide was then added, and the wells were incubated for 15 minutes at room temperature. The reaction was then stopped by adding 100 μL NH_2SO_4. The optical absorbance of the wells was read by microplate spectrometer (PowerWave XS; Bio-Tek) at 492 nm. The lower detection limit of the glucagon assay was 0.21 ng/mL.

Statistical Analysis

All data were entered into statistical software (SPSS for Windows. ver. 15.0; SPSS Inc., Chicago, IL) for analysis. Analysis of categorical variables was performed with the χ^2 test, and comparisons of continuous variables were performed using the nonparametric Mann-Whitney U test. Nonparametric Spearman correlation analysis was performed to determine the correlation of plasma insulin and glucagon and the fatty acid concentrations and activities of the desaturases. $P \leq 0.05$ was considered statistically significant.

RESULTS

Subjects Demographics and Genotype Analysis

A total of 16 patients with BCD from 14 families were recruited, and all were of Chinese ethnicity. The mean ± SD age of the subjects was 49.5 ± 13.9 years (range, 31–75). There were nine (48.3%) women and seven (56.2%) men. In the control group, the mean ± SD age was 55.0 ± 18.6 years (range, 30–76), and there were six (56.2%) women and seven (43.8%) men. The control group was comparable with the BCD group in terms of mean age (Mann-Whitney U test, $P = 0.45$) and gender (χ^2 test, $P = 0.59$). The genotype and full-field electroretinography (ERG) findings of the patients with BCD are listed in Table 2. The genotype and specific phenotypes of patients numbered 1 to 15 have been reported previously and are summarized in Table 2.[12]

Serum Fatty Acid Concentrations

The mean percentage composition of the serum fatty acids in the patients with BCD and control subjects are displayed in Table 3. Among the 12 fatty acids evaluated, five fatty acids accounted for more than 80% of the serum fatty acid compositions. These included 16:0, 18:0, 18:1n-9, 18:2n-6, and 22:6n-3. The concentrations of two of fatty acids, 18:0 and 18:1n-9, were found to be significantly different between patients with BCD and control subjects. The concentration of 18:0 was significantly higher in patients with BCD compared with the controls, with concentrations of 18.28% and 13.52%, respectively (Mann-Whitney U test, $P = 0.007$). For the 18:1n-9 fatty acid, patients with BCD had significantly lower concentration compared with controls, with 10.97% compared with 14.88% (Mann-Whitney U test, $P = 0.007$).

TABLE 2. Genotype, Phenotype, and Full-Field ERG Findings in 16 Chinese Patients with BCD

