Novel *TULP1* Mutation Causing Leber Congenital Amaurosis or Early Onset Retinal Degeneration

Asimina Mataftsi,[1,2,3] *Daniel F. Schorderet,*[1,2,4] *Louisa Chachoua,*[5] *Myriam Boussalah,*[6] *Mohamed T. Nouri,*[7] *Daniel Barthelmes,*[8] *François-Xavier Borruat,*[2] *and Francis L. Munier*[2]

Purpose. To report a large, consanguineous Algerian family affected with Leber congenital amaurosis (LCA) or early-onset retinal degeneration (EORD).

Methods. All accessible family members underwent a complete ophthalmic examination, and blood was obtained for DNA extraction. Homozygosity mapping was performed with markers flanking 12 loci associated with LCA. The 15 exons of *TULP1* were sequenced.

Results. Seven of 30 examined family members were affected, including five with EORD and two with LCA. All patients had nystagmus, hemeralopia, mild myopia, and low visual acuity without photophobia. Fundus features were variable among EORD patients: typical spicular retinitis pigmentosa or clumped pigmented retinopathy with age-dependent macular involvement. A salt-and-pepper retinopathy with midperipheral retinal pigment epithelium (RPE) atrophy was present in the older patients with LCA, whereas the retina appeared virtually normal in the younger ones. Both scotopic and photopic electroretinograms were nondetectable. Fundus imaging revealed a perifoveal ring of increased fundus autofluorescence (FAF) in the proband, and optical coherence tomography disclosed a thinned retina, mainly due to photoreceptor loss. Linkage analysis identified a region of homozygosity on chromosome 6, region p21.3, and mutation screening revealed a novel 6-base in-frame duplication, in the *TULP1* gene.

Conclusions. Mutation in the *TULP1* gene is a rare cause of LCA/EORD, with only 14 mutations reported so far. The observed intrafamilial phenotypic variability could be attributed to disease progression or possibly modifier alleles. This study provides the first description of FAF and quantitative reflectivity profiles in *TULP1*-related retinopathy. (*Invest Ophthalmol Vis Sci.* 2007;48:5160–5167) DOI:10.1167/iovs.06-1013

From the [1]IRO (Institute of Research in Ophthalmology), Sion, Switzerland; the [2]Department of Ophthalmology, Jules Gonin Eye Hospital, University of Lausanne, Lausanne, Switzerland; the [3]II Department of Ophthalmology Aristotle University of Thessaloniki, Thessaloniki, Greece; [4]Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland; [5]Service d'Ophtalmologie, CHU (Centre Hospitalier Universitaire) d'Hussein-Dey, Alger, Algeria; [6]Service d'Ophtalmologie, CHU Tlemcen, Tlemcen, Algeria; [7]CHU Beni Messous, Alger, Algeria; and the [8]Department of Ophthalmology, University of Bern, Inselspital, Bern, Switzerland.

Supported by Grant 32.111948 from the Swiss National Science Foundation (FLM, DFS).

Submitted for publication August 27, 2006; revised February 28, 2007; accepted September 12, 2007.

Disclosure: **A. Mataftsi**, None; **D.F. Schorderet**, None; **L. Chachoua**, None; **M. Boussalah**, None; **M.T. Nouri**, None; **D. Barthelmes**, None; **F.-X. Borruat**, None; **F.L. Munier**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Francis L. Munier, Jules Gonin Eye Hospital, Avenue de France 15, 1004 Lausanne, Switzerland; francis.munier@ophtal.vd.ch.

Leber congenital amaurosis (LCA) is an autosomal recessive retinal degeneration first described by Theodore Leber in 1869[1] as a congenital form of retinitis pigmentosa (RP). It represents the most common inherited cause of blindness in childhood and is characterized at or near birth by severe visual loss, wandering nystagmus, amaurotic pupils, and normal fundus. A severely reduced electroretinogram (ERG) was added as a diagnostic feature in 1956 by Franceschetti and Dieterlé.[2]

LCA, early-onset retinal degeneration (EORD), and RP may be considered a continuum of entities, in which LCA represents the extreme end of severity, as far as age of onset and functional outcome are concerned.[3–5] Based on behavior toward light during the first 2 years of life, Hanein et al.[3] divided patients with LCA/EORD into two main groups: (1) congenital or very early cone–rod dystrophy, and (2) severe yet progressive rod–cone dystrophy.

