Identification of Novel Mutations in Patients with Leber Congenital Amaurosis and Juvenile RP by Genome-wide Homozygosity Mapping with SNP Microarrays

Anneke I. den Hollander,[1] *Irma Lopez,*[2] *Suzanne Yzer,*[3] *Marijke N. Zonneveld,*[1] *Irene M. Janssen,*[1] *Tim M. Strom,*[4,5] *Jayne Y. Hehir-Kwa,*[1] *Joris A. Veltman,*[1] *Maarten L. Arends,*[1] *Thomas Meitinger,*[4,5] *Maria A. Musarella,*[6] *L. Ingeborgh van den Born,*[3] *Gerald A. Fishman,*[7] *Irene H. Maumenee,*[8] *Klaus Rohrschneider,*[9] *Frans P. M. Cremers,*[1] *and Robert K. Koenekoop*[2]

Purpose. Leber congenital amaurosis (LCA) and juvenile retinitis pigmentosa (RP) cause severe visual impairment early in life. Thus far, mutations in 13 genes have been associated with autosomal recessive LCA and juvenile RP. The purpose of this study was to use homozygosity mapping to identify mutations in known LCA and juvenile RP genes.

Methods. The genomes of 93 consanguineous and nonconsanguineous patients with LCA and juvenile RP were analyzed for homozygous chromosomal regions by using SNP microarrays. This patient cohort was highly selected, as mutations in the known genes had been excluded with the LCA mutation chip, or a significant number of LCA genes had been excluded by comprehensive mutation analysis. Known LCA and juvenile RP genes residing in the identified homozygous regions were analyzed by sequencing. Detailed ophthalmic examinations were performed on the genotyped patients.

Results. Ten homozygous mutations, including seven novel mutations, were identified in the *CRB1*, *LRAT*, *RPE65*, and *TULP1* genes in 12 patients. Ten patients were from consanguineous marriages, but in two patients no consanguinity was reported. In 10 of the 12 patients, the causative mutation was present in the largest or second largest homozygous segment of the patient's genome.

Conclusions. Homozygosity mapping using SNP microarrays identified mutations in a significant proportion (30%) of consanguineous patients with LCA and juvenile RP and in a small number (3%) of nonconsanguineous patients. Significant homozygous regions which did not map to known LCA or juvenile RP genes and may be instrumental in identifying novel disease genes were detected in 33 patients. (*Invest Ophthalmol Vis Sci.* 2007;48:5690–5698) DOI:10.1167/iovs.07-0610

From the [1]Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; the [2]McGill Ocular Genetics Center, McGill University Health Center, Montreal, Quebec, Canada; [3]The Rotterdam Eye Hospital, Rotterdam, The Netherlands; the [4]Institute of Human Genetics, German Science Foundation National Research Center for Environment and Health, Munich-Neuherberg, Germany; the [5]Institute of Human Genetics, Technical University, Munich, Germany; the [6]Department of Ophthalmology, State University of New York, New York; the [7]Department of Ophthalmology, University of Illinois at Chicago, Chicago, Illinois; the [8]Wilmer Eye Institute, John Hopkins University, Baltimore, Maryland; and the [9]Department of Ophthalmology, University of Heidelberg, Heidelberg, Germany.

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Corresponding author: Anneke I. den Hollander, Department of Human Genetics, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands; a.denhollander@antrg.umcn.nl.

Leber congenital amaurosis (LCA) represents a group of severe retinal dystrophies, characterized by congenital blindness, nystagmus, and a nondetectable electroretinogram (ERG). LCA is generally inherited in an autosomal recessive manner and is genetically heterogeneous. To date, mutations in 10 genes (*AIPL1*, *CEP290*, *CRB1*, *CRX*, *GUCY2D*, *LCA5*, *RDH12*, *RD3*, *RPE65*, and *RPGRIP1*) and two loci (1p36 and 14q24) were found to be associated with autosomal recessive LCA.[1–5] Mutations in each of these 10 genes have been identified in 0.1% to 20% of patients with LCA. Comprehensive mutation screening of all known LCA genes in a large cohort has not yet been performed, but it may currently be possible to determine the genetic basis of disease in approximately 60% of LCA cases.

LCA exhibits significant clinical overlap with juvenile retinitis pigmentosa (RP). However, juvenile RP is considered milder and does not have congenital onset of visual impairment. Patients with juvenile RP usually experience night blindness within the first years of life, progress to a gradual loss of peripheral visual field, and eventually lose central vision. Mutations in *CRB1*, *RDH12*, and *RPE65* can cause both juvenile RP and LCA.[6–8] In addition, mutations in *LRAT*, *MERTK*, and *TULP1* can cause juvenile RP, which in some cases may be clinically diagnosed as LCA.[9–11]

Patients affected by a recessive disease and born of a consanguineous union are likely to be homozygous for the disease-causing mutation and for polymorphisms in the region surrounding this mutation.[12] Homozygosity mapping is therefore an effective method for locating the responsible gene in consanguineous families. In rare recessive diseases, as in LCA, the frequency of homozygous mutations can also be relatively high in patients with no apparent consanguinity, due to a (distant) common ancestor who cannot be traced in the available family history. The average size of a homozygous region inherited

from a common ancestor living 10 generations ago measures 10 cM, a region that can readily be detected with high-density SNP microarrays.[13]

