Whole Exome Sequencing in Eight Thai Patients With Leber Congenital Amaurosis Reveals Mutations in the *CTNNA1* and *CYP4V2* Genes

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Purpose. Our goal was to describe the clinical and molecular genetic findings in Thai patients with Leber congenital amaurosis (LCA).

Methods. Whole exome sequencing (WES) was performed in eight unrelated patients. All genes responsible for inherited retinal diseases (IRDs) based on RetNet were selected for analysis. Potentially causative variants were filtered through a bioinformatics pipeline and validated using Sanger sequencing. Segregation analysis of the causative genes was performed in family members when available.

Results. Eleven deleterious variants, six nonsense and five missense, were identified in seven genes: four LCA-associated genes (*CEP290*, *IQCB1*, *NMNAT1*, and *RPGRIP1*), one gene responsible for syndromic LCA (*ALMS1*), and two IRDs-related genes (*CTNNA1* and *CYP4V2*). Clinical reassessment supported the diagnosis of syndromic LCA in those patients harboring potentially pathogenic variants in the *ALMS1*. Interestingly, two causative genes, *CTNNA1* and *CYP4V2*, previously reported to cause butterfly-shaped pigment dystrophy (BSPD) and Bietti's crystalline dystrophy (BCD), respectively, were detected in two other patients. These two patients developed rapid and severe visual loss in contrast to BSPD and BCD patients in previous studies. The results of this study demonstrate that causative variants identified in the *CTNNA1* and *CYP4V2* genes are also associated with LCA.

Conclusions. This is the first report describing the molecular genetics and clinical manifestations of Thai patients with LCA. The present study expands the spectrum of LCA-associated genes, which is a benefit for molecular diagnosis. The identification of mutations in the *CTNNA1* and *CYP4V2* genes requires further elucidation in larger cohorts with LCA.

Keywords: whole exome sequencing, Leber congenital amaurosis, molecular genetics, Thai, inherited retinal diseases

Leber congenital amaurosis (LCA; Mendelian Inheritance in Man [MIM] #204000) is the most severe form of inherited retinal diseases (IRDs), with onset in the first few months after birth or in early childhood. It has an estimated worldwide prevalence between 1:30,000 and 1:80,000.[1] The clinical hallmarks of LCA include wandering nystagmus, oculodigital sign, high hyperopia, photophobia, sluggish or near-absent pupillary responses, and severely reduced rod and cone responses on the electroretinogram (ERG). Leber congenital amaurosis is a clinically heterogeneous disease. Not only the ocular features were observed, but systemic manifestations, such as diabetes mellitus, renal failure, sensorineural hearing loss, and neurodevelopmental delay and intellectual disability, have also been reported in the patients who were initially diagnosed with LCA. Some syndromic diseases, including Alström syndrome, Bardet-Biedl syndrome, Batten disease, Jourbert syndrome, and Senior-Loken syndrome, exhibit additional clinical presentations that overlap with LCA.[1] Leber congenital amaurosis is also a genetically heterogeneous disease, showing both autosomal recessive (ar) and autosomal dominant (ad) inheritance patterns. Mutations in at least 24 genes have been implicated in nonsyndromic LCA (RetNet; in the public domain, https://sph.uth.edu/retnet/sum-dis.htm): 22 genes for arLCA (*AIPL1*, *CABP4*, *CEP290*, *CLUAP1*, *CRB1*, *CRX*, *DTHD1*, *GDF6*, *GUCY2D*, *IFT140*, *IQCB1*, *KCNJ13*, *LCA5*, *LRAT*, *NMNAT1*, *PRPH2*, *RD3*, *RDH12*, *RPE65*, *RPGRIP1*, *SPATA7*, and *TULP1*) and three genes for adLCA (*CRX*, *IMPDH1*, and *OTX2*). The gene *CRX* displays both patterns of inheritance. Disease-causing genes have been identified in approximately 70% of LCA cases.[1]

Due to the clinical and genetic heterogeneity of LCA, molecular diagnostic testing is necessary to provide information for diagnostic accuracy. Recently, whole exome sequencing (WES), an efficient and cost-effective molecular diagnosis tool, has been used to identify disease-causing genes in LCA.[2–7]

This is the first report of WES being conducted in Thai patients with LCA. We performed WES in eight unrelated Thai patients clinically diagnosed with LCA. Eleven potentially causative variants, five novel and six known, in seven genes were identified. Remarkably, two genes, *CTNNA1* and *CYP4V2*, previously reported in other adult-onset inherited retinal degenerations, have been demonstrated to be associated with severe congenital visual impairment.