Poor vision may either be present at birth or develop progressively during childhood or adolescence. Fundus examination is usually normal at birth and retinopathy does not become evident for several years. Extinguished ERGs before the age of 1 year can be used as a criterion to distinguish LCA from EORD.[6] Associated clinical signs such as posterior polar cataract and keratoconus may be part of the phenotype. Refractive error also seems to be a distinct feature, as patients with mutation in certain genes are very hyperopic, whereas other mutated genes are associated with emmetropia or high myopia.[3]

To date, nine genes (*GUCY2D*, *CRB1*, *RPE65*, *RPGRIP1*, *AIPL1*, *CRX*, *RDH12*, *IMPDH1*, and *CEP290*)[3–9] and three loci (LCA3, LCA5, and LCA9)[3] have been reported to be associated with LCA. *LRAT* and *TULP1* have also been implicated in LCA,[3] although controversy remains regarding their phenotypic associations, and other researchers consider them to be a cause of juvenile or early-onset RP rather than LCA.[4–6] It has been shown, that all LCA genes except *RPGRIP1* are also associated with other retinal dystrophies, and that different mutations in one gene can cause alternate phenotypes.[4] Moreover, some patients with LCA have been found to have inherited mutations in more than one of these LCA-associated genes, suggesting modifier alleles.[10]

To add to the complexity, the reported genes participate in a wide variety of retinal pathways, are expressed in separate retinal cells, and have diverse putative functions. The exact pathophysiological role of most of them is as yet unknown.[4,11]

Efforts have been made to define clinical subtypes and to establish gene-specific phenotypes as well as LCA carrier phenotypes, so as to guide the genetic testing strategies and counseling.[3,12,13] Recognizing distinct clinical subtypes and correlating them with a specific genetic background is a necessary step into the understanding of this heterogeneous group of disorders.

We report a large Algerian family exhibiting a novel *TULP1* mutation and its variable phenotypic expression, ranging from typical LCA to EORD.

METHODS

Patients

This study adhered to the tenets of the Declaration of Helsinki (1983 Revision). Informed consent was obtained from all subjects after the nature and possible consequences of the study had been explained.

All accessible members of the family had a complete clinical examination, including refraction, best corrected visual acuity, Goldmann perimetry, color vision testing (Ishihara plates or Farnsworth D-15 hue test), pupillary responses, biomicroscopy, tonometry, and funduscopy after pupillary dilatation. Fluorescein angiography was performed on the propositus (subject VII3) and two other affected members (subjects VI25 and VI26). Macular optical coherence tomography (OCT; Stratus OCT 3; Carl Zeiss Meditec Inc., Dublin, CA), fundus autofluorescence (FAF) measurement with a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiogram [HRA 2], Heidelberg Engineering, Heidelberg, Germany), and photopic and scotopic full-field electroretinography (ISCEV standards[14]) were available only for the proband investigated in Lausanne, the remainder of the family being examined in Algeria.

For analysis of retinal lamination irregularities raw data of each line scan were exported from the OCT device. Light reflection profiles (LRPs) of each line scan were analyzed (IGOR Pro 5.05a; Wavemetrics Inc., Lake Oswego, OR).[15–17] A 6-year-old normal subject was used as the control.

DNA Analysis

Genomic DNA was extracted from peripheral blood of the family members by using a standard procedure. Fifty-one unrelated healthy individuals of Algerian ancestry were used as control subjects. Linkage to the 12 autosomal recessive loci that were known at the time of investigation was tested by using polymorphic genetic markers flanking the region of each gene by approximately 2.5 Mb (Table 1a). Homozygosity mapping was performed because of consanguinity and our hypothesis that this family shared a unique mutation by descent.

Thirteen primer sets were used to amplify all 15 exons of *TULP1* (tubby-like protein 1) and their intronic junctions in two unaffected obligate carriers (Table 1b). The primers were designed from adjacent intronic sequences 50 to 70 bp from the splice site, using Primer3 software (provided in the public domain by the Whitehead Institute, Massachusetts Institute of Technology, Cambridge, MA; http://frodo.wi.mit.edu/cgibin/primer3/primer3_www.cgi). All exons were PCR-amplified and sequenced on both strands (Big Dye Terminator, ver. 1 or 3) and electrophoresed (model 3100 genetic analyzer; Applied Biosystems [ABI], Rotkreuz, Switzerland).