In this study we analyzed 93 patients (82 isolated patients and 4 small families, each with 2 to 4 affected sibs) with LCA and juvenile RP for homozygous regions using SNP microarrays. Thirty-three patients were born from consanguineous marriage or in populations with a high degree of consanguineous unions. For 60 patients, mainly from The Netherlands or Germany or of French Canadian descent, no consanguinity was reported. The patients included in the study represent a highly selected patient group, as known mutations in the known LCA genes had been excluded with the LCA mutation chip, or a significant number of LCA genes had been excluded by comprehensive mutation analysis. Known LCA and juvenile RP genes residing in homozygous regions were analyzed for mutations. This approach successfully identified homozygous mutations in 12 patients, including seven novel mutations in the *CRB1*, *RPE65*, and *TULP1* genes. In most patients, the disease-causing mutation was present in the longest or second longest homozygous segment of the patient's genome. Significant homozygous regions were detected in 33 consanguineous and nonconsanguineous patients, which did not map to known LCA or juvenile RP genes and may be instrumental to identify novel LCA genes. In fact, we have recently demonstrated the effectiveness of using these homozygous regions to identify new LCA genes. One family with four affected siblings was instrumental in identifying the *CEP290* gene as an important cause of LCA, and two unrelated patients with LCA recently allowed us to identify the *LCA5* gene.[3,5]

Materials and Methods

Patient Samples

Ninety-three patients with LCA and juvenile RP were included in the study, including 82 unrelated patients and 4 small families consisting of two to four affected sibs. The diagnosis of LCA was given to patients with blindness or severe visual impairment from birth or at least before the age of 6 months, onset of nystagmus within the first few weeks of life, and a nondetectable electroretinogram (ERG) before the age of 1 year. Patients were deemed to have juvenile RP when the onset of visual loss, nystagmus, and/or nyctalopia occurred later in life, but before the age of 6 years. The ERG responses of patients with juvenile RP were either nondetectable at the time of presentation or significantly reduced.

Thirty-three (35%) patients were born of a known consanguineous union, or originated from populations with a high rate of consanguineous marriages, such as those of the Middle East and Turkey. For 60 patients, no consanguinity was reported. Thirteen of the nonconsanguineous patients were from the French Canadian founder population of Quebec, 25 were from The Netherlands, 13 were from Germany, and 9 were from several other countries (Canada, United States, Italy, and Africa).

In 65 patient samples all known mutations in the known LCA genes were excluded with a genotyping microarray based on arrayed primer extension (APEX) technology (Asper Ophthalmics, Tartu, Estonia).[14,15] This analysis, however, did not include the *CEP290*, *LCA5*, and *RD3* genes, since these genes were not known at that time. In the remaining 27 patients, the involvement of at least four of the known LCA genes was excluded by single-strand conformation analysis (SSCA)[16] or denaturing high-performance liquid chromatography (DHPLC). One consanguineous patient (31863) from Afghanistan had not been analyzed for mutations.

Informed consent was obtained from all participating individuals consistent with the tenets of the Declaration of Helsinki. The institutional review boards of the participating centers approved the study.

SNP Microarrays

DNA samples for SNP analysis were purified (QIAamp DNA Mini Kit; Qiagen, Valencia, CA). Most of the consanguineous patients were genotyped for 11,555 SNPs (GeneChip Human Mapping 10K Array; Affymetrix, Santa Clara, CA). French-Canadian patients were genotyped for 116,204 SNPs (GeneChip Human Mapping 100K Set, consisting of a 50K Hind and a 50K Xba array; Affymetrix). The remaining patients were genotyped for 262,000 SNPs (GeneChip Mapping 250K Nsp Array; Affymetrix). In addition, seven consanguineous patients who showed only a few or no homozygous regions with the 10K array were also analyzed at higher resolution with the 250K array. Array experiments were performed according to protocols provided by the manufacturer. Arrays were scanned and genotypes were called as described.[17]

The 10K SNP genotypes were analyzed for homozygous regions (ExcludeAR sheet; Excel, Microsoft, Redmond, WA).[18] Chromosomal segments were accepted as homozygous if they contained ≥39 consecutive homozygous SNPs, since the likelihood that this would occur by chance is less than 1:100.[18] These segments correspond to regions of (on average) 8 Mb and larger. The 100K and 250K SNP data were analyzed with the software package CNAG,[19] and chromosomal segments were accepted to be homozygous if the loss-of-heterozygosity (LOH) score was ≥15. The LOH score is a measure for the likelihood of a stretch of SNPs to be homozygous based on the population SNP allele frequencies. An LOH score of ≥15 corresponds to regions of (on average) 4 Mb and larger.

Mutation Analysis

Known LCA and juvenile RP genes that resided in homozygous chromosomal segments were analyzed for mutations. Primers for amplification of the coding exons and splice junctions were described previously,[6] or were designed with ExonPrimer (http://ihg.gsf.de/ihg/ExonPrimer.html/ provided in the public domain by the Institute for Human Genetics, Technical University of Munich, Germany) and Primer3.[20] Primer sequences and PCR conditions can be requested from the authors. PCR products were purified with 96-well filter plates (Multiscreen HTS-PCR; Millipore, Bedford, MA) or by gel extraction (QiaQuick Gel Extraction Kit; Qiagen). Sequencing was performed with dye terminator chemistry (BigDye Terminator, ver. 3 on a 3730 or 3100 DNA Analyzer; Applied Biosystems, Inc., [ABI], Foster City, CA).

Ophthalmic Examinations

Ophthalmic files of all patients were studied extensively, and additional information was collected prospectively. The examination included best corrected visual acuity, refraction, ERG according to ISCEV (International Society for Clinical Electrophysiology of Vision) protocol, Goldmann perimetry using V4e and I4e targets, and fundus photography.

Results

Quantification of Homozygosity in Consanguineous and Nonconsanguineous Patients with LCA and Juvenile RP

SNP genotypes of 93 LCA and juvenile RP patients were analyzed for homozygous segments in their genome, including 82 unrelated patients and four small families consisting of two to four affected sibs. Thirty-three (35%) patients were born of a known consanguineous union or originated from populations with a high rate of consanguineous marriages, such as the Middle East and Turkey. For 60 patients (mainly from The Netherlands or Germany or of French Canadian descent), no consanguinity was reported.

