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RESEARCH ARTICLE

ACLINICAL AND GENETIC FEATURES IN CHINESE BIETTI CRYSTALLINE DYSTROPHY (BCD) FAMILIES WITH *CYP4V2* MUTATIONS.

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Abstract

Bietti crystalline dystrophy (BCD) is a rare autosomal recessive inherited disorder characterized by crystals in retinal pigment epithelium. The purpose of this study was to identify the genetic defect in Chinese families with BCD and analyze their clinical features. The clinical examination and genetic studies were performed in three BCD families at Tongji University Hospital. Eye examination included fundus fluorescein angiography, slit lamp, fundus photograph, optical coheres tomography and visual acuity examination has been carried out. Blood samples were collected from three pro-bands and their family members. Genomic DNA extracted from whole blood used for polymerase chain reaction (PCR) to identify genetic defect. Fundus examination revealed that the presence of tiny yellowish-sparkling crystals at the posterior pole of the fundus and atrophy of the retinal pigment epithelium in some patients. The PCR results also showed that three different mutations in the three families including Family C with compound heterozygous mutations of deletion c.802-8_810del17bpinsGT and insertion c.1062dupA, Family B with compound heterozygous mutations of a splicing mutation c.1091-2A>G and deletion mutation c.802-8_810del17bpinsGC and Family A with homozygous deletion mutation c.802-8_810del17bpinsGC. In this study, the PCR result also identified CYP4V2 as the disease causative gene in three Chinese families with BCD. Overall, these findings broaden the spectrum of *CYP4V2* mutations that cause BCD and characterize phenotypic features of the disease in Chinese families.

Introduction

Bietti crystalline dystrophy (BCD) is a rare autosomal recessive retinal degenerative disease first pronounced by Bietti in 1937, a disorder in which numerous small, yellow or white crystal-like deposits of fatty (lipid) compounds accumulate in the light-sensitive tissue that lines on the retina (Ghosh et al., 2016). The deposits damage the retina,

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resulting in progressive vision loss which is mostly occurs in Chinese and Japanese populations (Gupta et al., 2010 ; Yin et al., 2016). However, it has also been recognized around the world (Yin et al., 2014). Beitti crystalline dystrophy is characterized by multiple small glistening intra retinal crystal deposition scattered all over the posterior pole related with progressive degenerate of the retinal pigment epithelium (RPE) and choroidal sclerosis. It is linked with numerous mutations in CYP4V2 gene (Song et al., 2013 ; Ng et al., 2016). In the early stages of the disease, the RPE, chorio capillaris complex is the first structure involved, while the functions of rods and cones are still preserved. The crystalline deposits are associated with atrophy of the RPE and chorio capillaris, pigment clumping and sclerosis of the choroidal vessels. Gradually, the deterioration of functional parameters in relation to the progression of retinal has damaged.

Although the BCD symptoms are inadequate to the visual system, crystalline inclusions are found throughout the body as well as in lymphocytes and skin fibroblasts (Lai et al., 2007; Yin et al., 2016). Clinically, the illness shows itself between the second and fourth decades of life and slowly but surely progresses. Patients have decreased vision, nyctalopia, and early-stage para central scotoma, which typically lead to obvious visual impairment, peripheral visual field loss, and legal blindness in the fifth and sixth decades of life (Kojima et al., 2012). The disease is caused by mutations in the CYP4V2 gene (Yokoia et al., 2011). The protein product of the CYP4V2 gene is predicted to be a member of cytochrome p-450 family and may play a role in lipid metabolism. The CYP4V2 gene is expressed widely in human heart, brain, placenta, lung, liver, lymphocyte, retina and retinal pigment epithelium (RPE) (Furusato et al., 2010).

The disease is characterized by reduced conversation the fatty acid precursors in to n-3 polyunsaturated fatty acid caused by deregulation in lipid metabolism because of deficient lipid binding elongation or desaturation (Nakano et al., 2012). Biochemical analysis of cultured lymphocytes from patients with BCD showed abnormally high levels of triglycerides and cholesterol with the absence of two fatty acid binding proteins (Tian et al., 2015). It is assumed that the protein encoded by the gene plays a role in fatty acid and corticosteroid metabolism (Rossi et al., 2012). Related to previous study, different researchers showed that CYP4V2 gene is causing the occurrence of BCD disease (Haddad et al., 2012; Mamatha et al., 2011). On the other hand, some study in China showed that CYP4V2 gene is not the cause of BCD disease (Song et al., 2013). Thus, the present study was designed to investigate whether CYP4V2 gene is the cause of BCD or not.

