Detection of Variants in 15 Genes in 87 Unrelated Chinese Patients with Leber Congenital Amaurosis

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Abstract

Background: Leber congenital amaurosis (LCA) is the earliest onset and most severe form of hereditary retinal dystrophy. So far, full spectrum of variations in the 15 genes known to cause LCA has not been systematically evaluated in East Asians. Therefore, we performed comprehensive detection of variants in these 15 genes in 87 unrelated Han Chinese patients with LCA.

Methodology/Principal Findings: The 51 most frequently mutated exons and introns in the 15 genes were selected for an initial scan using cycle sequencing. All the remaining exons in 11 of the 15 genes were subsequently sequenced. Fifty-three different variants were identified in 44 of the 87 patients (50.6%), involving 78 of the 88 alleles (11 homozygous and 56 heterozygous variants). Of the 53 variants, 35 (66%) were novel pathogenic mutations. In these Chinese patients, variants in GUCY2D are the most common cause of LCA (16.1% cases), followed by CRB1 (11.5%), RPGRIP1 (8%), RPE65 (5.7%), SPATA7 (4.6%), CEP290 (4.6%), CRX (3.4%), LCA5 (2.3%), MERTK (2.3%), AIPL1 (1.1%), and RDH12 (1.1%). This differs from the variation spectrum described in other populations. An initial scan of 55 of 215 PCR amplicons, including 214 exons and 1 intron, detected 83.3% (65/78) of the mutant alleles ultimately found in these 87 patients. In addition, sequencing only 9 exons would detect over 50% of the identified variants and require less than 5% of the labor and cost of comprehensive sequencing for all exons.

Conclusions/Significance: Our results suggest that specific difference in the variation spectrum found in LCA patients from the Han Chinese and other populations are related by ethnicity. Sequencing exons in order of decreasing risk is a cost-effective way to identify causative mutations responsible for LCA, especially in the context of genetic counseling for individual patients in a clinical setting.

Introduction

Leber congenital amaurosis (LCA, MIM 204000) is a severe form of inherited retinal dystrophy, characterized by severe visual loss at or near birth, Franceschetti’s oculo-digital sign, searching or wandering nystagmus, and pigmentary retinopathy [1,2]. Visual acuity is rarely better than 20/400 [3]. Fundus changes are extremely variable, ranging from a normal appearance to an obvious pigmentary retinopathy similar to retinitis pigmentosa. Electroretinogram (ERG) recordings are usually extinguished or severely subnormal. In most cases, LCA shows an autosomal recessive pattern of inheritance. However, several families with autosomal dominant LCA have been well documented [2]. The prevalence of LCA is around 1–2 per 80,000 live births, accounting for approximately 20% of cases of inherited blindness among children and more than 5% of all congenital retinopathies [4]. Currently, 16 loci for LCA have been mapped, at which mutations in 15 genes have been identified as being responsible for the disease: GUCY2D [5], CRB1 [6], RPE65 [7], RPGRIP1 [8], AIPL1 [9], LCA5 [10], CRX [11], LRAT [12], TULP1 [13], RDH12 [14], CEP290 [15], RD3 [16], SPATA7 [17], IMPDH1 [18], and MERTK [19].

It has been estimated that mutations in these 15 genes are responsible for about 65% of all LCA cases [2]. The most frequently mutated genes in published studies are CEP290 (15%), followed by GUCY2D (12%), CRB1 (10%), RPE65 (6%), AIPL1 (5.3%), RPGRIP1 (4.2%), LCA5 (1.8%), CRX (1.0%), and MERTK (0.6%). However, the full frequency spectrum of variation in these 15 genes has not been evaluated in East Asia, an area containing one third of the world’s population. In this study, we performed a comprehensive evaluation of variation in these 15 genes in 87 unrelated Han Chinese patients with LCA.