TABLE 1. Primers

a. Primers Used for Homozygosity Mapping

Gene	Position (Mb)	Microsatellite	Forward Primer	Reverse Primer
TULP1	36.3	D6S291	CTCAGAGGATGCCATGTCTAAAATA	GGGGATGACGAATTATTCACTAACT
	32.7	D6S1666	CTGAGTTGGGCAGCATTTG	ACCCAGCATTTTGGAGTTG
RPE85	66.7	D1S198	GACTTCACCATCAACGCCTG	CAGGAAAGTGGATGTGACGA
	71.3	D1S2895	TCCTCAGCCATGTGGAA	TAGCAGCCCAAACTGACTAA
CRX	55.46	D19S904	ACAAGAATTGCTTGAACCTGG	GCTCCATTTCGGAGATGTTA
	51.7	D19S412	TGAGCGACAGAATGAGACT	ACATCTTACTGAATGCTTGC
AIPL1	3.75	D17S1828	TGCACTCACAGATTTGCC	TTAAGCCAGTTCGGATTTG
	8.75	D17S786	GTGAAAGTGACATGTTTTCCA	AGGATTTGGGCTCTTTTGTA
GUCY2D	5.91	D17S1832	ACGCCTTGACATAGTTGC	TGTGTGACTGTTCAGCCTC
	10.05	D17S1879	GAAGTTTACGAAAATTGCTGTC	AAGGGTAGTTCTGCGTGC
RPGRIP1	19.9	D14S261	CCAGCAGCACTATTCAGACT	ACTCCTTCCCACAATTTCTC
	23.4	D14S972	TTAACGCATAACAGCCAAGA	TCTGACTGCCTCCATGA
CRB1	195.3	D1S413	GCCAAGCCTGAGATCAAAAT	ACTTGAACAGATTGGGATTG
	190.7	D1S412	TAGGACTTTTCAACTTCCACAG	ATAGGCACAGAATCAATGAATG
LRAT	158.7	D4S413	ATATAGTGCTCCAGAAACAGAAATG	GAAGAATTGTAGTATAGTTTGTCTCC
	147.1	D4S1586	GCATGTACCATTGCCAGG	CCCAGAGTGCTGATGTGTG
	142.5	D4S424	GCGCTCTTGGTATATGGTACAG	TGTGGGCAACGTCACTC
RDH12	64.2	D14S1046	CATTTGGAGTTGAGTGGTTAGA	CCTCTGTGGATTCTGGGA
	68.5	AFM273xb9	CCCAGATCGCNCCATT	ATGGCTCTGAGGCGGG
LCA3	77.7	D14S74	TGTACCACTACCTGAGTTGAGTC	CAGGATATTGTTACACTGCCTAAG
LCA9	9.5	D1S450	GCTCCAATGTCCAAGGG	GGGTACTCAGATGGCTGGT
	17.5	D1S3669	TTTTGTTTCTTGATCTGGGC	TGTTAAACTTTTCACTGAGGTATAA
LCA5	84.4	D6S1609	TATAGCACAGAGCCTGATACACTTG	TAGCCATTTTGCCCAGTC
	93.4	D6S1274	CAAATTTCAGATTACATGCAGC	GGCATGTAGGCATACTCTGG

b. *TULP1* PCR Conditions

Exon	Forward Primer	Reverse Primer	Annealing Temperature (°C)	Use of Betain
1 + 2	TTGAGATTCTTCCCCCAGTG	GACCTGTCCCACCCCTAGAC	58	No
3	GTTATTTCTGGCGGCTCAAG	CTTCTACACCAACCCCAACC	55	Yes
4	GGAGATCCCTAGGGTGAGGA	GAAAAGTGGGGGCTATTTGA	58	No
5	AACTGGGCTCCCCAGATAAG	CTCAGTGAGACGCCAAGC	58	No
6 + 7	TAGGCAGGACAAGAGGGTTG	TGGCAAACTCCTTACCTAGCA	55	Yes
8	ATGGGGTCGACTCTCTTGTG	CACCCTCTAGGCTCCCAAGT	58	No
9 + 10	CTCCCCAGAGCCTCCTAACT	GTGAGCTGCAGGGAGAAATC	58	No
11	CAAACGGGCCTGATTTCTC	ACTGTGGTGGGTGCTCTACC	58	No
12 + 13	TAAACTGCTTGGCTGTGTGG	GATGGATGTGCTCAGGGAGT	60	Yes
14	ATGAAGATGTCCAGGCCACA	TGGTGTGTGTGTGTGTGAGG	62	Yes
15	GAGATAGAAGAAGGGATGGGTGT	AGTTTTCCGCGGGAGCTT	55	Yes
15b		AAGGTTGAGGGCACTGTCTG	58	No

RESULTS

Phenotype

The pedigree of the family strongly suggests autosomal recessive inheritance (Fig. 1). Seven individuals (three females, four males) were found to be affected. None was obese or showed other endocrine disorders or hearing impairment, with the exception of the oldest patient (VI24), who was deaf-mute after acute meningitis at the age of 2 years.