As expected, the majority (28/33; 85%) of consanguineous patients carried significant homozygous regions in their genomes— on average, 6.5 homozygous regions with an average

size of 18.7 Mb. These homozygous regions covered ~4.1% of their genome. Approximately half (31/60; 52%) of the nonconsanguineous patients also carried significant homozygous regions, but a lower number (average, 2.8) and smaller (average, 8.8 Mb) than those in consanguineous patients. The homozygous regions covered ~0.9% of the genomes of nonconsanguineous patients.

Identification of Mutations in Known LCA and Juvenile RP Genes

LCA and juvenile RP genes that resided in the identified homozygous regions in the remaining 59 patients were analyzed for mutations. We found that in 19 patients, one or more known LCA or juvenile RP gene(s) resided in the homozygous segments documented by the SNP array. The *CRB1* gene resided in a homozygous segment in six patients, the *TULP1* gene in four patients; the *LRAT*, *MERTK*, and *RPE65* gene each in three patients; the *RDH12* gene in two patients; and the *AIPL1*, *CRX,* and *GUCY2D* in one patient. No patients were homozygous for the chromosomal region containing the *RPGRIP1* gene. The *CEP290*, *LCA5*, and *RD3* genes were not analyzed, since they were identified during the course of the study.[3–5]

Sequence analysis of the known LCA genes in the respective patients identified 10 different homozygous mutations in 12 patients (Table 1). Ten of the 12 patients in which homozygous mutations were identified were born of consanguineous unions. The average size of the homozygous region containing the disease-causing mutation was 24 Mb (range, 8–41 Mb). In 10 patients, the disease gene was present in the largest or second largest homozygous segment of the patient's genome (Table 1).

Seven mutations are novel, including three *TULP1* mutations (c.718+2T>C, c.999+5G>C, and p.Leu461Val), three *CRB1* mutations (p.Gln362X, p.Cys939Tyr, and p.Cys1332Phe) and one *RPE65* mutation (c.289_290dupA). The *RPE65* missense mutation (p.Asn321Lys) identified in patient 27233 has been identified in several patients with LCA.[21,22] This patient had not been analyzed with the LCA mutation chip, which contains this mutation, but was comprehensively screened for mutations in four LCA genes (*AIPL1*, *CRB1*, *CRX*, and *RPGRIP1*). The *CRB1* mutation (p.Cys948Tyr) identified in patient 27235 is also not novel.[6] This patient was comprehensively screened for mutations in four LCA genes, but not for the *CRB1* gene.

In patients 27241 and 27266 identical homozygous haplotypes surrounding the *LRAT* gene were identified by 100K SNP analysis, spanning 118 SNPs and 2.3 Mb of genomic DNA. In both patients, the same homozygous frameshift mutation (c.217_218delAT) was subsequently identified in the *LRAT* gene. The identical haplotypes suggest that this mutation represents a founder mutation in the French-Canadian population. We did not identify this mutation in the remaining French-Canadian patients. Of interest, the mutation was recently identified in a French LCA family,[23] supporting the hypothesis that it may represent a founder mutation originating from France.

The three missense mutations in the *CRB1* gene—p.Cys939Tyr, p.Cys948Tyr, and p.Cys1332Phe—all affect conserved cysteine residues in the EGF-like domains. These residues are involved in the formation of disulfide bridges, and the mutations are predicted to affect correct folding of the CRB1 protein.[16,24] The splice site mutation c.718+2T>C in the *TULP1* gene identified in two patients from Afghanistan (27208 and 31863) is expected to have a severe effect on splicing, since the invariable dinucleotide of the splice donor site of intron 7 is affected. The splice site mutation identified in patient 21978 severely affects the splice donor site of intron 10 of the *TULP1* gene. The splice prediction score of this splice site dramatically decreases from 0.82 to 0.21 (NNSPLICE, ver. 0.9[25]). The *TULP1* missense mutation p.Leu461Val changes an amino acid residue that is completely conserved in TULP1 orthologues and TULP family members.[26]

Clinical information of patients with *TULP1*, *CRB1*, and *LRAT* mutations are summarized herein and in Tables 2, 3, and 4, respectively. No detailed clinical data were available for the two patients with *RPE65* mutations (27279 and 27233).

Clinical Characteristics of Patients with *TULP1* Mutations

Patient 27208 (Fig. 1A) presented at age 6 with nyctalopia. She had a mild speech impediment besides her retinal dystrophy but was otherwise healthy. Her audiogram was within normal limits. Her pupils were briskly reactive, and a fine horizontal nystagmus was noted with a large (40 prism diopter [PD]) exotropia. The visual fields showed relative preservation of the V4e and I4e isopters (Fig. 1B). Nine years later, at age 17, the acuities remained at 20/200, and the visual fields were unchanged. The retinal appearance changed toward diffuse bone spicule pigmentation.

Since heterozygous carrier parents of LCA offspring can present with certain clinical symptoms and signs,[27–29] the

Table 1. Mutations Identified in LCA and Juvenile RP Genes Residing in Homozygous Regions