Methods

LCA Patient cohort

Eighty-seven unrelated patients with LCA were recruited at the Pediatric and Genetic Clinic in the Eye Hospital of Zhongshan
Half Mutations Detected in 9 Exons of 15 LCA Genes

| Gene | Total number of exons | Exons for initial scan | Exons for subsequent sequencing |
|------|-----------------------|------------------------|--------------------------------|
| GUCY2D | 19 | 2, 3, 8–12,15–17 | All remaining |
| CRB1 | 12 | 2,6,7,9,11 | All remaining |
| RPRGIP1 | 24 | 2–4,12,15,16, 21–23 | All remaining |
| SPATA7 | 12 | 8,11,12 | All remaining |
| RPE65 | 14 | 3,4,9,10 | All remaining |
| CRX | 3 | 3 | All remaining |
| LCA5 | 7 | 1,2,7 | All remaining |
| AIPL1 | 6 | 2,5,6 | All remaining |
| RDH12 | 7 | 5 | All remaining |
| CEP290 | 54 | IVS26+1665, 36, 41 | All remaining |
| MERTK | 19 | 4,7,14,15,19 | All remaining |
| IMPDH1 | 17 | 6–8 | None |
| LBRAT | 3 | 2 | None |
| RD3 | 3 | 2 | None |
| TULP1 | 15 | 12–14 | None |

| doi:10.1371/journal.pone.0019458.0001 |

**Table 1. The exons and one intron sequenced in our initial screen and in subsequent analyses.**

**Results**

Fifty-three different variants were identified in 44 of the 87 patients (50.6%), involving 78 of the 88 alleles (11 homozygous and 56 heterozygous) (Tables S2 and S3). These include 8 homozygous or 12 compound heterozygous cases, 5 cases in whom two mutations in one gene and a single mutation in a second gene were identified (triplicated), 3 patients in whom two mutations in different genes were identified (digenic), and 16 patients in whom only a single heterozygous variant was identified. Of the 53 variants identified, 35 (66%) were novel and predicted to be pathogenic, 9 (17%) were known to be pathogenic, and 9 (17%) were novel neutral or unknown effects. An initial scan of 55 amplicons (out of 215 in total, 25.6%) detected 83.3% (65/78) of the mutant alleles detected by the full scan. Sequencing the remaining exons in 11 of the 15 genes detected only 13 additional mutant alleles (16.9%).

The percentage of patients who had variants in the 15 genes were, in decreasing order: **GUCY2D** 16.1% (14/87), **CRB1** 11.5% (10/87), **RPRGIP1** 8% (7/87), **RPE65** 5.7% (5/87), **SPATA7** 4.6% (4/87), **CEP290** 4.6% (4/87), **CRX** 3.4% (3/87), **LCA5** 2.3% (2/87), **MERTK** 2.3% (2/87), **AIPL1** 1.1% (1/87), and **RDH12** 1.1% (1/87) (all variants were taking into account for those patients who had variants in more than one gene). For individual genes, the majority of variants were found in one exon (**RPE65**, **CRX**, **LCA5**, **AIPL1**, and **RDH12**), two exons (**CRB1**, **RPRGIP1**, **SPATA7**, **CEP290** and **MERTK**), or three exons (**GUCY2D**) (Table 2). Further analysis revealed that 9 exons (4.19% fragments sequenced, 9/215) contained 50.8% (39/78) of the variant alleles detected in this study (Table S4). Polymorphisms detected in these genes are shown in Table S5. Clinical data for the 44 patients in which variants were found are listed in Table S6.

**GUCY2D**

Fifteen **GUCY2D** variants (including 13 novel variants) were identified in 14 LCA patients (Tables S2 and S3). Variants were found in the homozygous, compound heterozygous, and heterozygous states in 4, 5, and 5 patients, respectively. Of these 14 patients, 6 also had variants in other genes, carrying 2, 3, 4 variant alleles overall. The c.164C>T [p.T55M] variant was found in four patients (one homozygote and three heterozygotes), suggesting an existence of mutation hot spot at this site, since each of those patients have different local SNP haplotypes (data not shown). The
c.935C>T (p.T312M) and c.2302C>T (p.R768W) mutations have been described previously. The most frequently mutated 10 exons contained 86.9% (20/23) of the mutant alleles detected by sequencing all 19 coding exons. Variants were most frequent in exon 2 (7 variants found in 6 patients), followed by exons 11 and 12. Besides, several polymorphisms were detected, including c.61T>C (p.W21R), c.154G>T (p.A51S), and c.2101C>T (p.P701S), respectively (Table S5). The c.61T>C (p.W21R) was reported as a polymorphism [21] even though PolyPhen predicted (p.P701S) it to be possibly damaging. No variants were found in exons 1, 3, 5, 10, or 19 (Table 2).