Materials and Methods

Subjects and Clinical Assessment

The study protocol was approved by the ethics committees of the institutional review board of Siriraj Hospital Mahidol University and adhered to the tenets of the Declaration of Helsinki. Eight unrelated patients with a clinical diagnosis of LCA were recruited from the Division of Ophthalmology, Siriraj Hospital, Bangkok, Thailand. All participants underwent detailed ophthalmologic examinations, including best-corrected visual acuity (BCVA), slit-lamp biomicroscopy, dilated-pupil fundus examination, cycloplegic refraction, full-field ERG, flash visual evoked potential (FVEP), and optical coherence tomography (OCT) when available. After informed consent was obtained from the patients or their parents, venous whole blood samples were collected for molecular genetic testing.

Whole Exome Sequencing (WES) and Bioinformatics Analysis

Genomic DNA preparation, exome capture, Illumina sequencing, and variant-calling pipelines were performed as described previously.[8] Annotation of variants obtained from each patient was performed according to the wANNOVAR web server (in the public domain, http://wannovar.wglab.org/index.php).[9]

Variant Identification

Initially, variants identified in 24 candidate genes for LCA were selected for data analysis and variant filtering. For patients with no mutations, 233 IRD-associated genes reported in the RetNet database were further analyzed. By following the American College of Medical Genetics and Genomics (ACMG) guidelines,[10] the nucleotide sequence variants identified in this study were classified into five classes: pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign. Variants were classified as causative based on patterns of inheritance of each gene with the following criteria: (1) missense or nonsense variant, (2) reported as deleterious by at least two out of three prediction tools: PolyPhen2 (in the public domain, http://genetics.bwh.harvard.edu/pph2/), SIFT (in the public domain, http://sift.jcvi.org/), and MutationTaster (in the public domain, http://www.mutationtaster.org/), (3) located at the donor or acceptor splice site, (4) not observed in the dbSNP database built 138 (in the public domain, http://www.ncbi.nlm.nih.gov/projects/SNP/), 1000 Genomes Project (in the public domain, ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp), Exome Variant Server (EVS; in the public domain, http://evs.gs.washington.edu/EVS/), or Exome Aggregation Consortium (ExAC; in the public domain, http://exac.broadinstitute.org/). However, the disease-associated variants in genes that are inherited as ar traits may also be present in the heterozygous state in publicly available databases. Mutations specified in the Human Gene Mutation Database (HGMD; in the public domain, http://www.hgmd.cf.ac.uk/ac/index.php) or those characterized as deleterious variants in previous studies were considered as disease causing. HOPE (in the public domain, http://www.cmbi.ru.nl/hope/) was used for the prediction of protein structures and functions of missense variants.[11] The Variant Call Format (VCF) output files that detail the deleterious variants were loaded into the Integrative Genomics Viewer (IGV; in the public domain, http://www.broadinstitute.org/igv/) to visualize the exome coverage and base calls. Multiple sequence alignments of the amino acid residues across species were generated using the ClustalW online tool (in the public domain, http://workbench.sdsc.edu/).

Sanger Sequencing and Segregation Analysis

Sanger sequencing confirmed predicted deleterious variants in the candidate genes. Specific primers were designed with the Primer3 program (in the public domain, http://bioinfo.ut.ee/primer3/) using NCBI37/hg19 as the reference sequence. Sanger sequencing also performed segregation analysis of pathogenic or likely pathogenic variants in available family members. Novel nucleotide substitution variants that were likely to be deleterious and also segregated within the families were subsequently screened in 130 ethnically matched control subjects by denaturing high-performance liquid chromatography (Transgenomics, Omaha, NE, USA).

Results

WES was performed to identify the disease-causing genes in eight unrelated Thai patients with LCA. According to our criteria for variant analysis, we identified 11 different single-base substitutions (6 nonsense and 5 missense) in seven genes associated with LCA, syndromic LCA, and other IRDs. A summary of the clinical information, fundus images, family pedigrees, and the pathogenic variants identified in this study are shown in Table 1, Figure 1, Figure 2, and Table 2, respectively. However, the fundus photograph for patient LCATH4 was not available. The fundus description in Table 1 is only based on a fundoscopic examination. All nucleotide alterations, except for the variant detected in patient LCATH5 and LCATH7, segregated in trans in the available family members without disease manifestations.

Pathogenic Variants Identified in LCA-Associated Genes

Pathogenic variants in four candidate genes for LCA (*CEP290*, *IQCB1*, *NMNAT1*, and *RPGRIP1*) were identified in four of eight patients (Table 2). Patient LCATH1, a 6-month-old male, harbored novel compound heterozygous nonsense variants, p.Gln123Ter and p.Glu543Ter, in the *CEP290* gene. Patient LCATH2 is a 4-year-old female, who carried a novel homozygous variant, p.Lys315Ter, in the *IQCB1* gene. Patient LCATH3, a 13-month-old male, carried previously reported compound heterozygous missense mutations, p.Arg66Trp[12] and p.Val212Met,[13] in the *NMNAT1* gene. Patient LCATH4, a 6-month-old male, harbored a previously reported homozygous variant for p.Arg371Ter[14] in the *RPGRIP1* gene.