Patient (family) No.	Age	Genetic Mutation	Visual acuity	Fundus Findings	Scotopic ERG	Maximum Response ERG	Photopic ERG	30-Hz Flicker ERG
1 (1)	44	IVS6-8del17bp/insGC + IVS8-2A>G	RE: 20/40 LE: 20/200	CD, BSP	Reduced amp./ normal i.t.	Reduced amp./ normal i.t.	Reduced amp./ normal i.t.	Reduced amp.
2 (2)	59	IVS6-8del17bp/insGC + W244X	RE: 20/40 LE: 20/30	CD, CA	Reduced amp./ normal i.t.	Low normal amp./normal i.t.	Low normal amp./normal i.t.	Reduced amp.
3 (3)	53	Homozygous IVS6-8del17bp/ insGC	RE: 20/25 LE: FC	CD, BSP, CA	Nonrecordable	Nonrecordable	Nonrecordable	Nonrecordable
4 (3)	52	Homozygous IVS6-8del17bp/ insGC	RE: 20/50 LE: 20/70	CD, BSP, CA	Nonrecordable	Nonrecordable	Nonrecordable	Nonrecordable
5 (4)	68	Homozygous H331P	RE: 20/50 LE: 20/400	CD, BSP, CA	Reduced amp./ normal i.t.	Reduced amp./ delayed i.t.	Reduced amp./ normal i.t.	Reduced amp.
6 (5)	75	IVS6-8del17bp/insGC + P396L	RE: 20/70 LE: 20/50	CD, CA	Reduced amp./ normal i.t.	Reduced amp./ normal i.t.	Reduced amp./ normal i.t.	Normal
7 (6)	30	IVS8-2A>G + D324V	RE: 20/30 LE: 20/30	CD, BSP, CA	Reduced amp./ normal i.t.	Normal amp./ normal i.t.	Normal amp./ normal i.t.	Normal
8 (7)	63	R400H + R400C	RE: FC LE: 20/400	CD, BSP, RVA, CA	Reduced amp./ delayed i.t.	Reduced amp./ delayed i.t.	Reduced amp./ normal i.t.	Reduced amp.
9 (8)	70	IVS6-8del17bp/insGC + Y219H	RE: 20/70 LE: 20/70	CD, BSP, CA	Reduced amp./ normal i.t.	Reduced amp./ normal i.t.	Reduced amp./ normal i.t.	Reduced amp.
10 (9)	31	IVS6-8del17bp/insGC + H331P	RE: 20/30 LE: 20/40	CD, BSP, CA	Nonrecordable	Nonrecordable	Nonrecordable	Reduced amp.
11 (10)	47	Homozygous H331P	RE: HM LE: 20/40	Few CD, BSP, RVA, CA	Reduced amp./ delayed i.t.	Reduced amp./ delayed i.t.	Reduced amp./ delayed i.t.	Reduced amp.
12 (10)	42	Homozygous H331P	RE: 20/30 LE: 20/30	Few CD	Reduced amp./ delayed i.t.	Reduced amp./ delayed i.t.	Reduced amp./ delayed i.t.	Reduced amp.
13 (11)	41	IVS6-8del17bp/insGC + P396L	RE: 20/40 LE: 20/25	CD, BSP, CA, retinal scarring	Reduced amp./ normal i.t.	Reduced amp./ delayed i.t.	Reduced amp./ delayed i.t.	Reduced amp.
14 (12)	40	Homozygous IVS6-8del17bp/ insGC	RE: 20/200 LE: HM	CD, BSP, CA	Nonrecordable	Reduced amp./ delayed i.t.	Reduced amp./ delayed i.t.	Reduced amp.
15 (13)	37	IVS6-8del17bp/insGC + IVS8-2A>G	RE: 20/25 LE: 20/30	CD, BSP	Nonrecordable	Reduced amp./ delayed i.t.	Reduced amp./ delayed i.t.	Reduced amp.
16 (14)	41	Homozygous H331P	RE: 20/50 LE: 20/70	CD, BSP, CA	Reduced amp./ delayed i.t.	Reduced amp./ delayed i.t.	Reduced amp./ delayed i.t.	Reduced amp.

RE, right eye; LE, left eye; HM, hand motion; FC, finger count; CD, crystalline deposits; BSP, bone spicule pigmentation; CA, choriocapillaris atrophy; RVA, retinal vascular attenuation; ERG, full-field electroretinogram; amp., amplitude; i.t., implicit times.

TABLE 3. Serum Fatty Acid Concentrations and Desaturase Activities in Patients with BCD Compared with Controls

Mean ± SD Fatty Acid Concentration (mol% of Total Fatty Acids)	BCD patients (n = 16)	Controls (n = 13)	P
16:0	35.41 ± 8.73	35.23 ± 5.87	0.71
16:1	0.76 ± 0.40	0.97 ± 7.34	0.71
18:0	18.28 ± 5.30	13.52 ± 1.81	0.007
18:1n-9	10.97 ± 4.15	14.88 ± 3.32	0.008
18:2n-6	25.38 ± 11.60	29.04 ± 4.56	0.17
18:3n-3	1.88 ± 0.92	1.33 ± 0.66	0.13
20:3n-6	0.23 ± 0.16	0.14 ± 0.11	0.08
20:4n-6	1.87 ± 2.18	1.31 ± 0.99	0.48
22:5n-3	0.23 ± 0.16	0.14 ± 0.11	0.08
22:6n-3	4.56 ± 2.37	3.22 ± 1.66	0.12
24:0	1.90 ± 2.20	1.31 ± 0.99	0.48
24:1	0.10 ± 0.14	0.00 ± 0.00	0.09
Δ-9 desaturase	0.71 ± 0.49	1.14 ± 0.37	0.004
Δ-5 desaturase	0.56 ± 0.82	0.47 ± 0.33	0.35
Total of saturated fatty acid	55.59 ± 11.98	50.07 ± 6.08	0.10
Total of monounsaturated fatty acid	11.82 ± 4.14	15.85 ± 3.96	0.012
Total of polyunsaturated fatty acid	32.58 ± 11.77	34.08 ± 5.60	0.75

Of the two desaturase activities calculated, the activity of Δ-9 desaturase was significantly lower in patients with BCD than in control subjects, with 0.71 and 1.14 respectively (Mann-Whitney U test, $P = 0.007$). There was no significant difference in the Δ-5 desaturase activity between patients with BCD and control subjects.