According to patients' histories, symptoms started either at birth (patients VII3 and VII4) or during infancy (patients VI9, VI10, VI24, VI25, and VI26). Age at diagnosis ranged from 3 to 12 years. All patients had night blindness and nystagmus, but none presented photophobia, and the oculodigital sign was not reported. Four patients retained enough vision to learn reading and writing (patients VI25, VI26, VI9, and VII3) whereas two subjects did not go to school, and one was still too young to attend school at the time of examination.

All tested eyes had a myopic refractive error, except for patient VI10 who presented mild hyperopia (Table 2). Visual acuity at examination ranged from perception of light to 20/100. The proband (VII3) was examined on two occasions (8 months apart), and during this interval, his visual acuity declined from 20/100 to 20/250 in the better eye. Visual fields were moderately to severely restricted in all subjects who managed to undergo the test, and only the V4e stimulus was seen. Color vision was severely disturbed without a specific axis of confusion. The pupillary reflex was extremely sluggish in most of the patients. None presented signs of keratoconus.

In the proband, full-field electroretinography showed completely extinguished rod- and cone-driven responses. An annulus of increased perifoveal FAF, as depicted by HRA, was present at approximately 2° to 5° eccentricity (Fig. 2).

Macular OCT scans in the proband showed an abnormal lamination due to the underlying degenerative disease. The nerve fiber layer and inner plexiform layer seemed to be preserved (Fig. 3), but the structures of the outer retina were nondetectable. The compared LRPs between control and patient revealed a complete loss of the reflective structures that seem to correspond to the outer nuclear layer and the highly reflective layer between inner and outer photoreceptor segments. Retinal thickness in the analyzed OCT scans was significantly reduced (290 ± 11 μm in the normal subject vs. 95 ± 8 μm in the patient). This finding can mostly be explained by the degenerative loss of structures in the outer retina (Fig. 3).

Funduscopic findings varied significantly across and within the sibships, mainly in an age-dependant manner. The youngest patient had no detectable maculopathy. Patients VII3, VI9, and VI10 had an indistinct foveolar reflex (Fig. 4). All three older affected patients presented pronounced maculopathy. In the two sisters (VI25 and VI26), an annulus of pinpoint perifoveal yellow deposits, similar to those seen in the periphery, surrounded a dull fovea (VI25, Fig. 4b). In subject VI24, geographic atrophy had ensued (Fig. 4c).

The optic disc was healthy-colored in younger patients, whereas moderate to waxy disc pallor was evident in the three older ones (subjects VI26, VI25, and VI24). The latter patients also presented an annulus of peripapillary RPE atrophy in both eyes (Fig. 4b, 4c). This feature did not seem to correspond to a myopic crescent, as it was equally large temporally and nasally, and it was more pronounced in the eldest patient (VI24), who was equally or less myopic than his sisters (VI25 and VI26), at least in the left eye (right eye refraction and funduscopy were not possible in VI24, because of very dense cataract).

Pigmentary retinopathy and retinal vessel attenuation were present in all but the youngest subject (VII4). Patients VI25, VI26, and VI9 presented severely sclerosed arteries and multiple occluded or ghost vessels in the periphery and occasionally at the posterior pole (Fig. 4d, subject VI9). Pigment spicules