Pathogenic Variants Detected in Syndromic LCA-Associated Gene

Deleterious truncating variants in gene associated with syndromic LCA were observed in two patients (Table 2). Two

Table 1. Clinical Information for Eight Thai Patients With LCA

Patient	Sex	Age of Onset	Presenting Symptoms	Age at Initial Examination	BCVA		Lens		Fundus	ERG	FVEP	Refraction		Extraretinal Associations
					RE	LE	RE	LE				RE	LE	
LCATH1	Male	4 mo	History of not fixing on and following objects	6 mo	Wandering eye	Wandering eye	Clear	Clear	Pink disc, artery attenuation, mild RPE changes midperiphery, no bone spicules	NR	Mildly decreased amplitude, RE; moderately decreased amplitude, LE	NA	NA	-
LCATH2	Female	1 y	Nystagmus	4 y	6/24	5/60	Clear	Clear	Pink disc, artery attenuation, generalized RPE changes, no bone spicules, macular sparing	NR	Normal	+3.75 − 4.00 × 15°	+5.75 − 4.75 × 170°	Two simple cysts in the right kidney
LCATH3	Male	1 y	History of holding things too closely to his face, mild nystagmus	13 mo	Wandering eye	Wandering eye	Clear	Clear	Pale disc, artery attenuation, generalized RPE changes with bone spicules, maculopathy with RPE atrophy and pigment clumps	NR	Mildly delayed latency and decreased amplitude, BE	+6.25 − 3.50 × 180°	+0.75 − 2.25 × 9°	-
LCATH4	Male	Birth	History of not fixing on and following objects	7 mo	Not fixing on and following	Not fixing on and following	Clear	Clear	Mild pale disc, generalized RPE changes, no bone spicules	NR	Normal	NA	NA	Neurodevelopmental delay
LCATH5	Male	Birth	History of blurred vision since birth	6 y	6/120	6/240	Clear	Early PSC	Pale disc, artery attenuation, generalized RPE changes, no bone spicules	NR	NA	+7.25 − 2.50 × 7°	+7.50 − 2.50 × 14°	Obesity, hearing loss, myocarditis, asthma and pneumonia
LCATH6	Male	7 mo	Blurred vision in daylight, photophobia	6 y	6/240	6/240	Clear	Early PSC	Pale disc, artery attenuation, generalized RPE changes, no bone spicules	NR	Normal	+6.25 − 3.50 × 180°	+6.25 − 3.50 × 180°	Obesity, hearing loss, hypertriglyceridemia
LCATH7	Male	Birth	History of not fixing on and following objects	5 mo	Not fixing on and following	Not fixing on and following	Clear	Clear	Pale disc, artery attenuation, generalized RPE changes with bone spicules, maculopathy with RPE atrophy and pigment clumps	NR	Normal	NA	NA	-

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Table 1. Continued

Patient	Sex	Age of Onset	Presenting Symptoms	Age at Initial Examination	BCVA		Lens		Fundus	ERG	FVEP	Refraction		Extraretinal Associations
					RE	LE	RE	LE				RE	LE	
LCATH8	Male	1 y	Nystagmus, photophobia	2 y	Not fixing on and following	Not fixing on and following	Clear	Clear	Pale disc, artery attenuation, generalized RPE changes, no bone spicules	NR	Moderately decreased amplitude, BE	NA	NA	-

BCVA, best-corrected visual acuity; RE, right eye; LE, left eye; BE, both eyes; PSC, posterior subcapsular cataract; RPE, retinal pigment epithelium; ERG, electroretinogram; FVEP, flash visual evoked potentials; NA, not available; NR, nonrecordable.

patients, LCATH5 and LCATH6, demonstrated pathogenic variants in the *ALMS1* gene, a causative gene for Alström syndrome (AS). Molecular findings in patient LCATH5, a 6-year-old male, showed compound heterozygous nonsense variants; a reported mutation, p.Ser1299Ter,[15] and a novel variant, p.Glu2681Ter. Due to the lack of DNA samples from additional family members, we were unable to confirm the segregation of these two variants in this family. Patient LCATH6, a 6-year-old male born to consanguineous parents, carried a reported homozygous nonsense mutation of p.Ser1299Ter[15] in the *ALMS1* gene. Cosegregation analysis confirmed that p.Ser1299-Ter variants were inherited from heterozygous carrier parents (Fig. 2).

