

Mutation Survey of Known LCA Genes and Loci in the Saudi Arabian Population

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PURPOSE. The purpose of this study was to perform a comprehensive survey of all known Leber congenital amaurosis (LCA) genes and loci in a collection of 37 consanguineous LCA families from Saudi Arabia.

METHODS. Direct PCR and sequencing were used to screen 13 known LCA genes (*GUCY2D*, *CRX*, *RPE65*, *TULP1*, *AIPL1*, *CRB1*, *RPGRIP1*, *LRAT*, *RDH12*, *IMPDH1*, *CEP290*, *RD3*, *LCA5*). In addition, families without mutations identified were further screened with STR markers around these 13 known LCA genes and two loci.

RESULTS. Disease-causing mutations were identified in nine of the 37 families: five in *TULP1*, two in *CRB1*, one in *RPE65*, and one in *GUCY2D*. Mutations in known genes only accounted for 24% of the Saudi families—much less than what has been observed in the European population (65%). Phenotype-genotype analysis was carried out to investigate the LCA disease penetrance for all families whose mutations identified. All identified mutations were found to segregate perfectly with the disease phenotype. On the other hand, severity of the disease varies for different patients carrying the same mutation and even within the same family. Furthermore, based on homozygosity mapping with both STR and SNP markers, one family is likely to map to the *LCA3* locus.

CONCLUSIONS. These results underscore the importance of studying LCA disease families from different ethnic backgrounds to identify additional novel LCA disease genes. Furthermore, perfect segregation between mutation and disease indicates that LCA is fully penetrant. However, phenotypic variations among patients carrying the same mutation suggest that at least some of the variations in the clinical phenotype is due to modification from the genetic background, environment, or other factors. (*Invest Ophthalmol Vis Sci.* 2009;50:1336–1343) DOI:10.1167/iovs.08-2589

Leber congenital amaurosis (LCA; Mendelian Inheritance in Man [MIM] #204,000) was first described in 1869 by Theodor Leber as an “intrauterine” form of retinitis pigmentosa.¹ Among the most common genetic causes of visual impairment in infants and children, LCA accounts for more than 5% of all

retinal dystrophies.^{2,3} The clinical phenotype of LCA is usually severe and is characterized by several visual perturbations identifiable at birth or within the first year of life, including infantile nystagmus, various fundus changes, and minimal or absent responses on the electroretinogram, each of which occurs with an autosomal recessive mode of inheritance.⁴ In addition, numerous variations in retinal pathology have been defined in LCA patients, including heterogeneity in retinal appearance, refractive errors, photoaversion, nyctalopia, and the oculodigital sign.^{3–5} These phenotypic variations suggest a heterogeneous genetic nature of the clinical disorder.

To date, at least 14 genes associated with LCA have been identified (Table 1). These genes are involved in various genetic pathways, including retina development (*CRB1*⁶ and *CRX*^{7–9}), phototransduction (*GUCY2D*² and *AIPL1*¹⁰), vitamin A metabolism (*RPE65*,^{11,12} *LRAT*,¹³ and *RDH12*^{14,15}), protein transport (*TULP1*,¹⁶ *RPGRIP1*,^{17,18} *CEP290*,¹⁹ and *LCA5*²⁰), and RPE phagocytosis (*MERTK*²¹). In addition, the function of *RD3* remains unclear.^{20,22} Furthermore, in rare cases, certain mutations in *CRX* and *IMPDH1*, which is involved in guanine nucleotide synthesis, have been shown to cause a dominant form of LCA.^{23,24} Finally, two loci (*LCA3* at 14q24 and *LCA9* at 1p36) have been linked to LCA, but the underlying mutations remain unknown. Several mutation survey studies of subsets of these genes from LCA patients have been reported.^{5,19,25–27} Cumulatively, mutations in these genes account for ~65% of LCA patients, with *CEP290* accounting for the most (21%), followed by *GUCY2D* (11.8%), *CRB1* (8.6%), *AIPL1* (5.6%), *RPE65* (4.4%), *RPGRIP1* (4.1%), *RDH12* (4%), *TULP1* (1.7%), *CRX* (1.5%), *LRAT* (1.1%), and *RD3* (1.1%), respectively. The frequency of mutations in the recently cloned lebercilin gene (*LCA5*) has not been studied in detail but probably accounts for less than 3% of all cases.²⁰ However, since most of the samples in these surveys are of European descent, the distribution of mutations in LCA patients from other ethnic populations is not necessarily represented.

In contrast to the heterogeneity of a given retinal degenerative disorder, different retinal dystrophies share many common clinical features. For example, although many persons with LCA may have normal or near normal fundi as infants, retinal examinations of some LCA patients evolve over time to exhibit pigmentary retinopathy similar to retinitis pigmentosa (RP). Consistent with this observation, four known LCA disease genes, including *CRX*, *CRB1*, *RPE65*, and *TULP1*, have also been linked to the clinical appearance of RP in other families.²⁸ Interestingly, different mutant alleles of *CRX* lead to different clinical presentations, including RP, LCA, and cone rod dystrophy, possibly due to differences in severity between alleles and modification by other factors in the genome.²³ Similar phenomena have also been observed with most of the known LCA genes. Mutations in some LCA disease genes also lead to cone rod dystrophy (*CRX*, *AIPL1*, *GUCY2D*, *RPE65*, and *RPGRIP1*), retinal dystrophy (*RDH12*), and Bardet-Biedl syndrome (BBS; *CEP290*). Taken together, these results suggest that the molecular mechanisms underlying different retinal dystrophies may often be shared. Therefore, the study of LCA provides valuable insights and understanding of other retinal dystrophies.