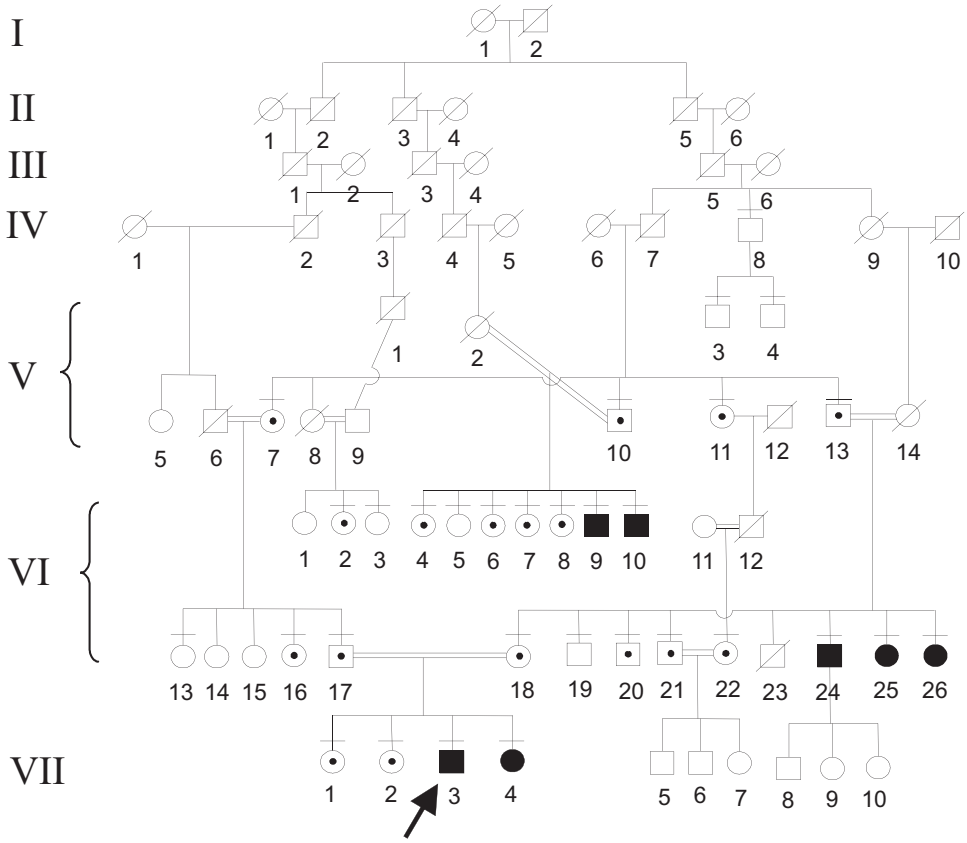

FIGURE 1. Simplified depiction of the studied pedigree, comprising seven (VII) generations and revealing multiple consanguineous couples (linked by a *double line*). *Arrow*: proband; *circles*: females; *squares*: males; *filled symbols*: affected; *symbols* with *center dot*: healthy carriers; *slashed symbols*: deceased. A *horizontal dash* above the symbol indicates that the individual was examined.

TABLE 2. Clinical Characteristics of the Homozygotes

Patient	Age at Visit (y)	Nystagmus	VA (OD, OS)	Goldman VF (V4e Stimulus) OD, OS	Refraction OD, OS (D)	Optic Disc	Anterior Segment	Macula	Vessels	Periphery
VII4	3	Intermittent	F + F F + F	Not measurable	C-0.50 × 180° C-2.0 × 180°	Tilted optic discs + atrophy of temporal sector	Normal	Unremarkable	Normal	No lesions detected
VII3	8	Horizontal-rotational	20/500 20/250	Approximately 60° H, 40° V in both eyes	S + 1.50 C-2.50 × 10° S + 1.50 C-3.0 × 160°	Healthy-colored disc	Normal	Indistinct foveal reflex; no deposits	Slightly attenuated	Few pigment clumps, shiny white spots at mid periphery; discrete diffuse RPE mottling, salt-and-pepper appearance
VI10	20	Rotational	CF CF	Not measurable	S + 2.50 S + 3.00	Healthy-colored disc	Normal	Indistinct foveal reflex; no deposits	Attenuated	Multiple prominent osteoblasts; pronounced pigment migration around vessels
VI9	26	Horizontal	HM PL	Not measurable	Not measurable Not measurable	Healthy-colored disc	Normal	Indistinct foveal reflex; no deposits	Very attenuated, few ghost vessels	Multiple pigment clumps, shiny white spots at mid periphery; pronounced pigment migration around vessels
VI26	33	Rotational	20/1000 HM	15° H, 15° V 10° H, 10° V	S-5 C-2 × 180° S-6 C-2 × 180°	Waxy pallor; peripapillary RPE atrophy	Posterior subcapsular cataract	Annulus of yellow pinpoint perifoveal deposits	Severely attenuated; Multiple completely fibrosed arteries	Discrete diffuse RPE mottling
VI25	37	Vertical	20/1000 20/1000	25° H, 20° V 40° H, 20° V	S-3 C-2 × 180° S-2 C-3 × 180°	Pale optic disc with peripapillary RPE atrophy	Posterior subcapsular cataract	Annulus of yellow pinpoint perifoveal deposits	Very attenuated	Few pigment clumps, shiny white spots at mid periphery
VI24	44	Horizontal	CF HM	Not measurable	Not measurable S-3 C-1 × 180°	Mild disc pallor; pronounced peripapillary RPE atrophy	Posterior subcapsular cataract, dense nuclear sclerosis OD	Well-defined geographic atrophy (diameter 300 μm); no deposits	Very attenuated; Sclerosis of large choroidal vessels	Areas of accentuated retinal mottling; pronounced chorioretinal atrophy along the temporal arcades

F + F, fixes and follows target; H, horizontal; V, vertical; RPE, retinal pigment epithelium; VA, visual acuity (best corrected, for distance); HM, hand movements; PL, perception of light; CF, counting fingers; VF, visual field.