Pathogenic Variants Identified in Other IRDs Genes

In our study, we identified two patients carrying three deleterious variants in two genes (*CTNNA1* and *CYP4V2*) associated with other types of retinal degenerations. Patient LCATH7, a 5-month-old male, harbored a novel heterozygous missense variant, p.Gly353Cys, in the *CTNNA1* gene, which causes butterfly-shaped pigment dystrophy (BSPD) (Table 2). The p.Gly353Cys variant is predicted to be deleterious by multiple in silico algorithms and was not observed in all public variant databases and a cohort of 130 control subjects. Cosegregation analysis was performed only in the mother. The result showed that the unaffected mother carried normal alleles. Additionally, a novel single heterozygous missense variant, c.C5414T (p.Pro1805Leu), which was predicted to be deleterious was also identified in the *KIAA1549* gene associated with ar retinitis pigmentosa (RP).

Genetic analysis in patient LCATH8, a 2-year-old male from a consanguineous family, revealed the compound heterozygous missense variants, p.Glu79Asp and p.Met123Val, in the *CYP4V2* gene (Table 2). Both variants were previously reported in a patient with Bietti crystalline corneoretinal dystrophy (BCD).[16] Furthermore, a novel single heterozygous nonsense variant, c.C10819T (p.Arg3607Ter), in the *ALMS1* gene associated with an arAS has also been found.

Clinical Reassessment of Patients Harboring Pathogenic Variants in Syndromic LCA-Associated Gene and Other IRDs Genes

To elucidate the association between the clinical and genetic findings for the ambiguous cases, clinical re-evaluation was performed in patients LCATH5 and LCATH6 who carried pathogenic variants in syndromic LCA-associated gene (*ALMS1*), and patients LCATH7 and LCATH8 who carried pathogenic variants in other IRDs genes (*CTNNA1* and *CYP4V2*). The results showed that patients LCATH5 and LCATH6 had systemic manifestations consistent with the clinical phenotype of AS (Table 1). Both patients demonstrated the key features of AS including childhood obesity, sensorineural hearing loss, and retinal dystrophy. Patient LCATH5 developed myocarditis, asthma, and pneumonia while patient LCATH6 had hypertriglyceridemia and normal echocardiogram.

Mutations in the *CTNNA1* gene have been reported in BSPD, with onset in young to middle-aged adults and a BCVA between 20/20 and 20/200.[17] Patient LCATH7, with the *CTNNA1* variant, was unable to fix and follow an object at the age of 5 months (Table 1). Fundus photographs demonstrated atrophic macula with circinate pigmentation in both eyes similar to the wings of a butterfly (Fig. 1). Follow-up examination at 8-years old showed wandering eye movement with sunken eyes.

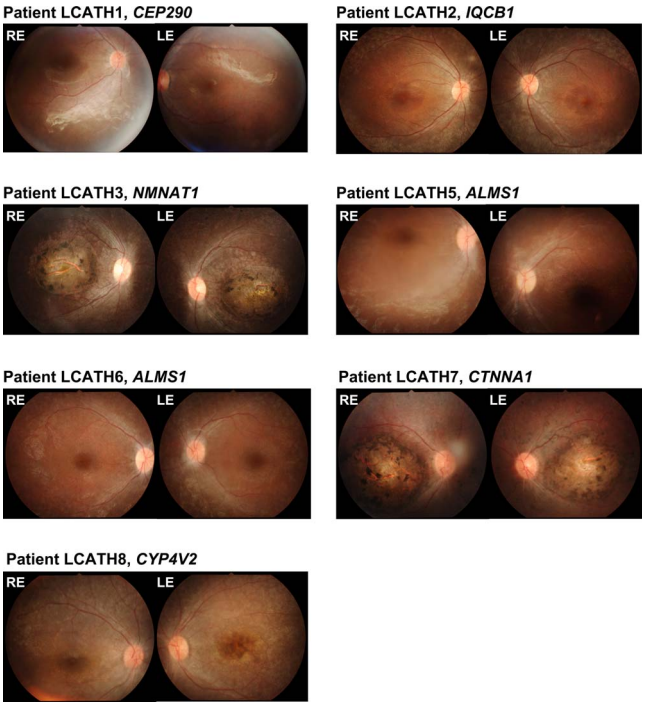

FIGURE 1. Fundus photograph results for seven LCA patients (LCATH1-LCATH8, with an exception of the patient LCATH4). The corresponding patient number together with the disease-causing gene is shown above each photo. Variable fundus findings were recorded among the patients, ranging from generalized RPE changes to with and without bone spicules. Macular atrophy with pigment accumulation was also observed in two patients, LCATH3 and LCATH7. Further clinical information for these patients is listed in Table 1. RE, right eye; LE, left eye.