Material and Methods:-

Patients and clinical studies:-

The Tenth People Hospital and Tongji University Teaching Hospital were selected for this study. Tenth People Hospital is located in Shanghai. This hospital give serves as teaching for medical college students of Tongji University and provide health care services for outpatient and in- patients cases. A total of six patients from three unrelated families were recruited for one year (January 1, 2017 to January 1, 2018) at Tongji University who underwent ophthalmology diagnosis. Aamong the three families, two families were found from Tongji University Hospital and one family from Tenth People Hospital.

For each patient, ophthalmic examinations including best corrected visual acuity (BCVA), slit lamp biomicroscopy, fundus photography, fundus autofluorescence and optical coherence tomography (OCT) were performed at Tongji University Hospital Ophthalmic Genetic Laboratory. This study was approved by Internal Review Board of Ophthalmology Department at Tenth People Hospital of Tongji University and followed tenets of Declaration of Helsinki and the Guidance of Sample Collection of Human Genetic Disease by Ministry of Public Health of China. Informed agreement was obtained from the participating individuals or their guardians before gathering of clinical and molecular data.

Molecular genetic studies:-

For polymerase chain reaction (PCR), the blood samples were collected by venipuncture. Then, genomic DNA from the whole blood sample was isolated by relax Gene blood DNA system with special DNA extraction buffer system from 2-4mL whole blood samples (Haddad et al., 2012). The PCR inhibitors such as proteins, lipid and other impurities were maximally removed in two efficient wash steps. Pure DNA is then eluted in water or the buffer provided with the kit. Phenol extraction and ethanol perception were not required in the current protocol followed. The genomic DNA isolated following the kit was of high quality and serves as an excellent template to amplify the CYP4V2 gene. As shown in (Table 1), all exons and the flanking introns of the gene were amplified by PCR using previously reported primers (Haddad et al., 2012). The PCR products were purified and then

sequenced on an automated sequencer ABI 3730 Genetic Analyzer (ABI, Foster City, USA). The results were analyzed with Laser gene SeqMan software (DNASTAR, Madison, USA) and compared with a reference sequence (Gene Bank accession number: NM_207352), 100 control chromosomes were screened to exclude nonpathogenic polymorphisms.

Table 1:- PCR Primers for the 11 exons of the *CYP4V2* gene

Exons	Primers	
	Forward	Reverse
Exon 1	CGTAGAGCAACCTCGCAG	ACAAGCAGCGGGTTCCT
Exon 2	CTCTCTACCTGGCTTCCTCTA	TCTGGTGGATAACAAGTGCT
Exon 3	TCCACTTGGTTCCTGGTTTA	GCCTTTCTCCTCCTTTCTGA
Exon 4	TTGTCATTCTGCCAAAAGC	GTAGAACCGCGCTGAAGA
Exon 5	GAAGAACAGGAACAGGGAGT	ACACGAGACAATGAGAAACAC
Exon 6	TAGCCTCTAAGACAATCATCG	GCACTTAATACCACCAAACTG
Exon 7	TGTATTTTCACAAGAGCCTATG	AATGTGTCTACTGCTGTGCC
Exon 8	GGCTTGTTTCCTTGTTTGT	GCCTTCCTGCTCATTACAC
Exon 9-10	AGCCCCCACTGCTCTTTC	CACTGTGAGAAACCCACCATC
Exon 11	CTCCTTCCACCTACTGCG	TGGCAGGCACCTGTAAT

Result:-

Clinical findings:-

A total of six chines patient clinical and genetically diagnosis with BCD from three unrelated family member were recruited in to this study including five male and one female (Figure 1 and Table 2). Except Family B (II:3), all affected patients were identified male individuals (Figure 1).

In the present study, the most common initial clinical sign and symptoms of crystalline retinal deposition, decrease visual acuity, night blindness and nyctalopia were observed at the age ranges of 26 to 41 (36±6.6) years (Table 2). It has been reported that the BCD disease usually occurs during the 2nd and 4th decade (Xiao et al., 2011; Nakano et al., 2012; Ng et al., 2016) which agrees with our result. Visual acuity rang of the patient from 0.1 to 0.8 with the onset of symptom characterized by multiple white small glistering intra retinal crystal deposit scattered throughout the posterior pole. For instance, participant from Family C (II:3) was showed typical white- yellowish crystals deposit at the posterior pole of the fundus with decreased visual acuity and nyctalopia (Figures 2), suggesting that the disease is BCD. Moreover, the clear appearance also indicated that retinal degeneration and choroidal sclerosis were significant in the current study participants. Optical coherence tomography investigation also confirmed that the intra retinal crystals deposit is BCD disease (data not shown). Fundus fluorescein angiography investigation was also showed crystalline deposited which confirmed the disease is BCD in the study participants.

