Next-generation Sequencing Extends the Phenotypic Spectrum for LCA5 Mutations: Novel LCA5 Mutations in Cone Dystrophy

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We aim to characterize the clinical features and genetic causes for two affected siblings from a Chinese family with cone dystrophy (CD). Two patients and four unaffected family members were recruited and received complete ophthalmic examinations. Genomic DNA was isolated from the peripheral blood samples from all patients. Targeted next-generation sequencing (NGS) approach followed by intrafamilial cosegregation and in silico analyses were employed to determine the genetic defects. Ophthalmic evaluations finalized the clinical diagnosis of CD for the two patients in this family, both of whom presented macular atrophy with no remarkable changes in the peripheral retina. Comprehensive genetic screening approach revealed biallelic missense mutations in the Leber congenital amaurosis 5 (LCA5) gene, p.[Ala212Pro];[Tyr441Cys], as disease causative for this family. Both mutations were novel. The first substitution was predicted to eliminate a hydrogen bond and alter the tertiary structure of lebercilin, protein encoded by LCA5. We for the first time report novel biallelic LCA5 mutations in causing CD. Our study extends the phenotypic and genotypic spectrums for LCA5-associated retinopathies and better illustrates its genotype-phenotype correlations, which would help with better genetic diagnosis, prognosis, and personalized treatment for CD patients.

Mutations in the Leber congenital amaurosis 5 (LCA5) gene (MIM 611408) are reported to cause one to two percent of patients with Leber congenital amaurosis (LCA). LCA is the most severe form of inherited retinal dystrophies (IRDs). Its typical features include blindness or severe visual impairments within the first year of life, congenital nystagmus, sluggish or absent pupillary responses, photophobia, and high hyperopia1,2. Since its first identification by den Hollander in 2007, LCA5 mutations have been widely reported as LCA causative in multiple ethnic groups3–13. Recent studies also indicate the disease causing roles of LCA5 mutations in two Asian families with early-onset retinal dystrophy (EORD) and a Spanish family with retinitis pigmentosa (RP)12,13. Patients from the two families with EORD showed dystrophy and pigmentation in the peripheral retina with their fovea spared and central vision preserved, while patients from the RP family showed very poor central vision with remarkable intrafamilial phenotypic diversity.

LCA5, mapped to chromosome 6q14.1, contains 9 exons and encodes lebercilin, a widely expressed ciliary protein with high evolutionary conservation5,13. Despite the ubiquitous expression of lebercilin, lebercilin defects only cause retinal dystrophies, suggesting its important role in keeping regular retinal functions. Herein, according to a comprehensive genetic screening approach, we describe two novel LCA5 mutations in a previously unreported correlation with cone dystrophy (CD) in two affected siblings from a Chinese family with autosomal recessive inheritance pattern.

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Results
Clinical Manifestations. Two patients, two unaffected siblings and their asymptomatic parents were included in the present study with their pedigree presented in Fig. 1A and clinical details summarized in Table 1. The 13-year-old proband, YZ-II:4, noticed a decrease in her vision since infancy. Her 30-year-old elder sister YZ-II:1 reported similar onset age and visual symptoms. The other two siblings and their parents denied similar visual issues. No family history of visual problems was recorded. Both patients presented dyschromatopsia since early childhood. The proband also noticed rapid deterioration of central vision. The best corrected visual acuities (BCV As) dropped from 20/40 at age 6, when she was first diagnosed of CD, to 20/100 within one year, to 20/200 at age 10 and remained stable since then. Similar visual symptoms were reported by her elder sister, who suffered from rapid drop in central vision in her early 10 s and the disease became stable since her mid-10 s. Nyctalopia was not reported and no remarkable changes were revealed in the anterior segment by the slit-lamp examination. Funduscopy indicated retinal atrophy restricted to the macular region with fovea involved for both patients (Fig. 2A,B,G,H). Healthy vascular arcades and optic disk with no signs of peripheral involvement were revealed in the fundus of both patients. Consistent with the fundus photography, fundus autofluorescence (FAF) of the proband indicated hypofluorescent central area (Fig. 2C,D). Fundus fluorescein angiography (FFA) revealed speckled changes of increased fluorescence in the macular region (Fig. 2E,F). Outer nuclear layer (ONL), inner/outer segments (IS/OS), and retinal pigment epithelium (RPE) are significantly thinned as suggested by optical coherence tomography (OCT) presentations of both patients (Fig. 2K–P). Visual field (VF) tests showed diffused loss of central vision in the proband YZ-II:4 and central scotoma in patient YZ-II:1. Photopic responses were undetectable for both patients as revealed by electroretinography (ERG), while scotopic responses were residual. Pathologic responses were recorded by visual-evoked potentials (VEP) tests for both patients, including low amplitude of P100 wave and enlarged latency (implicit time). In summary, the two patients from family YZ presented similar CD phenotypes.