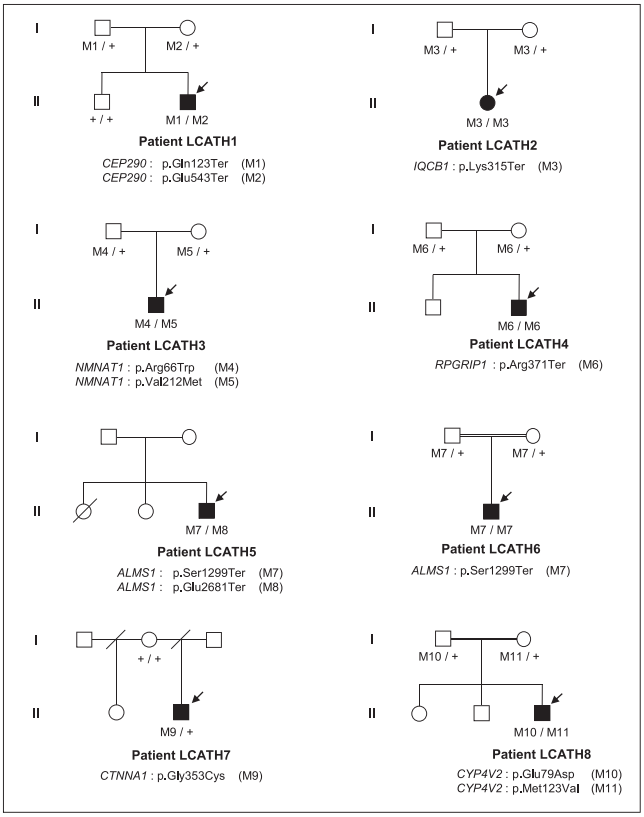

FIGURE 2. Pedigrees and genotypes for all eight patients, LCATH1 to LCATH8. Genotype data are shown below the patients and family members where applicable. *Filled symbols* with an *arrow* indicate the probands; *squares*: males, *circles*: females, *slashed*: separation or divorce, *double line*: consanguineous marriage, *slashed circle*: deceased. Normal alleles are indicated by '+', pathogenic variants identified in this study are indicated by '*M*'.

Mutations in the *CYP4V2* gene have been reported in BCD, with onset in young- to middle-aged adults. The presence of multiple glistening intraretinal crystals scattered throughout the fundus is the hallmark of this condition.[16] Patient LCATH8, who carried two previously reported mutations in the *CYP4V2* gene,[16] had a history of nystagmus and photophobia since age 1 year (Table 1). His visual acuity worsened rapidly from counting fingers at 5-years old to hand motion at the age of 7. Fundus examination disclosed generalized hypopigmented mottling of the RPE and patchy hyperpigmentation in the macular area (Fig. 1). We did not observe the typical crystals or lipid deposits normally shown in BCD.

DISCUSSION

Using WES, we identified pathogenic variants in all eight patients initially diagnosed with LCA. Among these patients, four harbored causative variants in the reported genes associated with LCA (*CEP290*, *IQCB1*, *NMNAT1*, and *RPGRIP1*), two carried causative variants in the gene responsible for syndromic LCA (*ALMS1*), and two were found to carry IRDs-associated genes (*CTNNA1* and *CYP4V2*).

Variable clinical expression in patients with the *CEP290* mutations have been reported, ranging from nonsyndromic LCA to severe systemic conditions, such as Joubert syndrome, characterized by retinal dystrophy and progressive cystic kidney disease; Bardet-Biedl syndrome, characterized by retinal degeneration, obesity, polydactyly-type limb anomalies, renal failure, cognitive impairment, hypogonadism, and genital anomalies; and Meckel syndrome, a lethal disease, characterized by central nervous system defect, bilateral renal cystic dysplasia, polydactyly, and hepatic developmental defects.[1] In this study, patient LCATH1 carried a novel compound heterozygous nonsense variants in the *CEP290* gene. The patient had a history of not fixing on and following objects since 4 months of age. Follow-up examination at the age of 6 years showed the same clinical symptoms (Table 1) without systemic manifestations. Because systemic abnormalities are not apparent until late childhood or adulthood, further renal function tests of patient LCATH1 should be done for surveillance and treatment planning.

Mutations in the *IQCB1* gene have been identified in both patients with Senior-Loken syndrome (SLS) and LCA.[18] In our study, patient LCATH2 harbored a novel homozygous nonsense variant in the *IQCB1* gene. The patient had a history of nystagmus developing in the first year of life. A follow-up investigation at the age of four years showed two simple cysts in the right kidney (Table 1). Regular follow-up examinations should be performed to determine the progressive renal function decline.