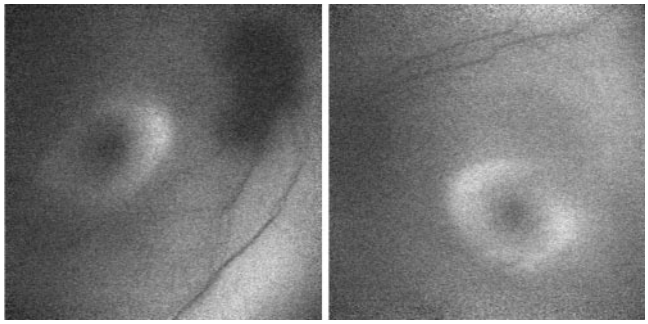

FIGURE 2. HRA imaging of the proband's macular area in the right and left eyes, depicting an annulus of increased perifoveal AF.

and clumps varied in intensity among patients. In sibs VI9 and VI10, pigment migration was predominantly paravenous (Fig. 4e, subject VI10). Pinpoint yellow-white deposits were also present in the peripheral retina in subjects VI25, VI26, VI9, and VII3 (Fig. 4d, subject VI9).

Fluorescein angiography confirmed the presence of peripapillary retinal pigment epithelial atrophy (subjects VI25 and VI26) and the absence of macular edema.

Fifteen of the 17 examined asymptomatic carriers of the mutation (mean age, 35 years; range, 14–85) had an unremarkable fundus appearance. Two heterozygous subjects (V13 and V10, age 66 and 64 years, respectively) presented a few drusen-like macular deposits, and V10 additionally presented age-related pigmentary macular changes.

Molecular Analysis

Homozygosity mapping excluded all the tested loci with the exception of *TULP1* located at 6p21.3 for which the parents were heterozygous, and all affected individuals were homozygous for the two analyzed markers. *TULP1* sequencing revealed five sequence modifications.

A small 6-base in-frame duplication, c.1593 to 1594dupTTCGCC (HSTULPS01-5, Retina International Mutation Database www.retina-international.org), was identified in exon 15 (Figs. 5a, 5b).[18] This change was homozygous in all seven affected members, heterozygous in their parents and 15 collaterals, and absent in 51 ethnic-matched control subjects. This mutation leads to a predicted duplication of two amino acids in the highly conserved C-terminal region of the protein (FA531-532dup; Fig. 5c). None of the 102 control chromosomes presented this change. In addition, this mutation was not observed in more than 500 index cases with various forms of RP.