Patient	Origin	Consanguinity	Gene	Homozygous Nucleotide Change	Homozygous Effect	Size of Disease Segment (Mb)	Ranking of Disease Segment	Number of Homozygous Segments	% of Genome Covered by Homozygous Segments
27208	Afghanistan	1st Cousins	*TULP1*	c.718+2T>C*	Splice defect	36	1st	2	1.9
31863	Afghanistan	1st Cousins	*TULP1*	c.718+2T>C*	Splice defect	34	3rd	11	9.4
21978	Turkey	3rd Cousins	*TULP1*	c.999+5G>C*	Splice defect	9	2nd	2	0.7
27232	Mexico	2nd Cousins	*TULP1*	c.1381C>G*	p.Leu461Val	28	1st	9	1.4
28603	Quebec	1st Cousins	*CRB1*	c.1084C>T*	p.Gln362X	29	1st	6	2.9
28606	Quebec	1st Cousins	*CRB1*	c.2816G>A*	p.Cys939Tyr	30	2nd	10	5.7
27235	Quebec	2nd Cousins	*CRB1*	c.2843G>A	p.Cys948Tyr	15	1st	2	0.6
21266	Germany	Not reported	*CRB1*	c.3995G>T*	p.Cys1332Phe	41	1st	12	6.2
27241	Quebec	Not reported	*LRAT*	c.217_218delAT	p.Met73AspfsX47	11	1st	2	0.7
27266	Quebec	2nd Cousins	*LRAT*	c.217_218delAT	p.Met73AspfsX47	8	2nd	4	1.1
27279	Quebec	Yes	*RPE65*	c.289_290dupA*	p.Arg97LysfsX33	11	6th	6	6.7
27233	Quebec	Yes	*RPE65*	c. 963T>G	p.Asn321Lys	36	2nd	11	9.1

*Novel mutations.

TABLE 2. Clinical Characteristics of Patients with *TULP1* Mutations at First Visit

Patient (Age, y)	Diagnosis	Sex	Onset, y	Night Blindness	Nystagmus	Refraction (SE)		VA		AS	Fundus	ERG
						OD	OS	OD	OS			
27208 (8)	Juv. RP	F	7	+	+	+1.00	+1.00	20/200	20/200	N	OP: mild temporal pallor PO: yellow perifoveal annular ring VE: narrow PE: RPE mottling	
31863 (5)	Juv. RP	M	1.5	+	+	+4.50	+4.00	20/100	20/100	N	OP: temporal pallor PO: yellow perifoveal annular ring VE: narrow PE: very fine, diffuse RPE mottling	ND
27232 (5)	Juv. RP	M	3	+	+	−5.00	−6.50	10/180	10/160	N	VE: arteriolar attenuation	ND
21978 (2)	Juv. RP	M	2	+	+	Plan	Plan	20/60	20/60	N	OP: pink VE: mild attenuation PE: RPE mottling, with round, atrophic lesions of the RPE with bone spicules	Rod-cone pattern

SE, spherical equivalent; VA, best corrected visual acuity; AS, anterior segment; ERG, electroretinogram; ND, non-detectable; N, normal; OP, optic disc; PO, posterior pole; VE, vessels; PE, periphery.

possible phenotype of *TULP1* carriers was investigated. Both heterozygous parents were asymptomatic and had normal ERG testing and visual acuities, but the mother had striking and easily discernible multiple, superior, and inferior subretinal yellow-white dots just outside of the vascular arcades (Fig. 1C).

After detecting the homozygous *TULP1* mutation in patient 27208, we identified another Afghan patient (31863) with juvenile RP a few years later, who was not known to be related to the first patient. Clinical features (Table 2) in this patient were similar to the phenotype of patient 27208, and we therefore postulated a *TULP1* mutation. Remarkably, mutation analysis of the *TULP1* gene identified the same homozygous splice site mutation (c.718+2T>C) in this patient. Patient 31863 was healthy, except for a mild developmental delay. He started "headbobbing" at age 3 years. The pupils were briskly reactive, and there were no paradoxical pupils. We found vertical nystagmus and a small headturn.

Patient 27232 presented with nyctalopia, photoaversion, head bobbing, and side and central visual difficulties of 1 to 2 years' duration at age 5 years. He was found to have a fine, small, horizontal, pendular nystagmus.

Patient 21978 presented with exotropia, nystagmus, and night blindness at the age of 2 years. At age 11 years, the visual acuity remained stable. The anterior segments then showed cortical lens opacities in both eyes. The optic discs were pink, and there was mild attenuation of the arterioles and some RPE mottling. There were distinct round atrophic lesions of the RPE with bone spicules in the periphery (Fig. 2A). The ERG was nondetectable. His younger brother developed similar symptoms at age 5. Acuities were 20/30 OD and 20/70 OS, with +4.00 −3.00 × 180° refraction OU. Retinal aspects were similar to those of the affected sib (Fig. 2B). His ERG also revealed a rod–cone pattern.

Clinical Characteristics of Patients with *CRB1* Mutations

History showed that the vision of patient 28603 remained stable until age 19. Then a rapid decline occurred. There was no preserved para-arteriolar retinal pigment epithelium (PPRPE). Temporal fields in both eyes were found on perimetry (V4e target). The retinal phenotype was striking, with extensive nummular pigmentation in the posterior pole.

Patient 28606 presented at age 10 years, with a history of visual difficulties since birth. She had ureteral hypoplasia and had undergone surgery for it as an infant. On examination she also had amaurotic pupils, pendular nystagmus, and enophthalmos. There was no PPRPE. At age 14, her vision dropped to hand motion.

Patient 27235 has two siblings with retinal dystrophy and two cousins with retinal dystrophy and hearing loss. This patient was first seen at age 66 years and reported better eyesight during the day than at night. On examination, we found pendular horizontal nystagmus and no light perception vision. The eyes were microphthalmic.

In patient 21266, visual field testing was performed at age 8 and showed a concentric restriction of 10° to 20° on Goldmann perimetry (III4e)5. At that age, her visual acuity was counting fingers in both eyes.

Clinical Characteristics of Patients with *LRAT* Mutations

Patients 27241 and 27266 were French-Canadian patients who were not known to be related to each other. For as long as patient 27266 could remember, she had had visual difficulties with nyctalopia and did not experience photophobia. Her funduscopic findings at age 23 years (Table 4) also showed normal RPE and retinal appearance in the posterior pole, then a sharp demarcation to an area with significant retinal hypopigmentation outside the arcades and to the periphery (Fig. 3A). Goldmann visual fields were 10° with the V4e target. Over the next 10 years, she maintained hand motion vision, but the VF declined to 5°, and she developed mild posterior subcapsular cataracts and a mild bull's eye maculopathy.