**CRB1**

Nine variants (7 novel variants) were detected in 10 patients (Tables S2 and S3), including 5 missense, 3 splicing, and 1 nonsense variant(s). The c.866C>T (p.T289M) variant in patient LH17 was reported as a pathogenic mutation based on a study of Italian patients [22], although it was predicted to be benign by Polyphen and tolerated by SIFT. However, it was found not to cosegregate with the disease in another study [23], suggesting it has a nonpathogenic role. The heterozygous variant c.4005+2T>G in LH29 was predicted to abolish the splicing site, with Automated Splice Site Analysis predicting that binding energy would be decreased to 0, and on that basis is predicted to be responsible for disease. The heterozygous 1903T>C (p.S635P) variant in patient LH15 was reported as a polymorphism based on a study of Italian patients [22], although it was predicted to be pathogenic and the c.995T>C (p.I332T) variant in patient QT654 was predicted to be neutral by both PolyPhen and SIFT. Most of these variants occurred within the RPGR interacting domain. The efficiency of the initial screening was 90.1% (10/11 alleles) (Tables 1 and 2). In addition, three known polymorphisms were detected in LCA patients (Table S5). Variants were most frequent in exon 3 (Table 2), and no variation was found in exons 1, 2, 6, 8–11, 16–20, 22, and 24.

**RPE65**

Three variants were detected in 5 patients, including one known (c.1059_1060insG/p.K354EfsX11) [24] and two novel variants (c.295G>A/p.V99I, c.997G>C/p.G333R) (Table S2). The two novel variants were predicted to be neutral by Polyphen and SIFT. Four patients carried the same heterozygous c.295G>A (p.V99I) variant that is absent in 96 normal controls. One patient was a compound heterozygote for two mutations (c.997G>C and c.1059_1060insG). Our initial scan of 4 of the 14 exons detected 100% of the variant alleles (6/6) identified after sequencing all 14 exons.

**SPATAt**

Three variants were found in 4 patients, including 2 novel and 1 known (Tables S2 and S3). Two variants were predicted to be pathogenic and the c.995T>C (p.I332T) variant was predicted to be neutral by Polyphen and SIFT. The variants were heterozygous in three patients and homozygous in one patient. Patient LH15 was heterozygous for a known 4 bp deletion in SPATAt and a common missense mutation in GUCY2D (p.T55M). Our initial scan of 3 of the 12 exons could find 60% of the mutant alleles (3/5) detected after all 12 exons were sequenced.

**CEP290**

First, we analyzed the genomic regions encompassing IVS6+1655, exon 36, and exon 41 of CEP290, where the c.2991+1655A>G is the most common mutation in Caucasians. However, no variation was detected in the 87 Chinese patients, except for a presumably neutral variant, c.5709+25A>G.

Five variants in the other CEP290 exons were subsequently detected in 4 patients, including 4 that were novel (c.367C>T, c.4897C>T, c.6766delC, and c.6787A>G) and 1 that was previously described (c.383_386delATAG). Two patients were

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**Table 2.** Frequencies of variant alleles detected in the individual exons of each gene.

| Gene | Exons in decreasing order (%) |
|------|-------------------------------|
| GUCY2D | Exon 2 (30.4) | Exon 11 (13.1) | Exon 12 (13.1) | Exon 3 (8.7) | Exon 4 (8.7) | Exon 8 (8.7) | Exon 9 (8.7) | Exon 16 (4.3) | Exon 17 (4.3) |
| CRB1 | Exon 6 (35.3) | Exon 11 (29.4) | Exon 9 (23.5) | Exon 7 (5.9) | Exon 4 (5.9) |
| RPGRIP1 | Exon 3 (36.3) | Exon 4 (18.2) | Exon 5 (9.1) | Exon 12 (9.1) | Exon 15 (9.1) | Exon 21 (9.1) | Exon 23 (9.1) |
| SPATAt | Exon 2 (40) | Exon 11 (40) | Exon 8 (20) |
| CEP290 | Exon 6 (50) | Exon 37 (16.7) | Exon 42 (16.7) | Exon 49 (16.6) |
| RPE65 | Exon 4 (66.6) | Exon 9 (16.7) | Exon 10 (16.7) |
| CRX | Exon 3 (66.7) | Exon 2 (33.3) |
| LCA5 | Exon 7 (50) | Exon 2 (50) |
| AIPL1 | Exon 6 (100) |
| MERTK | Exon 4 (50) | Exon 19 (50) |
| RDH12 | Exon 3 (100) |

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Five variants in the other CEP290 exons were subsequently detected in 4 patients, including 4 that were novel (c.367C>T, c.4897C>T, c.6766delC, and c.6787A>G) and 1 that was previously described (c.383_386delATAG). Two patients were
compound heterozygotes and another two were single heterozygotes. The effect of the c.6787A>G (p.S2263G) variant could not be predicted by PolyPhen but SIFT labeled it as damaging (Tables S2 and S3).