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TABLE 1. Human LCA Gene and Locus Summary

Gene	Gene	Chr	Genetics	Cloned
<i>GUCY2D</i>	Photoreceptor-specific guanylate cyclase	17p13.1	Recessive	1996
<i>CRX</i>	Photoreceptor-specific homeobox gene	19q13.32	Rec/Dom	1997
<i>RPE65</i>	Retinal pigment epithelium 65 Kd protein	1p31.2	Recessive	1997
<i>TULP1</i>	Tubby-like gene 1	6q21.31	Recessive	1998
<i>AIPL1</i>	Aryl hydrocarbon receptor-interacting protein like 1	17p13.2	Recessive	2000
<i>CRB1</i>	Crumbs homologue 1	1q31.3	Recessive	2001
<i>RPGRIP1</i>	Retinitis pigmentosa GTPase regulator interacting protein 1	14q11.2	Recessive	2001
<i>LRAT</i>	Lecithin retinol acyltransferase	4q32.1	Recessive	2001
<i>RDH12</i>	Retinal dehydrogenase 12	14q24.1	Recessive	2004
<i>IMPDH1</i>	Inosine monophosphate dehydrogenase 1	7q32.1	Dominant	2006
<i>CEP290</i>	Centrosome protein 290 Kd	12q21.32	Recessive	2006
<i>RD3</i>	Retina degeneration 3	1q32.3	Recessive	2006
<i>LCA5</i>	<i>Lebercilin</i>	6q11-16	Recessive	2007

Locus	Supporting Information	Chr	Genetics	Mapped
<i>LCA3</i>	Mapped with two Saudi Arabian families, one from this report	14q24	Recessive	1998
<i>LCA9</i>	Mapped with one Pakistani family	1p36	Recessive	2003

To assess the mutation distribution of LCA in a non-European population, we studied 37 consanguineous LCA families from Saudi Arabia. These families were screened for mutations in 13 known LCA genes (*GUCY2D*, *CRX*, *RPE65*, *TULP1*, *AIPL1*, *CRB1*, *RPGRIP1*, *LRAT*, *RDH12*, *IMPDH1*, *CEP290*, *RD3*, and *LCA5*, with the exception of *MERTK*, which is rare) by both direct sequencing and analysis of polymorphic simple tandem repeat markers (STRs). Surprisingly, only 24% (9/37) of the families were found to contain mutations in these 13 known LCA genes, much less than the 65% observed in populations of European descent. Among them, five families carry one identical mutation in *TULP1*, two families have the same mutation in *CRB1*, one family has a homozygous mutation in *RPE65*, and one family has a homozygous mutation in *GUCY2D*. Most of the mutant alleles identified (3/5) are novel, further underscoring the utility of studying multiple ethnic populations. In addition, STR markers have been used to potentially map these families to known LCA loci. A new *LCA3* family has been identified and has a different haplotype than the original *LCA3* family, which was also from Saudi Arabia.²⁹ Furthermore, phenotype-genotype correlation studies with the nine Saudi families whose mutations have been identified in our studies have been conducted. Given the large number of patients and unaffected individuals in these pedigrees (a total of 180 individuals, with 53 affected and 127 unaffected), we were able to assess the penetrance of LCA. By direct sequencing all members of these nine families, we found that mutations segregate with disease perfectly, suggesting that these LCA associated mutations are fully penetrant. Finally, we have found that the clinical phenotype of families with different mutations exhibit variable expressivity, supporting the idea that accurate molecular diagnosis is essential for developing effective treatments of these disorders.

MATERIALS AND METHODS

Patient Sample Collection

This research adhered to the tenets of the Declaration of Helsinki. LCA patient families were ascertained through the King Khaled Eye Specialist Hospital (KKESH) in Riyadh, Saudi Arabia. Ophthalmologic information for each individual was scored as LCA or unaffected by at least one ophthalmologist at the time of referral to the hospital for evaluation, and then reviewed independently and examined independently by one of us (RAL). Detailed family histories and pedigrees of these families were obtained and confirmed through interviews with appropriate

family members. Each subject, or a responsible adult on behalf of minors or wards, signed a Consent Statement for Participation approved by the Research Council at KKESH for these investigations and part of a larger program approved by the Baylor College of Medicine Affiliates Review Board for Human Subject Research. We obtained blood samples from subjects where appropriate. A total of 37 multi-generational consanguineous families with recessive LCA were collected; of 417 individuals from these families, 117 were affected. Pedigrees of the families with mutation identified in this study are shown in Figure 1. Once recruited, blood samples were collected from all available family members, and DNA was extracted with a blood genomic DNA extraction kit (Qiagen, Valencia, CA), following the protocol provided by the manufacturer.