Patient 27241 and his affected sister presented with a history of nyctalopia since the age of 2 years, poorly reactive pupils, and an accommodative esotropia. An interesting fundu-

TABLE 3. Clinical Characteristics of Patients with *CRB1* Mutations at First Visit

Patient (Age, y)	Diagnosis	Sex	Onset	Night Blindness	Nystagmus	Refraction (SE)		VA		AS	Fundus	ERG
						OD	OS	OD	OS			
28603 (26)	LCA	M		+	+	+8.00	+8.00	HM	HM	N	OP: swelling, with drusen VE: attenuation PO: atrophic foveal lesion, nummular pigment clumping PE: pigment clumping	
28606 (10)	LCA	F	Birth	−	+	+8.50	+8.50	20/400	20/100	N	OP: mild pallor, slightly swollen VE: narrow; diffuse, round stippling over the entire retina	
27235 (66)	LCA	M	Birth	+	+			LP	LP	Brunescent nuclear sclerotic and anterior and posterior subcapsular cataract	OP: pale VE: narrow PE: diffuse heavy bone spicule pigmentation	
21266 (5)	LCA	F	Birth	−	+			CF	CF		OP: tilted with slight prominence VE: normal PO: RPE changes and hyperpigmentation	ND

HM, hand motion; LP, light perception; CF, counting fingers. Other abbreviations as in Table 2.

scopic finding was the normal appearance of the posterior pole and the sharp demarcation with the peripheral retinal appearance (outside the arcades), which showed a striking grainy (salt and pepper) retinal degeneration (Fig. 3B). A year later (age 7 years) acuities were 20/70 OU, and at age 9, acuities were 20/100 OD and 20/200 OS. Goldmann visual fields were 75° (V4e) and 45° (I4e) OU at age six, and 65° (V4e) and 30° (I4e) with new central scotomas (to the II4e target) OU at age 9. His sister was very photophobic. Fine horizontal nystagmus was documented, and the pupils were sluggish. Retinal examination revealed pale optic discs, narrow blood vessels, and a marked translucency of the peripheral retina/RPE, with a

TABLE 4. Clinical Characteristics of Patients with *LRAT* Mutations at First Visit

Patient (Age, y)	Diagnosis	Sex	Onset	Night Blindness	Nystagmus	Refraction (SE)		VA		AS	Fundus	ERG
						OD	OS	OD	OS			
27266 (23)	LCA	F	2 mo	+	+	+4.00	+4.00	HM	HM	N	OP: pale with drusen VE: narrow PO: foveal umbo not well defined PE: hypopigmentation with bone spicules	
27241 (6)	Juv. RP	M	2 y	+	+	+9.00	+9.00	20/80	20/80	N	OP: normal VE: narrow PE: grainy degeneration	Rod-cone pattern
Sister of 27241 (4)	Juv. RP	F	2 y	+	+	+6.00	+6.00	20/100	20/100		OP: pale VE: narrow PE: grainy appearance with translucency of the RPE	ND

Abbreviations as in Tables 2 and 3.

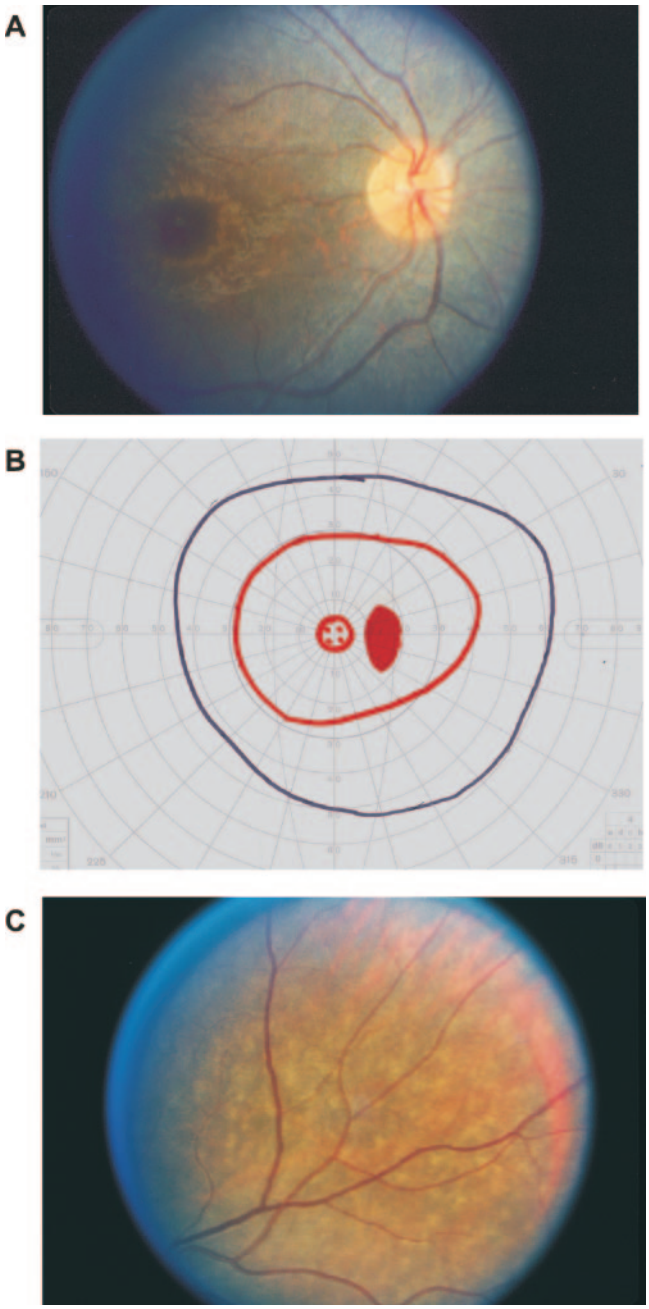

FIGURE 1. Clinical characteristics of patient 27208, who had juvenile RP caused by a homozygous splice site mutation (c.718+2T>C) in the *TULP1* gene. (**A**) Fundus photograph of the right eye at age 8 years illustrates the mild narrowing of the arterioles, mild RPE mottling, and the yellow perifoveal ring. (**B**) Goldmann visual field map showed the relative preservation of the I4e and V4e isopters, despite 20/200 acuity. (**C**) Fundus photograph of the mother of patient 27208 shows striking poorly defined, subretinal, yellowish flecks near the superior arcade.

grainy appearance (Fig. 3C). Four years later, at age 7 years, acuities were slightly decreased at 20/150, and the Goldmann visual fields were very different from those of her brother, as we measured 30° with the V4e and she did not see the I4e target.