CRX

Three heterozygous variants in CRX were identified in 3 patients (Tables S2 and S3). All 3 variations are predicted to be pathogenic. Of the 3, the c.541delG (p. A181PfsX5) variant in patient LH9 and the c.458delC(p.P153QfsX34) variant in RP178 were reported by our previously study [25,26].

LCAS

Two novel heterozygous variants in LCAS were identified in two patients, respectively (Tables S2 and S3). The 1820_1821delCA (p.Q607VfsX6) variant in exon 7 is predicted to cause a frameshift and the c.634G>T (p.A212S) variant in exon 2 is predicted to be benign by both PolyPhen and SIFT. Additionally, two known polymorphisms, p.D26A and p.G656D, were found in 35 and 32 patients, respectively (Table S5).

AIPL1

A novel homozygous variant, c.926_927insCCTGAACCG-CAGGGAGCT (p. E309DinsLNRREL), was identified in patient QT338 (Tables S2 and S3). In addition, a known polymorphism, c.268G>C (p.D90H), was detected in 31 patients (Table S5).

RDH12

One novel heterozygous c.236C>T (p.A79V) variant was detected in patient LH16, and was predicted to be benign (Tables S2 and S3). In addition, p.R161Q, a known polymorphism, was detected in 3 patients (Table S5).

MERTK

Two novel heterozygous variants in MERTK were identified in patients LH28 and RP143. Both of them are predicted to be pathogenic. Sequencing revealed that patient RP143 was heterozygous at two sites: c.2873C>T (p.R958L) in MERTK and c.6787A>G (p.S2263G) in CEP290 (Tables S2 and S3).

TULP1, RD3, LRAT and IMPDH1

Unlike the other 11 genes listed above, for which all coding exons were analyzed by sequencing, only those exons with previously reported variants were analyzed in TULP1, RD3, LRAT, and IMPDH1, due to the rarity of reported variants in the exons of these genes. No variants were detected in any of the exons screened in these genes, including exons 12–14 of TULP1, exon 2 of RD3, exon 2 of LRAT, and exons 6–8 of IMPDH1.

Discussion

Leber congenital amaurosis is the earliest occurring and most severe inherited retinal dystrophy. Since the initial identification of mutations to GUCY2D as a cause of LCA in 1996 [5], mutations in a total of 15 genes have been identified as being responsible for LCA, accounting for approximately 65%–70% of LCA cases [2,27,28]. However, the variant frequencies for these genes vary between different ethnic groups. In northern Pakistan, the genes most commonly mutated in LCA are RPGRIP1 (29% of families), AIPL1 (21% of families), and LCAS (21% of families); whereas in Caucasian populations, mutations in RPGRIP1, AIPL1, and LCAS account for only 4.2%, 5.3%, and 1.8% of LCA cases, respectively [2]. Although CEP290 is the most commonly identified mutant gene in Caucasian LCA patients, accounting for 15% of LCA cases [29], mutations in CEP290 have not been detected in LCA patients from Korea [30], Saudi Arabia [31], northern Pakistan [32], or southern India [33]. An understanding of the frequency spectrum of variants in these 15 genes in different populations will not only facilitate genetic diagnosis and genetic counseling for this disastrous disorder, but will also identify patients who may potentially benefit from gene therapy or other possible interventions [31]. Because the NPHP5 gene was only recently reported as related to LCA by Stone EM, et al [34], this gene was not included into our study.