Screening for Mutations

Direct PCR and sequencing were used to screen the exons and splicing sites of the 13 known LCA genes, along with the immediately flanking intronic sequences, for point mutations and other small-scale sequence changes. DNA from one affected individual from each family was sequenced for both strands of all exons, including splicing sites, of all known LCA genes. In addition, the intronic region that contains a mutation that results in splicing of a cryptic exon downstream of exon 26 in *CEP290* was also sequenced.¹⁹ Each exon or intron was individually amplified from leukocyte DNA samples by PCR with primer pairs (primer sequences available on request). Exons longer than 500 base pairs were split into multiple amplicons. Each amplicon is 300 to 500 base pairs in length and was sequenced directly in both forward and reverse directions (ABI3730; AME Bioscience Ltd., Sharnbrook, Bedfordshire, UK). Each read was aligned to the reference sequence and base changes identified with a sequencing program (Sequencher; Gene Codes Corporation, Ann Arbor, MI).

In addition to direct sequencing, families without mutations identified were further screened with STR markers around the 13 LCA genes and two loci. At least one marker near each gene and three markers near each locus were chosen from the GDB human genome database,³⁰ as listed in Table 2. Locations of these markers on the genome were determined by mapping the primer sequences to the human genome using bioinformatic software (BLAT; open source software). A universal primer was added to the end of each primer for fluorescent detection as described previously.³¹ The size of each PCR fragment was determined with a machine (ABI3730; AME Bioscience Ltd.) and the data were analyzed with genotyping software (GeneMapper; Applied Biosystems, Inc., Foster City, CA) by standard procedures.

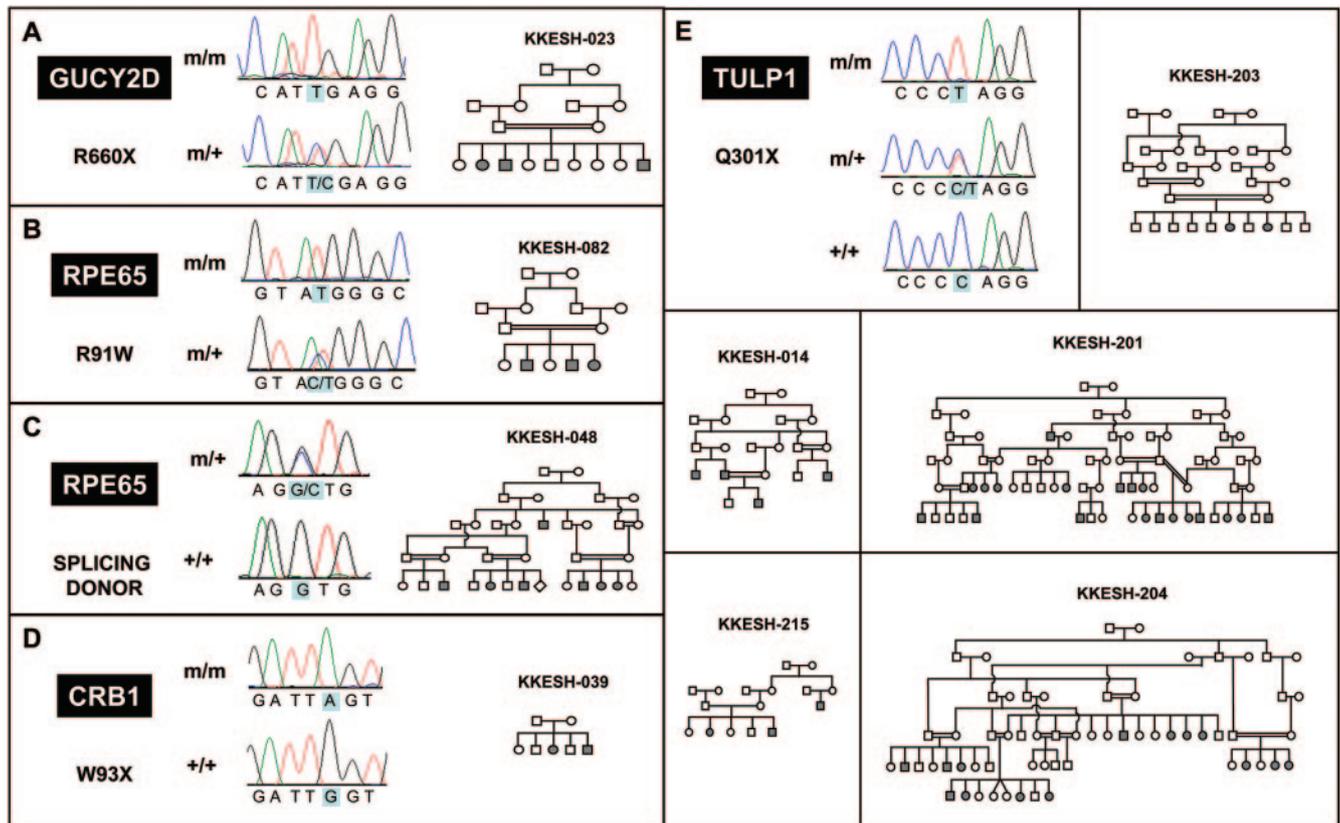


FIGURE 1. Pedigrees of nine families with mutations in known LCA genes. Pedigrees are drawn using the HaploPainter program.⁵⁵ Solid shapes represent affected members. DNA from patients whose identifiers start with *n* is not available.