Localization of New LCA Genes

During the course of this study, we identified two new LCA genes: *CEP290* and *LCA5*.[3,5] The *CEP290* gene was identified by homozygosity mapping in a consanguineous family that was included in this study and consisted of four affected sibs. The affected members of this family carried a homozygous intronic mutation (c.2991+1655A>G) that leads to the insertion of a cryptic exon in the *CEP290* mRNA and a premature stop codon in the CEP290 protein. Of note, we identified this mutation homozygously or compound heterozygously in 21% of our LCA cohort.[3]

Comprehensive mutation analysis of the *CEP290* gene was performed after the patients had been analyzed for homozygous regions with SNP microarrays. In the patients who were found to carry *CEP290* mutations, we could therefore go back to see whether they carried homozygous regions in their genomes. In four patients who were homozygous for the c.2991+1655A>G mutation, the homozygous region surrounding the mutation was very small—between 1.1 and 2.1 Mb. These regions were not detected as significant homozygous regions in our 250K SNP analysis, suggesting that the cutoff score used in our analysis (LOH score ≥15, corresponding to regions of 4 Mb and larger) might be too stringent. The shared homozygous haplotypes in these patients spanned only 1.1 Mb, covering 106 identical SNP calls on the 250K SNP array (rs7959909–rs7399104). In 14 patients the c.2991+1655A>G mutation was found heterozygously, together with a heterozygous deleterious mutation on the second allele.[3] In 10 of the 14 patients who were compound heterozygous for *CEP290* mutations, no significant homozygous regions were detected in the patient's genome.

Before this study, three chromosomal regions had been identified that contain unknown LCA genes: the LCA9 locus on 1p36,[30] the LCA5 locus on 6q11-q16,[31] and the LCA3 locus on 14q24.[32] Two patients who were included in our study were homozygous at the LCA5 locus: one consanguineous patient (27240) from Morocco and one patient (28609) from the United States without reported consanguinity. The homozygous region in the Moroccan patient overlapping with the LCA5 locus spanned 40 Mb and was the second largest of the eight homozygous segments identified in this patient. In the nonconsanguineous patient, the homozygous segment was 9 Mb and was the second largest of the five homozygous segments in the patient's genome. Analysis of a positional candidate gene within the overlapping homozygous regions recently allowed us to identify the *LCA5* gene.[5]

Of interest, in one nonconsanguineous patient (28604) we identified a 6-Mb homozygous region that partially overlapped the LCA9 locus. She is from the Mohawk tribe, a founder population of the original Canadians. This homozygous region was the only one identified in the patient's genome. Two patients exhibited large homozygous regions at the LCA3 locus: one consanguineous patient (28608) from Portugal and a patient from Turkey (20942). In the Portuguese patient, the homozygous region was 23 Mb and ranked fourth among nine homozygous segments. In the Turkish patient, the homozygous segment spanned 29 Mb and represented the second largest homozygous segment in the patient's genome.

Ten consanguineous and 20 nonconsanguineous patients with significant homozygous segments in their genomes were not homozygous for any of the known LCA genes and loci. These patients on average carried 3.9 homozygous fragments in their genomes, ranging from 1 to 51 Mb (average, 13.7 Mb).

DISCUSSION

In this study 93 patients (82 unrelated patients and four small families with two to four affected sibs) with LCA and juvenile RP were analyzed by SNP microarray for homozygous regions. The patients were either from consanguineous marriages or