Here, we performed comprehensive mutational analysis of 15 genes in 87 unrelated Chinese patients with LCA. Of the 15 genes, all coding exons in 11 genes were analyzed by cycle sequencing. For the remaining 4 genes which have only infrequently been identified as causal for LCA, only exons in which variants had previously been identified were sequenced. The percentage of patients who had variants in the 15 genes were, in decreasing order: GUCY2D 16.1% (14/87), CRB1 11.5% (10/87), RPGRIP1 8% (7/87), RPE65 5.7% (5/87), SPATA7 4.6% (4/87), CEP290 4.6% (4/87), CRX 3.4% (3/87), LCA5 2.3% (2/87), MERTK 2.3% (2/87), AIPL1 1.1% (1/87), and RDH12 1.1% (1/87). Thus, the frequencies of variants in different genes vary remarkably in different populations. GUCY2D was the gene most frequently mutated in Chinese patients while the genes found to be most frequently mutated in other populations were RPGRIP1 in people from northern Pakistan [32], CRB1 in the Spanish [28], and CEP290 in Caucasians [15]. The overall rate variant detection was 50.6% (44/87) in this study, which is comparable to the worldwide variations detection rate of 65% if the difference between the frequency of variants in CEP290 is taken into consideration (CEP290 mutations occur in 15% of Caucasian patients but in only in 6.4% of Chinese patients) [2].

Homozygous mutations were identified in a number of cases, often related to the family and population structure. For example, family LH22 was having homozygous T55M mutations in GUCY2D, families LH32 and QT585 showing homozygous S611P mutations in CRB1, and family RP208 showing a single base deletion in RPGRIP1 are from consanguineous matings in an isolated Chaoshan population. Families QT521 and QT608 showing splicing and M784R mutations in GUCY2D respectively, family LH24 with a R395X mutation in SPATA7 and family QT338 showing an 18 base pair insertion in AIPL1 are not known to be consanguineous, but are from isolated populations.

Identifying the most frequently mutated exons in these 15 genes will greatly facilitate detection of variation in LCA patients in a clinical testing. Our initial scan of 25.6% (55 of 215) of exons, based on the frequencies of variation in other populations, detected 83.3% (65/78) of all the mutant alleles we detected. This indicates that reducing the amount of labor and cost by 75% would still result in detection of over three quarters of the variation in our samples. Based on our study of 87 Chinese patients, sequencing only the 9 most polymorphic (4.18% of the 215 sequenced regions) exons (exons 2, 11, and 12 of GUCY2D; exons 6, 11, and 9 of CRB1; exon 4 of RPE65; exon 3 of RPGRIP1; and exon 6 of CEP290) would result in detection of 50.8% (39/78) of all variants found in this study. This would use less than 5% of the labor and cost of a comprehensive sequencing strategy and detect over 50% of the variation present in other groups of Chinese patients. Screening these exons in order of expected variation would be predicted to cut expenses significantly while still being accurate and comprehensive. Our results provide a key bridge between bench and bed side and should make genetic
diagnose of LCA in Chinese patients more accessible and practical. This should greatly enhance the clinical genetic counseling, diagnosis, and early intervention of LCA in the Chinese population. These results also highlight the importance of analyzing the causative genes and their exons in different ethnic groups in a systematic and population-specific fashion.

Another important point from our study is that some sequence variations might mistakenly be thought to be causative mutations for LCA if only a single individual gene were analyzed. For example, the heterozygous c.164C>T (p.T55M) variant in GUCY2D might be incorrectly labeled a common mutation for LCA because it was found in 3 unrelated patients with LCA but not in 96 controls, was predicted to be a pathogenic mutation by Polyphen, and was identified as a causative mutation in SIFT. This might be the case for patient LH22, who had homozygous c.164C>T mutations, but it may not cause LCA in patient QT453 since QT453 had homozygous nonsense mutations (G1226X) in CRB1. Even though both mutations themselves could be pathogenic, digenic mutations may not necessarily cause disease, since one of the phenotypically normal parents in families LH16, QT479, QT453, QT509, and RP2080 may carry digenic mutations. In this study, digenic variants were detected in 3 patients, including LH15 (GUCY2D and SPATA7), QT659 (CRB1 and LCA5), and RP143 (CEP290 and MERTK). Whether these mutations are indeed causative and what types of digenic variations may be responsible for LCA needs additional study. For the 5 patients (LH16, QT479, QT509, QT453, and RP2080) with 3 or 4 variants in two or 3 genes, their clinical phenotypes are comparable to patients with homozygous or compound heterozygous mutations in a single gene (Table S6), suggesting that the third or fourth mutant allele may not have an additive effect and may be more likely to act as a benign variant. In such cases, the 6 additional variant alleles in these 5 patients should not be counted toward the total number of mutant alleles and, therefore, the adjusted number of mutant alleles detected should be 72, not 78 (from 44 patients). In an earlier genotyping microarray study, Zernant, et al. determined 7.3% of LCA patients carry a third mutant allele [35], a greater fraction than would be expected by chance. On the other hand, single heterozygous variants were detected in genes known to cause autosomal recessive LCA in 16 patients (15 predicted to be pathogenic mutations and 1 predicted to be neutral). This might simply represent a failure to detect a second variant. Conversely, it is possible that neutral or silent variants or polymorphisms in the 15 genes sequenced here or in other genes might serve as disease modifier alleles with another major gene defect occurring simultaneously [36]. Additional comprehensive studies, or perhaps further work with animal models, are necessary to answer this question.