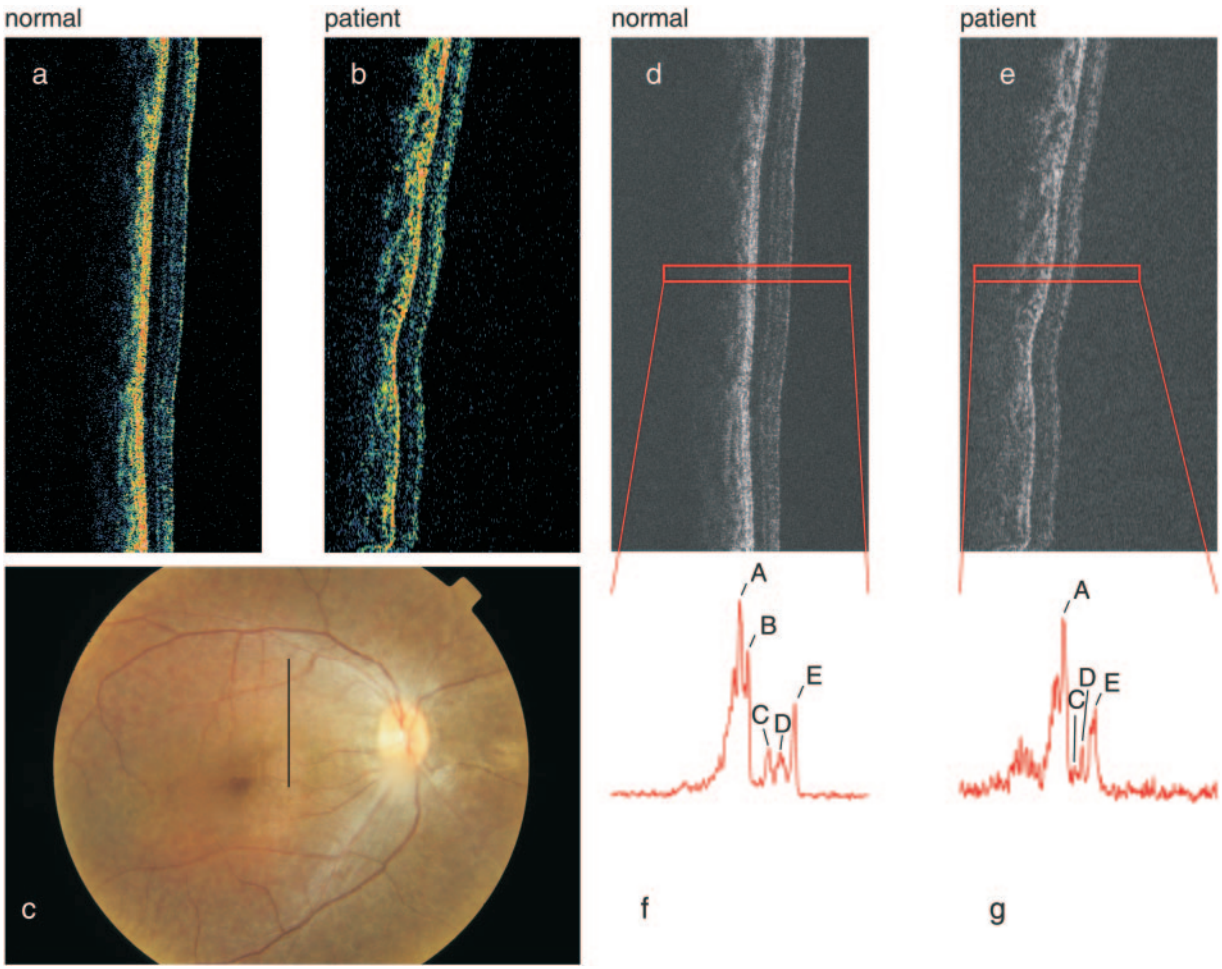

FIGURE 3. (**a**) A pseudocolor image of an OCT scan in a healthy age-matched control patient taken in the same place and direction as in the patient. (**b**) A pseudocolor image of patient VII3. (**c**) Scan location and direction. Images from the raw data of OCT scans of (**d**) control patient and (**e**) patient VII3. *Red rectangles*: the analyzed area. LRPs are shown for the (**f**) control subject and the (**g**) patient. Peaks in LRPs resemble: A, retinal pigment epithelium; B, highly reflective layer between inner and outer photoreceptor segments; C, outer plexiform layer; D, inner plexiform layer; E, nerve fiber layer. The outer limiting membrane was not detectable in the OCT scans. Distance B–C represents the outer nuclear layer, and distance A–E represents retinal thickness.