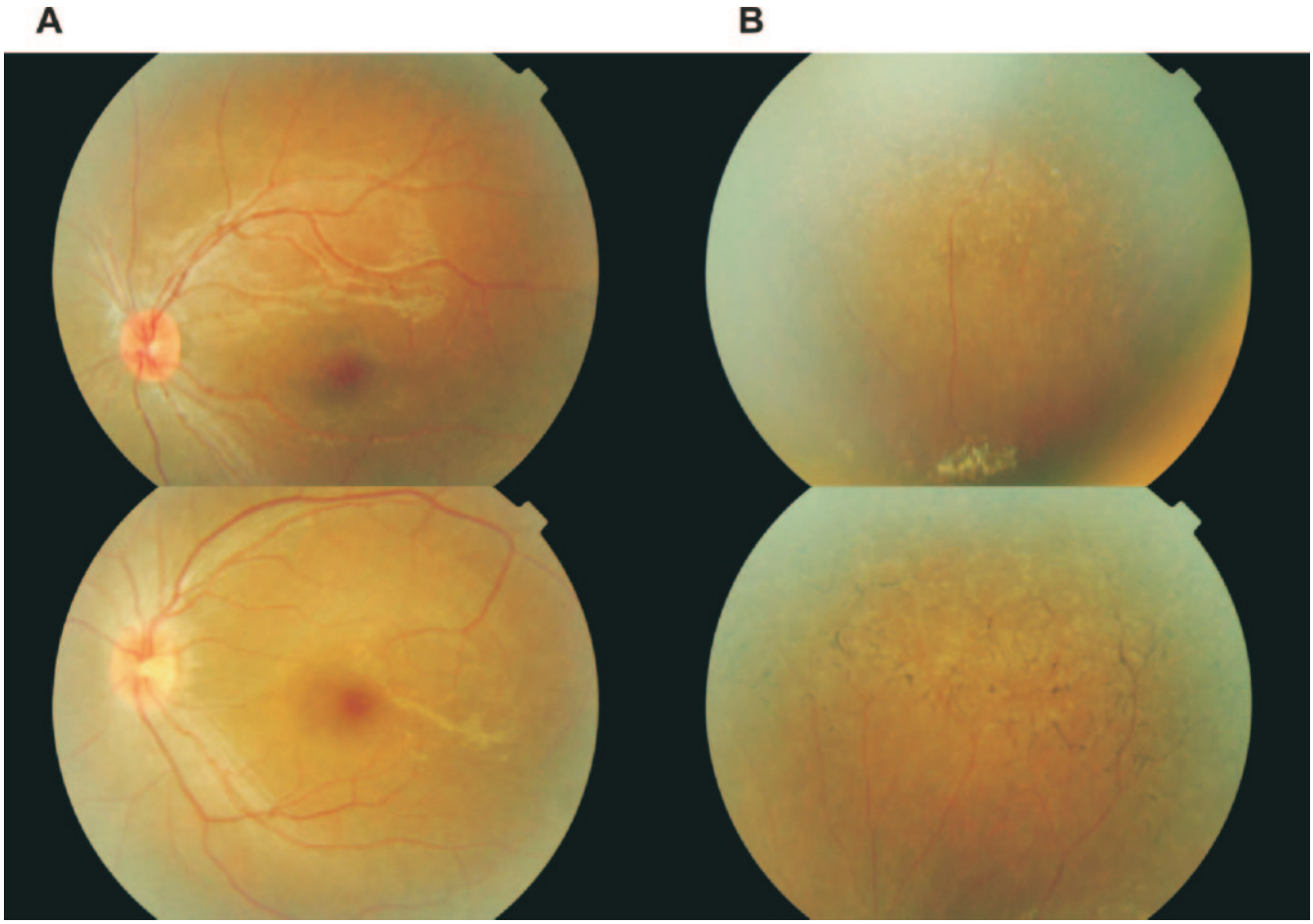

FIGURE 2. Fundus photographs of patient 21978 (**A**) and his affected brother (**B**), both of whom had juvenile RP caused by a homozygous splice site mutation (c.999+5G>C) in the *TULP1* gene. Both patients had relatively normal optic discs, mildly attenuated vessels, and relatively normal posterior poles. The youngest brother showed distinct, small, round lesions of RPE atrophy, whereas the older brother had more pronounced RPE atrophy with perivascular bone spicule pigmentation.

isolated populations (n = 33) or were from nonconsanguineous unions (n = 60). As expected, the majority (85%) of patients from consanguineous marriages and populations with a high rate of consanguineous unions in our cohort carried significant homozygous regions in their genomes. We found that the homozygous regions covered ~4.1% of their genomes. This percentage is most likely an underestimate of the total homozygosity in the genomes since the criteria for accepting a region to be homozygous were quite strict, to avoid the inclusion of false-negative homozygous regions and to exclude regions that are homozygous due to haplotype blocks that are frequent in the population. Strikingly, half of the patients without reported consanguinity also carried significant homozygous regions in their genomes. As expected, these patients carried a lower number of homozygous regions that were also smaller than those in consanguineous patients. The homozygous regions covered ~0.9% of their genomes. In patient 21266, the homozygous tracts covered more than 6% of the genome, but no consanguinity was reported. The occurrence of long homozygous tracts covering a substantial part of the genome in nonconsanguineous individuals has been reported.[33,34] Possible explanations could be that the parents have a relatively recent (unknown) common ancestor or that there is substantial inbreeding in the patient's population. Even if a patient is from an apparently outbred population, inbreeding can be high, because the patient's ancestors all lived in the same village or geographical area.

In 10 (30%) of 33 consanguineous patients, homozygosity mapping successfully identified a disease-causing mutation in one of the known LCA and juvenile RP genes, and in 2 (3%) of 60 patients with no reported consanguinity. The average size of the homozygous region containing the disease-causing mutation is 24 Mb, which is similar to the average size observed in a recent study.[12] Of note, the disease fragment was the largest or second largest segment in 10 of 12 patients. In a recent study, the longest homozygous segment was the disease-associated segment in 17% of the consanguineous individuals,[12] but the ranking of the other disease-associated segments was not shown. Although the number of patients analyzed in our study is small, our data suggest that the ranking of the disease segment can be helpful to predict which fragments may contain the disease gene.

The homozygous regions detected by SNP genotyping can also be used to identify new disease genes. During the course of this study we identified two new LCA genes: *CEP290* and *LCA5*.[3,5] In addition, we identified three patients who were homozygous at one of the two known LCA loci, which may be helpful in identifying the responsible genes. In 30 patients, we identified significant homozygous regions and excluded all known LCA and juvenile RP genes and loci. Although the homozygosity mapping approach used in this study may have missed compound heterozygous mutations in the currently known LCA and juvenile RP genes, at least a portion of them is