In conclusion, systematic analysis of the full frequency spectrum of variation in the 15 selected genes not only gives us an overview of the molecular etiology of LCA in Chinese but also provides useful biomarkers for genetic counseling. In the near future, patients in whom pathogenic mutations are identified could become potential participants for gene therapy, and thus, identification of efficient and effective diagnostic approaches based on the population genetics specific to the patient will become increasingly important.

Supporting Information

**Table S1 Primers used for sequencing.** This table listed 340 primers used to amplify genomic fragments of the GUCY2D, CRB1, RPE65, RPGRIP1, AIPL1, SPATA7, LCA5, CRX, RDH12, CEP290, IMPDH1, TULP1, and MERTK genes.

**Table S2 Variants detected in the 15 genes sequenced in 87 unrelated LCA patients and 96 healthy controls.** Mutations in each gene were listed separately. a) The value is the difference between the original value and substitution value. b) These changes are likely neutral variations based on computational prediction. c) Controversial, as some reports regard this mutation as a non-pathogenic mutation, den Hollander et al 2004.

**Table S3 Variants detected in the 15 genes sequenced in 87 unrelated LCA patients and 96 healthy controls.** Patients with mutations were separated into three groups. Group A: Patients with homozygous or compound heterozygous mutations in one gene. Group B: Patients with only one heterozygous mutation in one gene. Group C: Patients with Digenic or Triallelic or 4 variants. a) The difference between the original value and the substitution value. b) These variants are likely neutral variations based on computational prediction. c) Controversial, as some reports regard this mutation as an non-pathogenic mutation, den Hollander et al 2004.

**Table S4 Frequencies of the 78 variant alleles in individual exons.** The frequency of mutations in each exon was calculated based on either all mutant alleles in all 15 genes or those in individual gene.

**Table S5 Polymorphisms detected in 87 unrelated LCA patients and 96 healthy controls.** ND: not determined. a) The c.2101C>T (p.P701S) variant in GUCY2D has been reported to cosegregate with LCA in three pedigrees (Zernant et al., 2005). This variant was found at similar frequencies in the patients and the normal controls in our study (28 heterozygotes and 1 homozygote out of 87 LCA patients and 26 heterozygotes and 2 homozygote in 96 normal controls). The high frequency of this variant, and especially its occurrence in a heterozygous state in normal controls, argues strongly against its classification as a mutation (Valle et al., 2007).

**Table S6 Clinical data for the 44 patients who carry variants in the 15 sequenced genes.** FMB = First few months after birth *Cons = consanguinity marriage of parents. #The boy and his father are both affected. ¥Both of the twins are affected. PV = poor vision; PP = photophobia; NB: Night blindness; NYS = nystagmus; ODS = oculodigital sign; RN = roving nystagmus; AV = attenuated vessels; CRD = carpet-like retinal degeneration; PIG = pigment deposit; NFR = no foveal reflex; MA: Macular atrophy.

Acknowledgments

We thank all patients and controls for their participation.

Author Contributions

Conceived and designed the experiments: QZ JFH LL. Performed the experiments: LL XX. Analyzed the data: LL QZ JFH XJ. Contributed reagents/materials/analysis tools: SL XJ PW XG QZ. Wrote the paper: LL QZ JFH.
References

1. Leber T (1809) Uber retinitis pigmentosa und angeborene amaurose. Graefes Arch Klin Ophthalmol 13: 1–23.

2. den Hollander AI, Roepman R, Koenekep RK, Creemers FP (2002) Molecular genetics of Leber congenital amaurosis. Hum Mol Genet 11: 1169–1176.