RESULTS

Identification of Families Who Carry Mutations in Known LCA Genes

To screen for mutations associated with an LCA phenotype in the 37 families in our collection, we selected one affected patient from each family for direct DNA sequencing. By comparison with the reference sequence, five different mutations were identified in nine of the 37 families (Table 3 and Fig. 1), all of which are homozygous for the mutation. Of these nine families, five families (KKESH-014, -201, -203, -204, and -215) carry the same novel nonsense mutation at codon position 301 (Q301X) in *TULP1*. Two families, KKESH-048 and KKESH-082, carry mutations in *RPE65*. KKESH-082 has an *RPE65* mutation reported previously (R91W), while KKESH-048 carries a novel mutation at the splice donor site of exon 6 (GGT→GCT). In addition, KKESH-039 carries a novel nonsense mutation (W93X) in *CRB1*. Finally, a previously reported disease-causing mutation (R660X) in *GUCY2D* was found in family KKESH-023. In addition to the two previously known mutations, all three novel mutations identified in this study are also likely to be pathogenic. First, the nonsense mutations in *TULP1* and *CRB1* both occur early in the coding region and are predicted by conceptual translation to result in severe protein truncations. Similarly, the mutation at the splice donor site of exon 6 in *RPE65* causes inclusion of intron 6, leading to a frameshift and early termination. Second, clinical phenotypes observed in these subjects are consistent with the premise that these novel mutations are pathogenic. For example, patients from the KKESH-048 family that carry the mutation at the splicing site of exon 6 in *RPE65* exhibit a RP-like phenotype, a previously reported feature for patients with mutations in *RPE65* (Table

4). Finally, all mutations we have identified are likely to be disease-causing, since each identified mutant allele cosegregates with LCA and no unaffected person in any pedigree has two mutant alleles. All available affected and unaffected family members have been genotyped for the identified mutation by direct sequencing. Consistent with the pattern of consanguinity, all LCA subjects are homozygous for the identified mutant allele while unaffected individuals are either heterozygous for the mutant allele or homozygous wild type.

In addition to the five pathogenic mutations, five missense variations have also been identified during the initial sequencing of affected individuals (Table 3, section 2). These variations are likely to represent benign polymorphisms. First, three of these variations (marked with rs number) occur frequently in normal populations as common SNPs as documented in the dbSNP database.⁵² Second, although relatively rare, the other two novel variants do not segregate with LCA. Heterozygous affected and/or homozygous unaffected members have been found in the families, suggesting that these changes are not disease-causing.

LCA as an Early-Onset Genetic Disease with High Penetrance

LCA is considered an early-onset genetic disease with high penetrance. To assess the degree of penetrance of the disease phenotype, we conducted phenotype-genotype correlation studies with nine Saudi Arabian families with the mutations identified above. The pedigree of each family is shown as Figure 1. DNA from a total of 180 individuals (53 affected and 127 unaffected) from these nine families were genotyped for mutations identified above by direct sequencing of the causative mutations. Strikingly, all affected individuals are homozy-

TABLE 2. Microsatellite Markers for Known LCA Loci and Genes

Marker	Max Heterozygosity	Type	Range (bp)
LCA3 Locus			
D14S61	0.81	Dinucleotide Repeat	197-227
D14S1022	0.73	Dinucleotide Repeat	236-246
D14S68	0.91	Dinucleotide Repeat	148-172
LCA9 Locus			
D1S450	0.82	Dinucleotide Repeat	243-267
D1S244	0.82	Dinucleotide Repeat	285-296
D1S2667	0.83	Dinucleotide Repeat	224-272
Marker	Gene	Type	Range (bp)
Known LCA Genes			
D19S879L	<i>CRX</i>	Dinucleotide Repeat	217-265
GATA158H04L	<i>AIPL1</i>	Tetrinucleotide Repeat	174-194
D1S1660L	<i>CRB1</i>	Tetrinucleotide Repeat	226-250
D14S72L	<i>RPGRIP1</i>	Dinucleotide Repeat	257-271
D14S1069L	<i>RDH12</i>	Dinucleotide Repeat	200-220
D6S1645L	<i>TULP1</i>	Tetrinucleotide Repeat	226-252
D17S1844L	<i>GUCY2D</i>	Dinucleotide Repeat	227-249
D4S3049L	<i>LRAT</i>	Dinucleotide Repeat	210-236
D12S1719L	<i>CEP290</i>	Dinucleotide Repeat	223-239
D1S219L	<i>RPE65</i>	Dinucleotide Repeat	154-176
D7S530L	<i>IMPDH1</i>	Dinucleotide Repeat	106-118
D1S414L	<i>RD3</i>	Dinucleotide Repeat	185-205
D1S425L	<i>RD3</i>	Dinucleotide Repeat	92-108
D6S300L	<i>LCA5</i>	Dinucleotide Repeat	188-207
D6S1619L	<i>LCA5</i>	Dinucleotide Repeat	189-211
D6S286L	<i>LCA5</i>	Dinucleotide Repeat	206-232