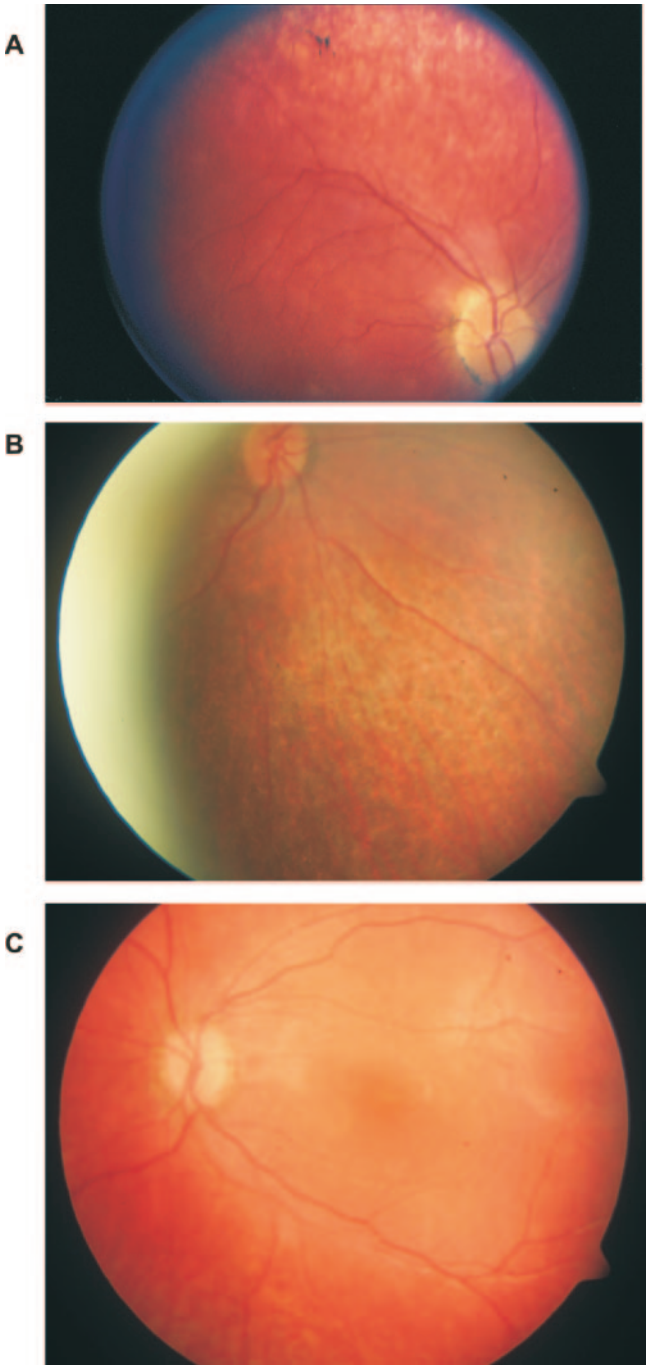

FIGURE 3. Fundus photographs of French-Canadian patients 27241 and 27266, both of whom carried the same homozygous frameshift mutation (c.217_218delAT) in the *LRAT* gene. (**A**) Patient 27266 with LCA at age 30 years is depicted. The photograph illustrates mild vessel narrowing, the relatively normal appearance of the disc and macula, and relative hypopigmentation with one bone spicule outside the vascular arcades. (**B**) Patient 27241 with juvenile RP at age 9 years is depicted. The photograph of the right inferior retina shows the mottled appearance of the RPE, vessel narrowing, relatively good disc color, and the absence of pigment degeneration. (**C**) Sister of patient 27241 at age 7 years. The photograph of the left retina illustrates the relatively normal appearance of the RPE and retina, with mild optic disc pallor and vessel attenuation. There is no evidence of pigment degeneration.

likely to have homozygous mutations in new LCA genes. The homozygous regions identified in these patients may be instrumental in identifying additional new LCA genes.

Homozygosity mapping is a well-known approach for identifying novel disease-causing genes in large to midsized consanguineous families, but it was not clear that it would work so well in very small families and isolated cases. In this study, we show that homozygosity mapping can be used very effectively not only for gene-finding, but also as a mutation screening tool. This result may have implications regarding the choice of which method to use for mutation screening of patients with LCA. LCA mutation chips (Asper Ophthalmics) are relatively cheap, contain all known disease-causing mutations in the known LCA genes, and are currently effective in approximately 60% of patients with LCA. We cannot determine what the efficiency of our homozygosity mapping approach would be in new patients diagnosed with LCA, since most of the samples analyzed in this study were prescreened with the LCA mutation chip. However, considering that 10K SNP arrays are currently cheaper than LCA mutation chips, homozygosity mapping with 10K SNP microarrays may be the best choice for mutation analysis in patients from consanguineous marriages, particularly when they originate from isolated populations that may have private mutations that are not present on the LCA mutation chip. If more than one known LCA gene is located in a homozygous segment, we would propose to prioritize sequencing of the genes based on the size of the homozygous regions.

Our clinical evaluations of the genotyped patients with LCA and juvenile RP reveal three important patterns. First, it may be possible to predict the causal LCA and/or juvenile RP gene from the retinal or other phenotypic aspect of disease. We correctly predicted the involvement of the *TULP1* gene based on the disease phenotype, which we believe is specific for *TULP1*—namely, early-onset nyctalopia with nystagmus; relative preservation of the isopters on kinetic perimetry, despite 20/200 visual acuity; and a perifoveal yellow annular ring. Also, patients with *LRAT* mutations can be recognized clinically, but overlap significantly with patients who harbor *RPE65* mutations.[23] Second, heterozygous carriers of recessive retinal dystrophy mutations may develop a subclinical phenotype that may point to the causal gene.[27–29] We found peculiar, previously unreported yellow subretinal lesions in one of the *TULP1* carriers. Much work remains to find an explanation for carrier phenotypes. Third, our work illustrates that LCA and juvenile RP overlap clinically and genetically, as we identified the identical *LRAT* mutation (c.217_218delAT) in both types of patients. The LCA patient was profoundly visually impaired from birth and remained relatively stable during our 33-year follow-up, whereas the two sibs with juvenile RP and the same *LRAT* genotype, clearly were born with vision, and presented, not only with visual loss but with nyctalopia, both of which progressed over time. Genetic and environmental factors may modulate the disease phenotype in *LRAT* patients, causing some to exhibit the LCA phenotype, whereas others develop juvenile RP.

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