3. Creemers FP, van den Hurk JA, den Hollander AI (2002) Molecular genetics of Leber congenital amaurosis. Hum Mol Genet 11: 1169–1176.

4. Perrault I, Rozet JM, Gerber S, Ghazi I, Lrowski C, et al. (1999) Leber congenital amaurosis. Mol Genet Metab 68: 200–208.

5. Perrault I, Rozet JM, Calvys P, Gerber S, Gurnuz A, et al. (1996) Retinal-specific guanylate cyclase gene mutations in Leber’s congenital amaurosis. Nat Genet 14: 461–464.

6. den Hollander AI, Heckenlively JR, van den Born LI, de Kok YJ, van der Velde-Visser SD, et al. (2001) Leber congenital amaurosis and retinitis pigmentosa with Coats-like exudative vasculopathy are associated with mutations in the crumb homologue 1 (CRB1) gene. Am J Hum Genet 69: 198–203.

7. Marløens F, Barel C, Grifﬁon JM, Zrenner E, Amalric P, et al. (1997) Mutations in PDE65 cause Leber’s congenital amaurosis. Nat Genet 17: 139–141.

8. Dryja TP, Adams SM, Grimsby JL, McGee TL, Hong DH, et al. (2001) Null RPGRIP1 alleles in patients with Leber congenital amaurosis. Am J Hum Genet 68: 1295–1298.

9. Solochi MM, Bowlie SJ, Sullivan LS, Blackshaw S, Cepko CL, et al. (2000) Mutations in a new photoreceptor-pigmented gene on 17p cause Leber congenital amaurosis. Nat Genet 24: 79–83.

10. den Hollander AI, Koenekep RK, Mohamed MD, Arts HH, Boldt K, et al. (2007) Mutations in LCA5, encoding the cilary protein lebercin, cause Leber congenital amaurosis. Nat Genet 39: 899–895.

11. Sauraop A, Wang QL, Wu W, Cook J, Coats C, et al. (1999) Leber congenital amaurosis caused by a homozygous mutation (R90W) in the homeodomain of CRX in the development of photoreceptor function. Hum Mol Genet 8: 299–305.

12. Thompson DA, Li Y, McHenry CL, Carlson TJ, Ding X, et al. (2001) Mutations in the gene encoding lecithin retinol acyltransferase with early-onset severe retinal dystrophy. Nat Genet 28: 123–124.

13. Hanie S, Perrault I, Gerber S, Tanguy G, Barbet F, et al. (2004) Leber congenital amaurosis: comprehensive survey of the genetic heterogeneity, clariﬁcation of the clinical deﬁnition, and genotype-phenotype correlations. Hum Mutat 23: 396–417.

14. Perrault I, Hanie S, Gerber S, Barbet F, Ducroc D, et al. (2004) Retinal dehydrogenase 12 (RDH12) mutations in leber congenital amaurosis. Hum Mol Genet 23: 1429–1436.

15. den Hollander AI, Koenekep RK, Yarr S, Lopez I, Arends ML, et al. (2006) Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber congenital amaurosis. Am J Hum Genet 78: 536–541.

16. Friedman JS, Chang B, Kannabiran C, Chakravarthy U, Singh HP, et al. (2006) Premature truncation of a novel protein, RDR3, exhibiting subnuclear localization is associated with retinal degeneration. Am J Hum Genet 78: 1059–1070.

17. Wang H, den Hollander AI, Moayed Y, Abulimiti A, Li Y, et al. (2009) Mutations in SPATA7 cause Leber congenital amaurosis and juvenile retinitis pigmentosa. Am J Hum Genet 84: 380–387.

18. Bowlie SJ, Sullivan LS, Mortimer SE, Hedstrom L, Zhu J, et al. (2006) Spectrum and frequency of mutations in IMPDH1 associated with autosomal dominant retinitis pigmentosa and leber congenital amaurosis. Invest Ophthalmol Vis Sci 47: 34–42.

19. Gal A, Li Y, Thompson DA, Weir J, Orth U, et al. (2000) Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause autosomal dominant retinitis pigmentosa. Nat Genet 26: 270–271.