gous for the mutations while unaffected siblings are either heterozygous for the mutations or completely wild type. Therefore, a perfect correlation has been observed between the diagnosis and the underlying genotype. Furthermore, the ages of affected individuals range from 1 year to 27 years old, while the unaffected individuals range from 1 year to 65 years old at the time of evaluation, suggesting that LCA is an early-onset genetic disease with complete penetrance in these families. Detailed clinical phenotypic data for each affected in these nine families are listed in Table 4.

Although patients with LCA all show early loss of vision, the clinical phenotype among them varies. Recent studies have

suggested that LCA can be classified into multiple categories and that each category may be caused by mutation in different sets of genes.^{3-5,25} Consistent with this idea, examination of the clinical phenotype of 53 affected individuals in these nine LCA families revealed that patients with mutations in different genes exhibit distinct phenotypes (Table 4). For example, patients carrying mutations in *TULP1* often also show a sandy granular RPE periphery phenotype. In contrast, patients carrying mutations in *GUCY2D* show relatively normal retinal appearance with no or minimal vascular attenuation. Among the three *GUCY2D* patients, one also shows navigational vision and another has "fix and follow only" vision. At the same time,

TABLE 3. Summary of Mutations and Variations Identified in Known LCA Genes

Family ID	Gene	Exon	Codon	Base Change	AA Change	Status
Mutations Identified						
KKESH-023	<i>GUCY2D</i>	10	660	C→T	R→Ter	Known
KKESH-082	<i>RPE65</i>	4	91	C→T	R→W	Known
KKESH-014	<i>TULP1</i>	10	301	C→T	Q→Ter	Novel
KKESH-201	<i>TULP1</i>	10	301	C→T	Q→Ter	Novel
KKESH-203	<i>TULP1</i>	10	301	C→T	Q→Ter	Novel
KKESH-204	<i>TULP1</i>	10	301	C→T	Q→Ter	Novel
KKESH-215	<i>TULP1</i>	10	301	C→T	Q→Ter	Novel
KKESH-039	<i>CRB1</i>	6	93	G→A	W→Ter	Novel
KKESH-048	<i>RPE65</i>	6	NA	GGT→GCT	Splice donor	Novel
Benign Variations Identified						
KKESH-087/-023	<i>GUCY2D</i>	12	782	T→A	L→H	rs8069344
KKESH-031	<i>RPGRIP1</i>	13	560	G→T	A→S	rs34263042
KKESH-001/-089	<i>RPGRIP1</i>	18	1033	G→C	E→Q	rs3748361
KKESH-041	<i>CRB1</i>	2	27	G→T	C→F	Novel
KKESH-087	<i>GUCY2D</i>	3	248	C→T	S→L	Novel