Serum Insulin and Glucagon Concentrations and Their Correlations with Fatty Acid Levels and Desaturase Activities

In the 16 patients with BCD, the mean ± SD serum insulin and glucagon concentrations were 16.5 ± 17.0 μIU/mL and 1.97 ± 3.26 ng/mL, respectively. In the control group, the mean ± SD serum insulin and glucagon concentrations were 17.6 ± 16.9 μIU/mL and 1.14 ± 1.10 ng/mL, respectively. There was no significant difference in the serum insulin and glucagon concentrations between the patients with BCD and control subjects (Mann-Whitney U test, $P = 0.56$ and $P = 0.23$, respectively).

In both the control and BCD groups, no significant correlation was found between serum insulin concentration and fatty acid concentrations or the activities of the two desaturases ($P > 0.05$). However, in the 13 control subjects, there was a significant positive correlation between glucagon concentration and total serum unsaturated fatty acid concentration (Spearman $\rho = 0.56$, $P = 0.049$). There was also a significant negative correlation between glucagon level and total serum

TABLE 4. Serum Fatty Acid Concentrations and Desaturase Activities in Patients with BCD with Homozygous or Heterozygous IVS6 or IVS8 Mutation in the *CYP4V2* Gene versus Other *CYP4V2* Mutations

Mean ± SD Fatty Acid Concentration (mol% of Total Fatty Acids)	Homozygous or Heterozygous IVS6 or IVS8 Mutation (n = 11)	Other *CYP4V2* Mutations (n = 5)	P
16:0	37.03 ± 9.59	31.85 ± 5.74	0.22
16:1	0.75 ± 0.47	0.77 ± 0.26	0.74
18:0	18.23 ± 4.99	18.39 ± 6.58	1.00
18:1n-9	11.54 ± 9.59	9.72 ± 5.03	0.83
18:2n-6	23.81 ± 11.82	28.83 ± 11.57	0.66
18:3n-3	1.95 ± 1.01	1.72 ± 0.77	0.66
20:3n-6	0.22 ± 0.16	0.26 ± 0.17	0.91
20:4n-6	1.50 ± 0.91	2.68 ± 3.82	0.83
22:5n-3	0.21 ± 0.15	0.26 ± 0.16	0.82
22:6n-3	4.40 ± 2.38	4.92 ± 2.56	0.91
24:0	1.53 ± 0.93	2.72 ± 3.86	0.91
24:1	0.12 ± 0.15	0.06 ± 0.13	0.58
Δ-9 desaturase	0.74 ± 0.52	0.66 ± 0.48	1.00
Δ-5 desaturase	0.47 ± 0.56	0.76 ± 1.30	0.91
Total of saturated fatty acid	56.78 ± 11.60	52.96 ± 13.78	0.91
Total of monounsaturated fatty acid	12.40 ± 3.91	10.55 ± 4.80	0.83
Total of polyunsaturated fatty acid	30.81 ± 12.68	36.49 ± 9.48	0.44

polyunsaturated concentration (Spearman $\rho = -0.59$, $P = 0.035$). In the 16 patients with BCD, no significant correlation was found between glucagon level and the concentrations of the fatty acids and the activities of the two desaturases ($P > 0.05$).

Fatty Acid Concentrations and Desaturase Activities Based on Genotype and Electroretinography

Further analysis was performed to evaluate the fatty acid concentrations and desaturase activities in patients with different genotypes and full-field ERG findings. Table 4 shows the fatty acid concentrations and desaturase activities in patients with homozygous or heterozygous IVS6 or IVS8 (intron) mutations in the *CYP4V2*, gene compared with other mutations. No significant difference was observed between the two groups. Table 5 shows the fatty acid concentrations and desaturase activities of patients with nonrecordable versus recordable full-field ERG, and analysis again showed no significant difference between the two groups. No significant difference was found in the proportion of nonrecordable full-field ERG between patients with homozygous or heterozygous IVS6 or IVS8 (intron) mutations in the *CYP4V2* gene compared with other mutations (Fisher exact test, $P = 0.99$).