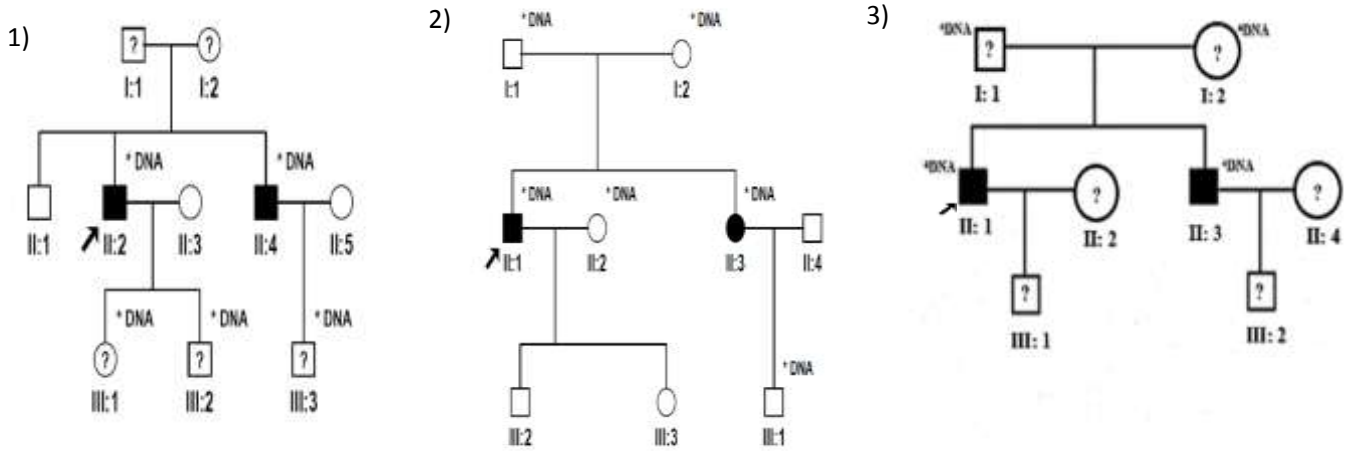

Figure 1:- Pedigrees of the three families with BCD affected individuals (1) Family A, (2) Family B and (3) Family C. The Squares and circles represent male and female, respectively. Filled symbols also indicate affected subjects with BCD and unfilled symbols represent normal family members. The arrow indicates the pro-band.

Table 2: Clinical and genetic features of patients with *CYP4V2* mutation

Family	Sex	Age (year)		First symptom	VA		Finding	Mutation
		Age	Onset		R	L		
A/II:2	M	38	28	DoV	0.2	0.5	CPR	c.802-8_810del17bpinsGC
A/II:4	M	31	30	DVA	0.3	0.7	CPR	c.802- 8_810del17bpinsGC
B/II:1	M	42	38	DVA/NB	0.1	0.3	CPR	c.1091-2A>G
B/II:3	F	45	41	DVA/Ny	0.2	0.4	CPR	c.802-8_810del17bpinsGC
C/II:1	M	29	26	DV/Ny	0.5	0.8	CPR	c.1062dupA
C/II:3	M	31	29	DV/Ny	0.4	0.6	CPR	c.802-8_810del17bpinsGT

DVA= decrease visual acuity, DoV=Distortion of vision, DV= decrease vision, NB= night blindness, Ny=Nyctalopia, CRP= crystal retinal pigment, RPE= retinal Pigment epithelium, CD= crystal deposit

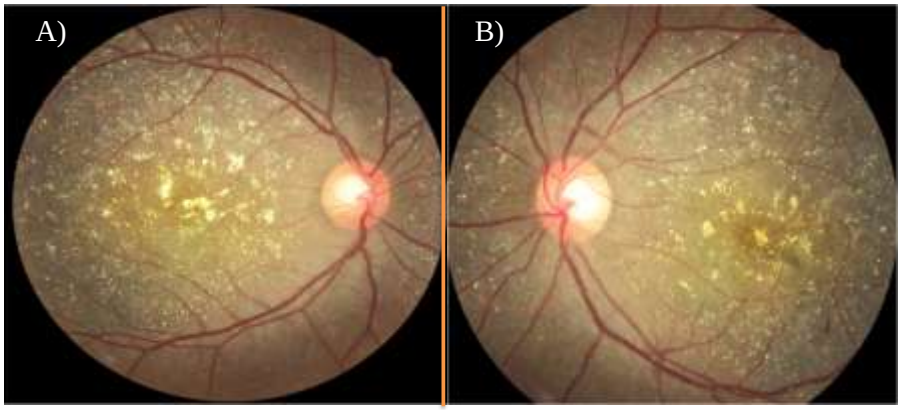

Figure 2:- Fundus photograph of Family C (II: 1) with *CYP4V2* mutation (c.1062dupA) of BCD disease (a) right eye and (b) left eye.