From previous reports, most of the patients with *NMNAT1*-associated LCA had the same phenotype, including severe visual impairment and central macular atrophy from birth.[19] In this study, patient LCATH3, who carried the reported compound heterozygous missense variants in the *NMNAT1* gene, showed consistent findings with generalized RPE changes, attenuated arterioles, pigment alteration, and demarcated circular atrophic area in the macula region, demonstrat-

Table 2. Pathogenic Variants Identified in This Study

Patient	Gene	MOI	Diagnosis	Variant Detected					Reference
				Chr	Exon	DNA Change	Protein Change	Zygosity	
LCATH1	*CEP290*	ar	LCA	12	6	c.367C>T	p.Gln123Ter	Heterozygous	Novel
					17	c.1627G>T	p.Glu543Ter	Heterozygous	Novel
LCATH2	*IQCB1*	ar	LCA	3	10	c.943A>T	p.Lys315Ter	Homozygous	Novel
LCATH3	*NMNAT1*	ar	LCA	1	3	c.196C>T	p.Arg66Trp	Heterozygous	Reported[12]
					5	c.634G>A	p.Val212Met	Heterozygous	Reported[13]
LCATH4	*RPGRIP1*	ar	LCA	14	9	c.1111C>T	p.Arg371Ter	Homozygous	Reported[14]
LCATH5	*ALMS1*	ar	AS	2	8	c.3896C>A	p.Ser1299Ter	Heterozygous	Reported[15]
					10	c.8041G>T	p.Glu2681Ter	Heterozygous	Novel
LCATH6	*ALMS1*	ar	AS	2	8	c.3896C>A	p.Ser1299Ter	Homozygous	Reported[15]
LCATH7	*CTNNA1*	ad	LCA	5	7	c.1057G>T	p.Gly353Cys	Heterozygous	Novel
LCATH8	*CYP4V2*	ar	LCA	4	2	c.237G>T	p.Glu79Asp	Heterozygous	Reported[16]
					3	c.367A>G	p.Met123Val	Heterozygous	Reported[16]

Nucleotide numbering reflects cDNA numbering with position 1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (in the public doman, http://www.hgvs.org/mutnomen). The initiation codon is codon 1. The nucleotide position of each gene was based on these GenBank cDNAs (accession number): *ALMS1* (NM_015120.4), *CEP290* (NM_025114.3), *CTNNA1* (NM_001903), *CYP4V2* (NM_207352.3), *IQCB1* (NM_001023570.3), *NMNAT1* (NM_022787.3), and *RPGRIP1* (NM_020366.3). MOI, mode of inheritance; Chr, chromosome.

ed at the age of 13 months (Fig. 1). Our results support the genotype–phenotype characterization in patients with *NMNAT1*-associated LCA.

Mutations in the *RPRGIP1* gene can cause both LCA and cone–rod dystrophy.[20] In this study, patient LCATH4 harbored a known homozygous nonsense variant, p.Arg371Ter, in the *RPRGIP1* gene. This patient had a history of not fixing on and following objects with oculodigital sign and developmental delay at age 7 months. Computed tomography scan shows focal atrophy of the frontal lobe. Neurodevelopmental delay has also been reported in three LCA patients with *RPGRIP1* mutations.[21,22] Although p.Arg371Ter was a reported disease-causing variant in an LCA patient,[14] no clinical data have been described. Therefore, we cannot compare phenotypic features between these two patients. From all of these data, our results are in accordance with previous findings[21] that disease-causing variants in the *RPGRIP1* gene can be observed in LCA patients with or without neurodevelopmental abnormalities.

The *ALMS1* gene was characterized as a causative gene for AS, a rare autosomal recessive syndromic form of LCA, with an estimated prevalence of less than 1 in 1,000,000.[23] In this study, two unrelated patients, LCATH5 and LCATH6, carried pathogenic variants in the *ALMS1* gene and had phenotypes consistent with AS. The null alleles, p.Ser1299Ter and p.Glu2681Ter, are localized in exons 8 and 10, respectively (Table 2). These findings, in accordance with previous studies, showed that most *ALMS1* mutations are either nonsense or frameshift mutations and are located in exon 16, exon 10, and exon 8.[23] Although both patients, LCATH5 and LCATH6, harbored the same pathogenic mutation (p.Ser1299Ter), the allelic state detected from each patient was different (i.e., heterozygous in LCATH5 and homozygous in LCATH6). The results from clinical re-evaluation imply that patient LCATH5 had a more severe phenotype with heart disease and chronic respiratory tract infection.