DISCUSSION

CYP4V2 is one of 63 members of the cytochrome P450 gene 4 family (CYP4) which catalyzes the ω-hydroxylation of saturated, branched chain, and unsaturated fatty acids.[18] Six CYP4 subfamilies have been identified in mammals: CYP4A, CYP4B, CYP4F, CYP4V, CYP4X, and CYP4Z.[18–20] Members of the CYP4B, CYP4A, and CYP4F subfamilies have been shown to have specificity in metabolizing short-chain (C7–C9), medium-chain (C10–C16), and long-chain fatty acids (C18–C26) respectively.[18] However, the exact specificities of CYP4V, CYP4X, and CYP4Z have yet to be determined.[20] *CYP4V2* is highly expressed in the human retina, kidneys, lungs, and liver and has been suggested to function in the prevention of lipotoxicity.[18] This finding is supported by the clinical observation of multiple refractile lipid-like crystals in retinas of patients with BCD, which may suggest abnormal lipid accumulation in the retina. Previous laboratory study has demonstrated that abnormality in fatty acid metabolism occurs in cells prepared from patients with BCD.[13] In addition, increased expression of the *CYP4V2* gene has been found in patients with colorectal cancer, and single nucleotide polymorphisms in the *CYP4V2* gene have been found to be associated with deep vein thrombosis.[21,22] These findings suggest that *CYP4V2* mutations in patients with BCD may result in a more generalized metabolic disorder rather than being limited purely to the eyes, as seen clinically.

In this study, we evaluated the serum fatty acid concentrations and estimated the desaturase activities in patients with BCD and control subjects. We found that the serum concen-

TABLE 5. Comparison of Serum Fatty Acid Concentration in Patients with BCD with Different Scotopic Full-Field ERG Characteristics

Mean ± SD Fatty Acid Concentration (mol% of Total Fatty Acids)	Nonrecordable Full-Field Scotopic Electroretinography (n = 7)	Recordable Full-Field Scotopic Electroretinography (n = 9)	P
16:0	35.99 ± 13.08	34.97 ± 3.79	0.54
16:1	0.70 ± 0.56	0.80 ± 0.28	0.30
18:0	15.68 ± 5.32	20.30 ± 4.58	0.14
18:1n-9	13.09 ± 4.42	9.32 ± 3.25	0.21
18:2n-6	26.73 ± 5.66	24.33 ± 9.02	0.54
18:3n-3	1.92 ± 1.25	1.84 ± 0.64	0.76
20:3n-6	0.18 ± 0.11	0.27 ± 0.19	0.41
20:4n-6	1.30 ± 0.93	2.31 ± 2.79	0.92
22:5n-3	0.18 ± 0.11	0.26 ± 0.18	0.41
22:6n-3	4.01 ± 2.13	4.99 ± 2.57	0.54
24:0	1.31 ± 0.94	2.35 ± 2.82	0.92
24:1	0.06 ± 0.10	0.13 ± 0.17	0.41
Δ-9 desaturase	1.00 ± 0.62	0.49 ± 0.21	0.25
Δ-5 desaturase	0.49 ± 0.68	0.62 ± 0.96	0.84
Total of saturated fatty acid	52.99 ± 16.14	57.62 ± 7.96	0.47
Total of monounsaturated fatty acid	13.84 ± 4.44	10.25 ± 3.30	0.21
Total of polyunsaturated fatty acid	33.17 ± 16.62	32.13 ± 7.21	0.47