TABLE 4. Clinical Phenotype of Patients with Mutations in Known LCA Genes

Family	Patient	Gene	Phenotype
KKESH-014	04	<i>TULP1</i>	Poor vision, nystagmus, hyperopic, vessel attenuation; diminished foveal and ILM macular reflexivities; fundus shows a gray depigmentation with pigment clumping.
	01		Atrophic macula with fovea spared; bone spicules in the mid periphery; Unstable nystagmus; peripheral cortical cuneiform spokes.
	10		Irregular coarse nystagmus; hyperopic astigmatism; nystagmus; vertically oval discs; 2/4 pallor; 2/4 vascular attenuation; coarse RP-like dense bone spicules beyond the arcade.
KKESH-201	11	<i>TULP1</i>	"RP" with macula spared.
	10		Disc elevation; macula spared; minimal vascular attenuation; sandy peripheral depigmentation; vision CF; horizontal nystagmus; small posterior subcapsular cataracts; vitreous syneresis and cells; midperipheral bone spicules.
	24		Disc elevation; macula spared; minimal vascular attenuation; sandy peripheral depigmentation; vision CF; horizontal nystagmus; small posterior subcapsular cataracts; vitreous syneresis and cells; midperipheral bone spicules.
	20		Nystagmus; minimal disc pallor; full discs; sandy granular RPE periphery.
	04		Pale discs; 3/4 vessel attenuation; full "pseudo-papilledema" discs; fine bone spicules.
	12		Small round pale discs; bone spicules in mid-periphery; vessel attenuation.
	06		+2 pallor of discs; +2 vessel attenuation; fine bone spicule in mid periphery; PVD OU; ERG nonrecordable.
	05		+5 D hyperopic; vertically oval discs; +2 pink pallor; slight elevation; +2 vessel attenuation; fine mid-peripheral bone spicules beyond the arcade.
	07		Nystagmus; poor vision to CF OU; disc hyperemia and temporal pallor; vessels mildly attenuated; bone spicules in midperiphery; CT normal; EKG normal; ERG nonrecordable.
	08		Jerk nystagmus; vision "follows objects poorly"; pseudopapilledema; CT normal; EKG normal; ERG nonrecordable; attenuated vessels; slightly pale discs.
KKESH-203	11	<i>TULP1</i>	Fundus shows "RP".
	12		No features of deafness, neurologic impairment or mental deficiency; normal EKG; CT scan normal; ERG nonrecordable; fundus shows pigmentary retinopathy with attenuated vessels.
	08		ERG nonrecordable; compound hyperopic astigmatism; diffuse optic nerve pallor attenuated vessels and RPE granularity; pendular nystagmus.
KKESH-204	10	<i>TULP1</i>	Irregular horizontal nystagmus; mild horizontal head titubation; onset at age 6 months; hyperopia $\approx +4.00D$; ERG nonrecordable; +2 disc pallor; mild vascular attenuation; atrophic foveal light reflexes; minimal RPE degeneration.
	07		High hyperopia OU: $+7.00 + 2.25 \times 90$; fundus periphery shows confluent outer retinal peripheral whitening and patchy dots at the posterior edge distantly similar to fundus albipunctatus.
KKESH-204	20	<i>TULP1</i>	Rotatory nystagmus; vertically oval discs; rosy pale discs; vascular attenuation; diffuse pigmentary degeneration.
	19		Microrotatory nystagmus; discs, vessels, and pigment same as sister.
	18		Microrotatory nystagmus; rosy orange discs; 2-3+ vessel attenuation; diffuse pigmentary changes without bone spicules.
	04		Rotatory nystagmus; high hyperopia $+5.50 + 1.50 \times 90$ OU; full elevated discs; +1-2 vessel attenuation; periphery brown grainy RPE; ERG nonrecordable.
	06		+8.25 hyperopic; minimal end-gaze nystagmus; macula normal; anterior to equator confluent outer retinal whitening.
	42		Rotatory nystagmus; rosy flat vertically oval discs; +1 vessel attenuation; fine midperipheral bone spicules.
	46		Rotatory nystagmus; flat rosy discs; +1 vessel attenuation; absent fovea and umbo; grey-brown peripheral RPE.
	48		Rotatory nystagmus; +1 elevated discs; +1 vessel attenuation; dirty brown peripheral granular RPE.
	26		Rotatory nystagmus; minimal hyperopia; rosy pale discs; typical RP in periphery.
	27		30 diopter XT; rotatory nystagmus; +4.00 D hyperopia; vascular attenuation, modest bone spicule midperiphery OU; $+6.00 + 0.74 \times 180$ OU.
KKESH-215	21	<i>TULP1</i>	Rotatory nystagmus; flat discs; vascular attenuation; midperipheral bone spicules.
	15		Rotatory nystagmus; 4-prism diopter XT; sluggish pupils; 2/4 vascular attenuation; rate bone spicules in periphery; +3.50 sphere hyperopia.
	04		Microrotatory nystagmus; +3.50 sph hyperopia; PVD and vitreous condensation OU; vascular attenuation; equatorial and peripheral dense pigmentary RP-like degeneration OU.
KKESH-082	07	<i>RPE65</i>	Microrotatory nystagmus; full elevated discs with grey corona; far peripheral retina shows confluent grey-white belt similar to family 204.
	04		Clear lenses; hypermetropic $+3.00 + 1.00 \times 90$ OU; Syneresis of vitreous; Microrotatory infantile nystagmus; Vertically oval discs, mild optic atrophy and vascular attenuation; peripheral depigmentation and NO bone spicules; ERG nonrecordable.
	06		Mild enphthalmos; clear lenses; clear vitreous; vertically oval discs, slight hyperopic optic nerve fullness; decreased retinal light reflexes; minimal peripheral pigment changes; infantile nystagmus $+1.50 + 1.00 \times 90$ OU; ERG nonrecordable.
	07		Enphthalmos; nystagmus; clear lenses; minimal peripheral pigmentary changes; decreased foveal light reflexes; hyperopic full discs and mild hyperemia; Minimal astigmatism, $+0.25 + 1.50 \times 90$ OU; ERG nonrecordable.

(continues)

TABLE 4 (continued). Clinical Phenotype of Patients with Mutations in Known LCA Genes