The *CTNNA1* gene encodes catenin alpha 1, which plays an important role in maintaining RPE integrity. Mutations in this gene cause BSPD, a group of ad pattern dystrophies of the RPE with an age at onset between 14 and 55.[17] In addition, the mouse model also exhibits the same ocular features as the human patients.[17] In this study, a likely disease-causing variant, p.Gly353Cys, in the *CTNNA1* gene was detected in patient LCATH7, who was diagnosed with isolated LCA at the age of 5 months (Table 1). This patient developed rapid and severe visual loss, which differs from previous studies in which patients with BSPD would experience a gradual loss of vision that is usually only apparent in old age. Although there was absence of cosegregation analysis in the patient's father, he has no symptoms of the disease. The glycine residue 353 of catenin alpha 1 is conserved among vertebrates. Based on the HOPE server, the p.Gly353Cys variant is predicted to alter protein structure of catenin alpha 1. Because only glycine is flexible enough to make the torsion angles for residue 353, amino acid substitution will force the local backbone into an incorrect conformation and will disturb the local structure. Additionally, the p.Gly353Cys variant was not found in the screening of 260 ethnically matched control chromosomes and all publicly available databases. In addition to the heterozygous *CTNNA1* missense variant, a novel heterozygous variant, p.Pro1805Leu, was also identified in the *KIAA1549* gene associated with autosomal recessive RP. Because this variant was found on only one allele and known variant in this gene has never been reported to modify the phenotype along with other genes, thus the p.Pro1805Leu in *KIAA1549* gene was classified as a VUS. However, based on current knowledge, we cannot exclude the possibility that this heterozygous missense *KIAA1549* allele may have some modifying effects on the phenotypic expression of the patient (LCATH7) to be early onset at birth which is different from previously reported patients with *CTNNA1* mutations.[17] From all of these data, we demonstrate for the first time that a heterozygous variant in the *CTNNA1* gene can lead to LCA with the fundus findings similar to BSPD.

Mutations in the *CYP4V2* gene are responsible for triggering BCD, a rare autosomal recessive disease with variable phenotypic features. The disease usually presents between the second and fourth decades. Severe visual impairment is observed by the fifth or sixth decades of life.[16] The *CYP4V2* gene encodes a member of the cytochrome P450 superfamily, which may play a role in fatty acid homeostasis in the eye.[24] The *CYP4V2* mutations can cause not only BCD but also RP.[25] Patient LCATH8, a 2-year-old male born to consanguineous parents, carried compound heterozygous missense mutations in the *CYP4V2* gene. These identical mutations have also been reported in a BCD patient from a consanguineous family.[16] However, the clinical manifestations were so different in that our patient developed symptoms very early in life. These results are consistent with previous reports showing that the same mutation in the *CYP4V2* gene can cause

distinctly different clinical manifestations in BCD patients[26] and RP patients.[25] Phenotypic variability between these patients may be due to the effect of modifier genes or epigenetics. In addition to the two *CYP4V2* mutations, a novel single heterozygous nonsense variant, p.Arg3607Ter, in the *ALMS1* gene associated with an arAS has also been identified. It is possible that this *ALMS1* variant may act as an extra allele affecting the LCATH8 patient's genome as a modifying factor leading to more severe phenotype (infancy onset) that was caused by *CYP4V2* mutations of which most phenotypes such as BCD and RP are adult onset.[25,26] This is the first time to report that *CYP4V2* mutations were not only responsible for BCD and RP, but also for LCA. Because an abnormality in serum fatty acid concentration was observed in BCD patients,[27] further clinical evaluation, especially the determination of the serum fatty acid profile of patient LCATH8, is required to confirm the genetic findings and clinical associations.

In conclusion, this study presents the molecular genetics and clinical features in Thai patients with LCA. Our study supports the data from previous studies that patients who were diagnosed with LCA may harbor mutations in other genes associated with IRDs. Furthermore, our data demonstrated that molecular diagnostic testing provides an accurate clinical diagnosis and can be used to guide clinical management of patients, especially those manifesting syndromic LCA. Finally, further analysis on large cohorts of LCA patients is necessary to confirm the association between the *CTNNA1* and *CYP4V2* genes and LCA.