20. Sunyavas S, Ramensky V, Koch I, Lathe W, Srd, Kendrahus AS, et al. (2001) Prediction of deleterious human alleles. Hum Mol Genet 10: 591–597.

21. Rozet JM PL, Gerber S, Ducroc D, Souied E, Munnich A, Kaplan J (2000) Functional analyses of retGC1 missense mutations identiﬁed in Leber’s congenital amaurosis. Investigative Ophthalmology & Visual Science 41: 8533.

22. Simonelli F, Zivello C, Testa F, Rossi S, Fazzi E, et al. (2007) Clinical and molecular genetics of Leber’s congenital amaurosis: a multicenter study of Italian patients. Invest Ophthalmol Vis Sci 48: 4204–4209.

23. den Hollander AI, Davis J, van der Velde-Visser SD, Zonneveld MN, Pierrottet CO, et al. (2004) CRB1 mutation spectrum in inherited retinal dystrophies. Hum Mutat 24: 355–369.

24. Bereta G, Kiser FD, Golczak M, Sun W, Heeon E, et al. (2008) Impact of retinal disease-associated RPE65 mutations on retinoid isomerization. Biochemistry 47: 9856–9865.

25. Zhang Q, Li S, Guo X, Guo L, Xiao X, et al. (2001) Screening for CRX gene mutations in Chinese patients with Leber congenital amaurosis and mutational phenotype. Ophthalmic Genet 22: 89–96.

26. Wang P, Guo X, Zhang Q (2007) Further evidence of autosomal-dominant Leber congenital amaurosis caused by heterozygous CRX mutation. Graefes Arch Clin Exp Ophthalmol 245: 1401–1402.

27. Yarr S, Leroy BP, De Barre E, de Ravel TJ, Zonneveld MN, et al. (2006) Microarray-based mutation detection and phenotypic characterization of patients with Leber congenital amaurosis. Invest Ophthalmol Vis Sci 47: 1167–1170.

28. Vallias P, Cantalapiedra D, Riveiro-Alvarez R, Wilke R, Aguierre-Lamban J, et al. (2007) Mutation screening of 299 Spanish families with retinal dystrophies by Leber congenital amaurosis genotyping microarray. Invest Ophthalmol Vis Sci 48: 3653–3661.

29. Perrault I, Delphin N, Hanie S, Gerber S, Dufier JL, et al. (2007) Spectrum of NPHP5/CEP290 mutations in Leber congenital amaurosis and delineation of the associated phenotype. Hum Mutat 28: 416.

30. Seong MW, Kim SY, Yu YS, Hwang JM, Kim JY, et al. (2008) Molecular characterization of Leber congenital amaurosis in Koreans. Mol Vis 14: 1429–1436.

31. Li Y, Wang H, Peng J, Gibbs RA, Lewis RA, et al. (2009) Mutation survey of known LCA genes and loci in the Saudi Arabian population. Invest Ophthalmol Vis Sci 50: 1336–1341.

32. McKe ﬀin M, Ali M, Mohamed MD, Booth AP, Bishop F, et al. (2010) Genotype-phenotype correlation for leber congenital amaurosis in Northern Pakistan. Arch Ophthalmol 128: 107–113.

33. Sundaresan P, Vijayalakshmi P, Thompson S, Ko AC, Fingert JH, et al. (2009) Mutations that are a common cause of Leber congenital amaurosis in northern America are rare in southern India. Mol Vis 15: 1781–1787.

34. Stone EM, Cideciyan AV, Aleman TS, Scheetz TE, Sumaroka A, et al. (2011) Variations in NPHP5 in patients with nonsyndromic leber congenital amaurosis and Senior-Loken syndrome. Arch Ophthalmol 129: 81–87.

35. Zernant J, Kuhl M, Dharmanaraj S, den Hollander AI, Perrault I, et al. (2005) Genotyping microarray (disease chip) for Leber congenital amaurosis: detection of modifier alleles. Invest Ophthalmol Vis Sci 46: 8032–8039.

36. Yoshida S, Yamaji Y, Yoshida A, Kusuhara R, Yamamoto K, et al. (2006) Novel triple missense mutations of GUCA2D gene in Japanese family with cone-rod dystrophy: possible use of genotyping microarray. Mol Vis 12: 1538–1546.