Molecular genetic finding:-

As shown in Figure 1, the pedigrees of the three unrelated familiels diagnosized for *CYP4V2* confirmed that all the families showed the *CYP4V2* gene mutation. Particularly, the *CYP4V2* gene mutuation has been observed inFamily A/II:2 and 4; Family B/II:1 and 3; and Family C/II:1 and 4. The two patients in Family A have been identified homozygous mutations while the rest four in Family B and C were confirmed heterozygous mutations. Except Family B/II:1 and Family C/II:1, all the mutations were also identified to be deletion types (Table 2). The six *CYP4V2* gene mutations (Table 2) include c.802-8_810del17bpinsGC (Family A/II:2), c.802- 8_810del17bpinsGC (Family A/II:4), c.1091-2A>G (Family B/II:1), c.802-8_810del17bpinsGC (Family B/II:3), c.802-8_810del17bpinsGT (Family C/II:1) and c.1062dupA (Family C/II:3). The c.802-8_810del17insGC mutation observed in the current study (66.6%) was the most common mutation in Chinese and Japanese populations, while the 8_810del17bpinsGC and c.1091-2A>G (16.7% each) mutations were only identified in the Chinese population.

Discussion:-

Bietti crystalline dystrophy is a progressive chorioretinal degeneration disease that has been demonstrated to be caused by mutations in the *CYP4V2* gene (Yokoia et al., 2011). In the current study, we reported the results of six Chinese patients from three unrelated family through clinically diagnosed and molecular genetic techniques. The examinations performed demonstrate that our patient meets all clinical criteria for BCD. The diagnosis was made on the basis of a history of night blindness, decreased visual acuity, visual field constriction, and supported by other clinical investigations. The results were confirmed diffuse clumping of typical yellowish-white glistening crystals and chorio capillary atrophy appearing towards the second to fourth decade of life (Rossi et al., 2011). In this study, affected patients have clinical investigation result like fundus photograph, fluorescein angiography and optical coherence tomography performed on each patient confirmed that posterior pole of the fundus white-yellowish sparking crystal deposits on the macular regions of the eyes. Additional, clinical results also showed that BCD patients have night blindness, decreased visual acuity, progressive visual field constriction and nyctalopia.

After confirmed BCD with clinical diagnosis, all patients were screened through molecular genetic analysis of the responsible mutation *CYP4V2* gene by direct sequencing method. The result was found to be two homozygous and

four heterozygous mutations. The c.802-8_810del17insGC mutation observed in the current study was the most common mutation in Chinese and Japanese populations, while the 8_810del17bpinsGC and c.1091-2A>G mutations were only identified in the Chinese population which agrees with other previous study (Xiao et al., 2011). Among the six *CYP4V2* mutant genes, the three mutations (c.1091_2A>AG, one c.802-8_810del17insGC and 1062inSA) were identified the pathogenic variant type (Table 2). The molecular study results confirmed that the BCD disease is caused by genetic inheritance that has not been associated with crystalline deposits such as primary hyperoxaluria type 1 and 2, cystinosis, Sjogren Larsson syndrome, drug toxicity (tamoxifen, methoxyflurane, canthaxanthin) and drug abuse (Abeshi et al., 2017). Moreover, the genetic studies carried out helps our participants crystal deposit is a genetic disorder which is not related with other autosomal recessive retinal disease that cause decreased vision, crystalline deposits and Nyctalopia (Astuti et al., 2015).

Conclusion:-

In the present study, we described the genetic and phenotypic characteristics of six Chinese patients affected with BCD. Results confirmed that c.802-8_810del17bpinsGC, and c.1091-2A>G are common mutations in Chinese patients with BCD. Another mutation was also found c.1062dupA that could result in a defective amino acid sequence which caused gross conformational change in the *CYP4V2* protein. So this study will be useful for BCD patient which could allow for genetic counseling to individuals and families with BCD to obtain information regarding the nature, inheritance and implications of their eye conditions.

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