Genetic Findings. Targeted NGS approach, summarized in Supplementary Table S1, was selectively conducted on patient YZ-II:1. Coverage of the targeted region reached 99.89%, and its mean depth
achieved 109.72-fold. A total of 2669 variants, including 2354 SNPs and 315 Indels were initially identified for patient YZ-II:1. Only 6 coding variants retained after filtration against the 6 SNP databases (Supplementary Table S2). Intrafamilial cosegregation analysis further confirmed that biallelic missense variants, LCA5 c.

Table 1. Clinical Features of Attainable Patients. — = not available; F = female; M = male; BCVA = best corrected visual acuity; O.D. = right eye; O.S. = left eye; N = normal; D = diminished; SR = slightly reduced; R = reduced; CV = central vision; CS = central scotoma. ∆Affected patients. *Low amplitude of P100 wave, enlarged latency (implicit time).

Figure 2. Fundus photographs, fundus autofluorescence (FAF) imaging, fundus fluorescein angiography (FFA), and optical coherence tomography (OCT) findings in the two patients and the asymptomatic mother from family YZ. (A,B) Fundus of patient YZ-II:4 indicates macular atrophy with loss of fovea reflex but no signs of peripheral involvement. (C,D) Oval hypofluorescent area in the maculae of both eyes is shown in the FAF of patient YZ-II:4. (E,F) FFA of patient YZ-II:4 notices speckled changes of increased fluorescence in the macular region of both eyes. (G,H) Similar to patient YZ-II:4, marked macular atrophy with fovea involved was also found in the fundus of both eyes of patient YZ-II:1. (I,J) The color fundus of the asymptomatic mother YZ-I:2. (K–P) OCT presentations of patients YZ-II:4 (K,L), YZ-II:1 (M,N), and their mother YZ-I:2 (O,P). The outer nuclear layer (ONL) and inner/outer segments (IS/OS) layers are vanished in the fovea and its surrounding maculae of patients YZ-II:4 and YZ-II:1 with no remarkable changes detected in the peripheral retina. Retinal pigment epithelium layer was significantly thinned in the macula of patient YZ-II:1 but was slightly changed in patient YZ-II:4. OD = right eye; OS = left eye.
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Targeted NGS Approach. Targeted NGS approach was selectively employed on patient YZ-II:1 for mutation identification using a previously described microarray targeting 180 IRDs causative and 9 candidate genes27–29. Library preparation, qualification, NGS on the Illumina HiSeq2000 platform (Illumina, Inc., San Diego, CA, USA), and bioinformatics analysis were performed as detailed previously30. Coverage and sequencing depth were further evaluated. Six SNP databases, including dbSNP137 (http://hapmap.ncbi.nlm.nih.gov), HapMap Project (ftp://ftp.ncbi.nlm.nih.gov/hapmap), 1000 Genome Project (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp), YH database (http://yh.genomics.org.cn/), Exome Variant Server (http://evs.gs.washington.edu/EVS/), and Exome Aggregation Consortium (http://exac.broadinstitute.org/), were subsequently used for the filtration process. Variants found homozygous or with a minor allele frequency (MAF) of over 0.01 in these SNP databases were then discarded. Intrafamilial cosegregation analysis and prevalence test in 150 additional controls were further conducted using Sanger sequencing with the primer information detailed in Supplementary Table S2.

In Silico Analysis. Vector NTI Advance 11 software (Invitrogen, Grand Island, NY) was applied to assess the evolutionary conservation of mutated residue by aligning the orthologous sequences of lebercilin in the following species, including Homo sapiens (ENSP00000376686), Pan troglodytes (ENSPTRP00000031394), Canis lupus familiaris (ENSCAFP00000004169), Bos taurus (ENSBTAP00000053829), Sus scrofa (ENSSSCP00000004819), and Mus musculus (ENSMUSP00000034791).

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Study design: X.C., X. Sheng and C.Z. Collected the samples and performed the experiments: X.C., X. Sun, Y.Z., H.L., Y.L., W.L. and Z.L. Data interpretation and analysis: X.C., X. Sheng, X. Sun, C.J. and S.D. Wrote the manuscript: X.C. and C.Z. All authors have read and approved the final manuscript.