Family	Patient	Gene	Phenotype
KKESH-048	21	<i>RPE65</i>	Micronystagmus; pigmentary granular dystrophy; foveal atrophy; disc atrophy; widespread vascular attenuation and grainy midperiphery.
	22		Micronystagmus; spared macula; +2/4 disc atrophy; +2/4 vascular attenuation; sandy RPE degeneration from inside the arcade to the periphery.
	15		No nystagmus; +2/4 vascular attenuation; +1/4 disc pallor; midperiphery sandy depigmentation; "nightblind" according to his father.
	04		Disc pallor' vessels attenuated; ERG nonrecordable.
	06		Non-recordable ERG; disc pallor, neuroepithelial atrophy; nystagmus.
KKESH-023	05	<i>GUCY2D</i>	Navigational vision; spectacles do not help; reads Braille; fundus exam (by others) "normal" at age 13 years.
	04		Poor vision from birth; ERG at age 10 years nonrecordable; vision 10/200 OU; pendular nystagmus; pupils sluggish to light; exotropia ≈30 diopters; hyperopic +4.00 D OU. Fundus "normal optic nerve and no pigmentary changes". Later examiner felt that there were minimally attenuated vessels and mild Optic Nerve pallor OU.
	11		Poor vision from birth; vision "fix and follow only"; refraction +6.00 sph OU; ERG nonrecordable; fundus normal to minimal vascular attenuation and diffuse RPE atrophy OU.
KKESH-039	05	<i>CRB1</i>	Bilateral enphthalmos; irregular infantile nystagmus; +3/4 optic atrophy; macular geographic atrophy without excavation; mild peripheral pigment clumping; far periphery normal.
	07		Bilateral enphthalmos; irregular conjugate nystagmus; hypermetropic hyperemic discs' +1-2 vascular attenuation; granular pigmentary dispersion in macula and salt + pepper mid-periphery; ERG nonrecordable.

the clinical phenotype among patients with the same mutation often varies. For example, we have identified five LCA families carrying the same novel nonsense mutation at codon position 301 (Q301X) in *TULP1* (Table 3). Not all patients show an RP-like phenotype, whereas several patients from the family KKESH-204 show a novel phenotype of retinal peripheral whitening and patchy dots at the posterior edge distantly resembling fundus albinopunctatus (Table 4).

Excluding Families from Mutations in Known LCA Genes Using STR Markers

It is possible that at least some of the remaining 28 families whose mutations have not been identified by direct sequencing of coding regions of known LCA genes actually carry disease-causative mutations in other functionally important parts of these genes, such as promoters or enhancers. To exclude all 13 known LCA disease genes from these families, we conducted further screening by STR markers (Table 2). As all 28 Saudi families are consanguineous, one can exclude a family from carrying a mutation in a known gene if an affected individual is heterozygous for an STR marker linked to that gene. At least one marker near each LCA disease gene was selected to genotype one or more affected individual from each family. Segregation of these markers with disease was examined for each pedigree and in each case at least one marker was found to be heterozygous in the affected individuals, therefore allowing us to exclude all 28 families from the 13 known LCA genes.

KKESH-060 Maps to the *LCA3* Locus

To test whether any of these 28 families who had been excluded from the 13 LCA genes' maps to either of the two known LCA loci (*LCA3* and *LCA9*) for which a specific gene has not been identified, three STR markers from each locus were tested with DNA from the affected individuals from these families (Table 2). At least one subject of each family was found to be heterozygous for one or more STR marker(s) for the *LCA9* locus, suggesting that none of the 28 families carry mutations in *LCA9*. In contrast, an affected individual from one family, KKESH-060, is homozygous for all markers from the *LCA3*

locus (Fig. 2). To determine whether this family is indeed linked to the *LCA3* locus, further genotyping of the other two affected and six unaffected members of the family was performed. We found that all three affected individuals are homozygous for all three STR markers in the *LCA3* locus, whereas all unaffected individuals are either heterozygous or completely wild type. In addition to poor vision, all three affected individuals have non-recordable ERGs, hypermetropic astigmatism, and infantile nystagmus. Two patients (KKESH-060-04 and KKESH-060-06) also showed neuroretinal atrophy and

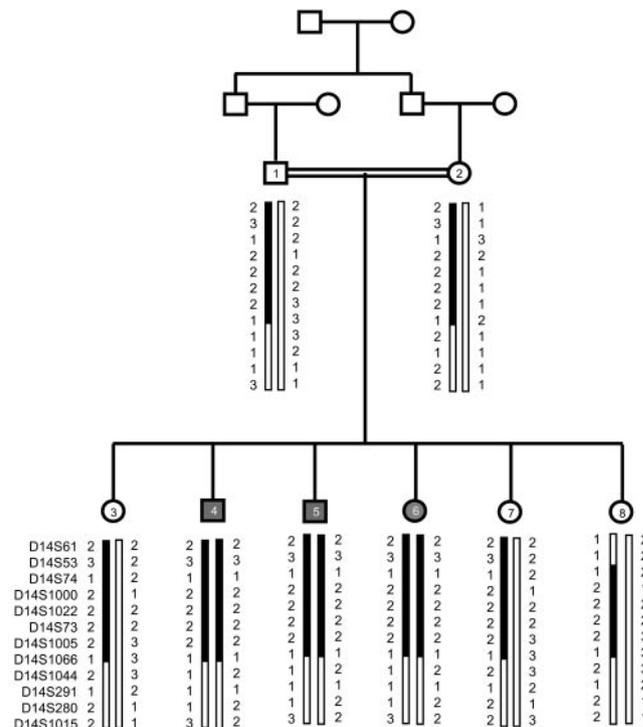


FIGURE 2. Pedigree and haplotype of KKESH-060 that mapped to the *LCA3* locus.

markedly attenuated vessels of the fundi at the time (age) of examination.