tration of the saturated 18:0 fatty acid was significantly higher in patients with BCD compared than in control subjects, whereas the concentration of the monounsaturated 18:1n-9 fatty acid was significantly lower in patients with BCD than in control subjects. Moreover, the concentration of total monounsaturated fatty acid was also significantly lower in the BCD group. These changes in fatty acid composition are associated with reduced desaturation of medium chain saturated fatty acid to monounsaturated fatty acid and imply a reduced activity of Δ-9 desaturase. The exact reason for the association of *CYP4V2* mutation and reduced Δ-9 desaturase activity is unclear. Δ-9 desaturase is also known as stearoyl-CoA desaturase (SCD) and is responsible for the last step of the synthesis of 18:1n-9 fatty acid from acetyl CoA.[23] SCD is of physiological importance for maintaining the composition of cell membrane phospholipids.[24] The expression of SCD is regulated by various hormones including insulin, as well as dietary n-6 and n-3 PUFAs, via two transcription factors: sterol regulatory elementary binding protein-1c and peroxisome proliferator activated receptor-α.[23] Therefore, it may be that the reduced Δ-9 desaturase activity observed in patients with BCD is secondary to the increase in dietary n-6 and n-3 PUFAs. However, our results showed that there was no significant difference in the level of various PUFAs except 18:1n-9, which has been shown to have no effect in suppressing SCD in vitro.[25] Therefore, the reduced Δ-9 desaturase observed in patients with BCD was unlikely to be due to the levels of various PUFAs but was directly associated with the *CYP4V2* mutation. A recent study has shown that *Drosophila* with mutation in the stearic ω-hydroxylase *CYP4g1* gene was found to cause imbalance in fatty acid desaturation, with a twofold increase in 18:1/18:0 fatty acid ratio.[26] It has been postulated that the human CYP4F subfamily may have similar function in regulating the 18:1/18:0 ratio by affecting the SCD activity or by competing for 18:0 substrates.[18] Therefore, it is likely that the reduced SCD activity with reduced 18:1/18:0 ratio observed in our patients with BCD was directly associated with mutation in the *CYP4V2* gene. Previous findings by Lee et al.[27] have also demonstrated that the fatty acid binding activities of two proteins of the molecular masses 32 and 45 kDa were abnormally low or missing in patients with BCD, and this may have resulted in fatty acid metabolism abnormalities. The exact mechanism of the reduced desaturation index is unclear but may be due to inhibition of SCD caused by the *CYP4V2* mutation via sterol regulatory elementary binding protein.[18] The finding of impaired desaturase activity in BCD is consistent with the findings in animal model of retinitis pigmentosa, in which miniature poodle with progressive rod–cone degeneration was found to have defective Δ-4 desaturase activity.[16]

Insulin has been found to induce the *CYP4F2* gene in primary human hepatocytes via sterol regulatory element binding protein 1c, which in turn leads to increased synthesis of SCD and conversion of 16:0 and 18:0 fatty acids to 16:1 and 18:1 fatty acids, respectively.[18] Therefore, fasting response with a reduced level of insulin and increased level of glucagon may reduce the synthesis of SCD and reduce the synthesis of unsaturated fatty acids. In our study, we also investigated the influence of fasting response among control subjects and patients with BCD. Correlation analysis in control subjects showed that increase glucagon level was significantly associated with higher total serum unsaturated and lower total serum polyunsaturated fatty acid concentrations. Similar correlation was not observed among the patients with BCD. Therefore *CYP4V2* mutation in BCD may be associated with an impaired fatty acid metabolism in response to fasting. Further studies to investigate the influence of fasting response on fatty acid metabolism associated with *CYP4V2* mutation are warranted.

In this study, we also performed subgroup analyses in an attempt to determine whether different genotypes or phenotypes are associated with differences in fatty acid composition or desaturase activities. Our findings showed no significant differences in the fatty acid composition or desaturase activities in patients with different genotypes or phenotypes. Further study with increase number of subjects and more diversified genotype will be useful in determining the relationship of fatty acids and different genotype and phenotype in patients with BCD.

One of the limitations of this study was the lack of a dietary survey for the patients in the two groups. Nonetheless, these patients all consume the usual southern Chinese diet with no restrictive vegan habits, and therefore our results should not be influenced significantly by dietary factors. Moreover, it has been shown that there was a lack of relationship between the content of monounsaturated fatty acids in the diet and the composition of serum fatty acid.[28] Therefore, the findings in which patients with BCD had a significantly higher 18:0 fatty acid concentration and lower 18:1n-9 concentration are unlikely to be related to dietary factors. Another limitation was the lack of direct measurement of desaturase activities in the subjects. However, direct measurement of desaturase activities can be difficult to perform, and therefore most studies have used fatty acid ratios for estimating desaturase activities and for understanding fatty acid desaturation.[16,29] Finally, the number of patients was limited in this study, and all were of Chinese descent. It is unclear whether the findings can be generalized to patients with BCD in other ethnic groups with different genetic mutations.

In conclusion, our results show that patients with BCD had increased serum concentration of unsaturated 18:0 fatty acid and reduced monounsaturated 18:1n-9 fatty acid concentration. These findings imply that there is reduced Δ-9 desaturase activity in patients with BCD. Such metabolic derangement is independent of *CYP4V2* genotype. Patients with BCD may also have abnormality in fatty acid metabolism in response to fasting. The findings confirmed and strengthened the hypothesis that BCD can result in systemic changes in fatty acid metabolism.

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