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References

1. den Hollander AI, Roepman R, Koenekoop RK, Cremers FPM. Leber congenital amaurosis: genes, proteins and disease mechanisms. *Prog Retin Eye Res*. 2008;27:391–419.
2. Wang X, Wang H, Cao M, et al. Whole-exome sequencing identifies ALMS1, IQCB1, CNGA3, and MYO7A mutations in patients with Leber congenital amaurosis. *Hum Mutat*. 2011; 32:1450–1459.
3. Chen Y, Zhang Q, Shen T, et al. Comprehensive mutation analysis by whole-exome sequencing in 41 Chinese families with Leber congenital amaurosis. *Invest Ophthalmol Vis Sci*. 2013;54:4351–4357.
4. Wang X, Wang H, Sun V, et al. Comprehensive molecular diagnosis of 179 Leber congenital amaurosis and juvenile retinitis pigmentosa patients by targeted next generation sequencing. *J Med Genet*. 2013;50:674–688.
5. Xu Y, Guan L, Xiao X, et al. ALMS1 null mutations: a common cause of Leber congenital amaurosis and early-onset severe cone-rod dystrophy. *Clin Genet*. 2016;89:442–447.
6. Xu Y, Xiao X, Li S, et al. Molecular genetics of Leber congenital amaurosis in Chinese: new data from 66 probands and mutation overview of 159 probands. *Exp Eye Res*. 2016;149:93–99.
7. Wang SY, Zhang Q, Zhang X, Zhao PQ. Comprehensive analysis of genetic variations in strictly-defined Leber congenital amaurosis with whole-exome sequencing in Chinese. *Int J Ophthalmol*. 2016;9:1260–1264.
8. Jinda W, Taylor TD, Suzuki Y, et al. Whole exome sequencing in Thai patients with retinitis pigmentosa reveals novel mutations in six genes. *Invest Ophthalmol Vis Sci*. 2014;55: 2259–2268.
9. Chang X, Wang K. wANNOVAR: annotating genetic variants for personal genomes via the web. *J Med Genet*. 2012;49:433–436.
10. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424.
11. Venselaar H, Te Beek TA, Kuipers RK, Hekkelman ML, Vriend G. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. *BMC Bioinformatics*. 2010;11:548.
12. Falk MJ, Zhang Q, Nakamaru-Ogiso E, et al. NMNAT1 mutations cause Leber congenital amaurosis. *Nat Genet*. 2012;44:1040–1045.
13. Wang H, Wang X, Zou X, et al. Comprehensive molecular diagnosis of a large Chinese Leber congenital amaurosis cohort. *Invest Ophthalmol Vis Sci*. 2015;56:3642–3655.
14. Zernant J, Kulm M, Dharmaraj S, et al. Genotyping microarray (disease chip) for Leber congenital amaurosis: detection of modifier alleles. *Invest Ophthalmol Vis Sci*. 2005;46:3052–3059.
15. Wang S, Zhang Q, Zhang X, Wang Z, Zhao P. Clinical and genetic characteristics of Leber congenital amaurosis with novel mutations in known genes based on a Chinese eastern coast Han population. *Graefes Arch Clin Exp Ophthalmol*. 2016;254:2227–2238.
16. Li A, Jiao X, 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.
17. Saksens NT, Krebs MP, Schoenmaker-Koller FE, et al. Mutations in CTNNA1 cause butterfly-shaped pigment dystrophy and perturbed retinal pigment epithelium integrity. *Nat Genet*. 2016;48:144–151.
18. Estrada-Cuzcano A, Koenekoop RK, Coppieters F, et al. IQCB1 mutations in patients with Leber congenital amaurosis. *Invest Ophthalmol Vis Sci*. 2011;52:834–839.
19. Hedergott A, Volk AE, Herkenrath P, et al. Clinical and genetic findings in a family with NMNAT1-associated Leber congenital amaurosis: case report and review of the literature. *Graefes Arch Clin Exp Ophthalmol*. 2015;253:2239–2246.
20. Hameed A, Abid A, Aziz A, Ismail M, Mehdi SQ, Khaliq S. Evidence of RPGRIP1 gene mutations associated with recessive cone-rod dystrophy. *J Med Genet*. 2003;40:616–619.
21. Khan AO, Al-Mesfer S, Al-Turkmani S, Bergmann C, Bolz HJ. Genetic analysis of strictly defined Leber congenital amaurosis with (and without) neurodevelopmental delay. *Br J Ophthalmol*. 2014;98:1724–1728.
22. Abouzeid H, Othman IS, Schorderet DF. A novel recessive RPGRIP1 mutation causing Leber congenital amaurosis. *Klin Monbl Augenheilkd*. 2016;233:456–459.
23. Marshall JD, Maffei P, Collin GB, Naggert JK. Alstrom syndrome: genetics and clinical overview. *Curr Genomics*. 2011;12:225–235.

24. Nakano M, Kelly EJ, Rettie AE. Expression and characterization of CYP4V2 as a fatty acid omega-hydroxylase. *Drug Metab Dispos*. 2009;37:2119–2122.
25. Wang Y, Guo L, Cai SP, et al. Exome sequencing identifies compound heterozygous mutations in CYP4V2 in a pedigree with retinitis pigmentosa. *PLoS One*. 2012;7:e33673.
26. Lai TY, Ng TK, Tam PO, et al. Genotype phenotype analysis of Bietti's crystalline dystrophy in patients with CYP4V2 mutations. *Invest Ophthalmol Vis Sci*. 2007;48:5212–5220.
27. Lee J, Jiao X, Hejtmancik JF, et al. The metabolism of fatty acids in human Bietti crystalline dystrophy. *Invest Ophthalmol Vis Sci*. 2001;42:1707–1714.

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