DISCUSSION

As one of the most severe causes of congenital blindness, both the clinical phenotype and the underlying molecular bases of the LCA disorders are very complex. To date, 14 genes and two loci have been linked to this disease, but these are estimated to account for only approximately 65% of all families of LCA.^{5,19,25-27} Therefore, identification of all genes underlying LCA and the development of accurate molecular diagnoses are essential for understanding disease mechanisms and designing proper intervention.

We found that the distribution of mutations in known LCA genes in subjects varies between populations. Previous mutation surveys of LCA patients have estimated that mutations in known LCA genes account for approximately 65% of all families.^{5,19,25-27} Since most surveys were conducted in Caucasian populations, the fraction of patients carrying mutations in the currently known LCA genes could differ in other populations. To assess this issue, we conducted a comprehensive survey for mutations in 13 known LCA genes (*GUCY2D*, *CRX*, *RPE65*, *TULP1*, *AIPL1*, *CRB1*, *RPGRIP1*, *LRAT*, *RDH12*, *IMPDH1*, *CEP290*, *RD3*, and *LCA5*) and two loci (*LCA3* and *LCA9*) in our LCA patient collection from Saudi Arabia. Interestingly, mutations in these 13 LCA genes were identified in only 24% of these families (9/37). This difference more likely reflects a difference in the populations rather than that a large portion of the Saudi families in our collection are related and share the same mutation. First, mutations in five known genes have been identified in nine families in our collection, and in most cases, only one or two families carry mutations in the same gene. Similarly, only two families have been mapped to the *LCA3* locus. Second, three of the five mutations are novel mutations, indicating that the allele distribution is different from previous studies. For example, despite the fact that 8.6% of all LCA cases are due to mutation in *CRB1* in previous surveys and more than 25 disease alleles have been reported, the 660R→Stop mutation identified in our study has not been identified previously.³³ Third, none of these 37 families carry mutations in *CEP290*, the most frequently mutated gene in LCA patients in the European population. While it has been reported that mutations in *CEP290* are involved in 21% of all cases in American families, no mutation has been found in this gene in our collection, despite both comprehensive sequencing and STR marker analysis. Interestingly, a recent survey of Italian LCA patients found that only 4% of patients have mutations in *CEP290*, further underscoring distinct mutation distributions even among different sub-populations of the same ethnicity. Fourth, based on our preliminary genome linkage scans, multiple novel LCA loci may be identified in the remaining 28 families who do not have mutations in the known LCA disease genes or loci, each of which is shared among only a few families (Chen R, unpublished data, 2008). Finally, historical information suggests that these Saudi families have widely divergent tribal origins (data not shown). Taken together, these results suggest that to uncover the entire complement of LCA disease genes, it is essential to examine diverse populations some of which may harbor different genetic variations and diverse mutant allele distributions.

We have confirmed that LCA, as selected here, is indeed an early-onset genetic disease with complete penetrance, consistent with previous reports. In our study, we have identified nine multi-generation, consanguineous families, including a total of 180 individuals with 53 affected and 127 unaffected members, carrying mutations in known LCA genes. All mem-

bers of these families have been genotyped for the causative mutations, and complete cosegregation between a homozygous mutant genotype and the disease phenotype has been observed (Fig. 1). Such a feature of LCA makes it amenable to identifying mutations with homozygosity mapping with consanguineous families such as the ones that we have reported here. Together with the large sequencing capacity offered by the next generation sequencing technologies, such as the 454 and Solexa platforms, it becomes possible to conduct positional cloning starting with even small consanguineous families.

With STR markers, we have identified another family in our collection, KKESH-060, whose disease maps to the *LCA3* locus at 14q24. One *LCA3* family, also from Saudi Arabia, has been reported previously.²⁹ A LOD score of 3 was obtained with this new family, and as in the previously reported *LCA3* family, all three affected members of this family are homozygous between STR markers *D14S61* to *D14S1066*, a 13.4 Mb interval. In addition, both families present a very similar clinical phenotype. Interestingly, this new *LCA3* family carries a different haplotype than the first *LCA3* family based on STR markers, suggesting different origin of the mutant alleles. The identification of this second *LCA3* family further supports the linkage between the *LCA3* locus and the disease and will provide valuable reagents for identifying the underlying causative mutant gene.

Complex genetic inheritance exists for many human disorders, including retinal diseases.³⁴ For example, digenic tri-allelic inheritance has been observed in families segregating the Bardet-Biedl syndrome phenotype where inheritance of a mutation in a second gene is required for an individual who has two mutations in the first gene to exhibit a clinical phenotype or to modify the severity of the primary phenotype.³⁴ However, we have not found evidence of tri-allelic inheritance in our inbred Saudi Arabian LCA families. No affected individuals from our cohort carry any coding or splicing mutations in other known LCA disease genes while all unaffected individuals are either wild-type or heterozygous for the mutation. Due to the relatively small sample size and homogenous genetic background, this result does not completely eliminate the possibility of complex inheritance for LCA in every instance.

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