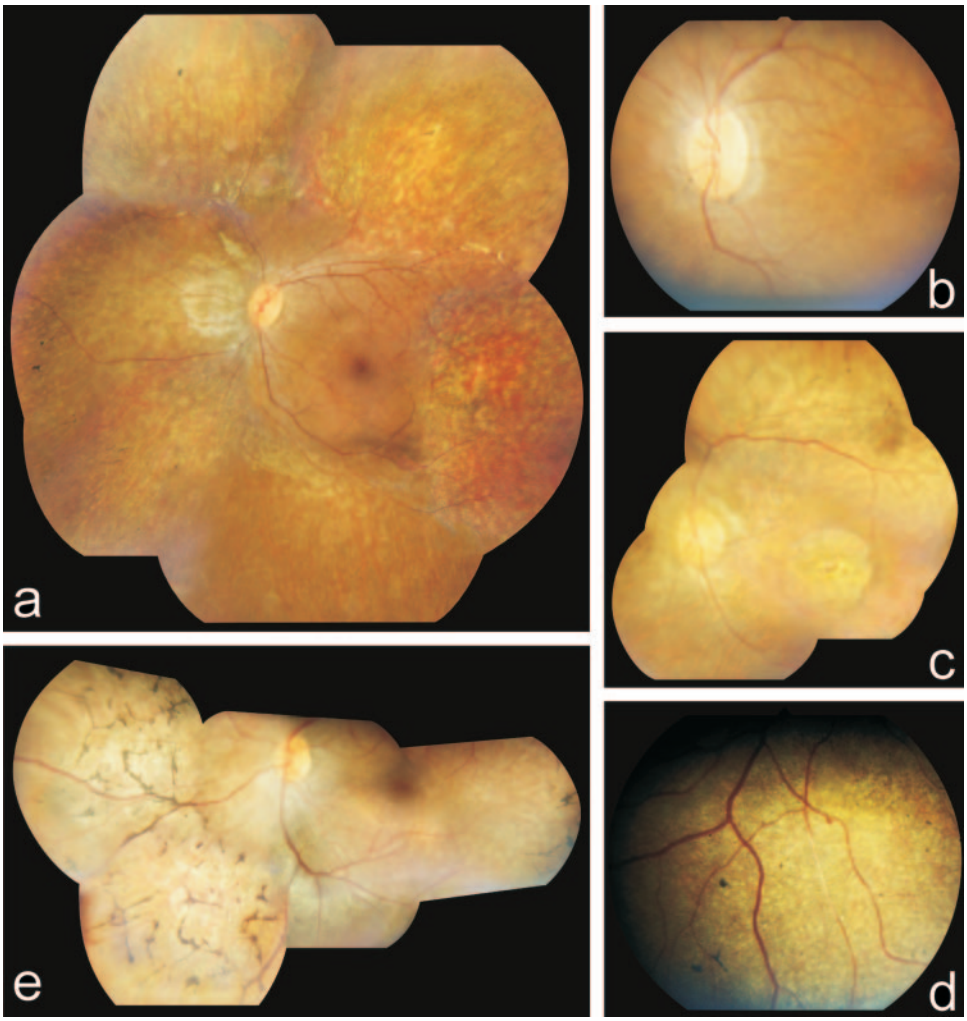

FIGURE 4. (**a**) Composite color photograph of the left fundus in the proband (VII3). The optic disc and macula were relatively preserved, vessels showed slight attenuation, and the RPE was diffusely altered, with formation of a few spicules in the mid periphery. (**b**) Color photograph of the left optic disc and macula in subject VI25. Vessel attenuation, yellow pinpoint deposits surrounding the dull fovea, and peripapillary RPE atrophy were noted. (**c**) Composite color photograph of the left fundus in the oldest affected family member (VI24), showing severe vessel attenuation, diffuse RPE atrophy with a distinct peripapillary large atrophic annulus, and geographic atrophy of the macula. (**d**) Color photograph in the mid periphery of the left fundus in VI9. Pinpoint white deposits and pigment clumps were present, as well as vessel occlusion, either established or developing. (**e**) Composite color photograph of the left fundus in VI10, showing pronounced pigment migration around vessels and well-preserved optic disc and macula.

A second sequence modification was identified in exon 14—namely, a G-to-A change (GCT to ACT)—leading to a predicted A496T amino acid substitution. This allele was found in the heterozygous state in the proband's father (VI17). Finally, three other polymorphisms were found, two of which are known single-nucleotide polymorphisms: I259T (ATA-to-ACA change) and K261N (AAG-to-AAC change) in exon 8. The former was present in the heterozygous state and the latter in the homozygous state in subject VI18. The third polymorphism, T454T (ACG-to-ACA change), in exon 14, was present in the heterozygous state in subject VI17.

DISCUSSION

In this study, we investigated a large Algerian family with multiple consanguineous loops, comprising seven patients (age range, 3–44 years) with LCA/EORD. We were able to exclude linkage to 11 of 12 known loci associated with this phenotype and to establish linkage to markers flanking the *TULP1* gene by homozygosity mapping. Screening this gene revealed a novel mutation, FA531-532dup, that cosegregated with the disease in the homozygous state and was absent in 102 control chromosomes belonging to normal individuals of Algerian origin.

Previous linkage analysis in a large Dominican pedigree with autosomal recessive (ar)EORD mapped the disease to 6p21.3.[19] This locus, known as RP14, was later shown to harbor mutations in the *TULP1* gene.[20,21] *TULP1* belongs to a highly expressed retinal family of tubby genes, including *TULP2* and *TULP3*. *TULP1* mutations are known to cause retinal degeneration with a wide range of severity, from congenital blindness/LCA to adult-onset RP.[3,22] The most commonly described phenotype is severe, early-onset retinal degeneration.

Although the specific function of *TULP1* is not yet known, recent studies have shown that the protein is expressed in both rods and cones.[23] It may act as a transcription factor[24] or be implicated in protein trafficking,[25] such as rhodopsin transport.[26]

TULP1 appears to be a rare cause of LCA, as mutations of this gene are reported in only 1.7% of patients with LCA.[3] A total of only 14 disease-causing alleles of *TULP1* have been reported in 11 index patients, including seven missense mutations (Arg400Trp, Gly368Trp, Arg420Pro, Phe491Leu, Ile459Lys, Phe382Ser, and Lys489Arg),[3,20,22,27] five splice-site mutations (c.99+1G>A, IVS2DS G-A,+1, IVS14-6C-A, IVS4-2delAGA, and IVS14DS G-A,+1),[3,20,21,22,28,29] one nonsense mutation (Glu402X),[3] and one frame-shift deletion (c.937delC).[28] These changes were observed in four simplex and seven multiplex pedigrees of autosomal recessive retinitis pigmentosa (arRP) or EORD and were present in populations of different origins, including Spanish. Of note, the present Algerian family is of Andalusian origin and immigrated to the region of Tlemcen in the 15th century at the time of the Reconquista.

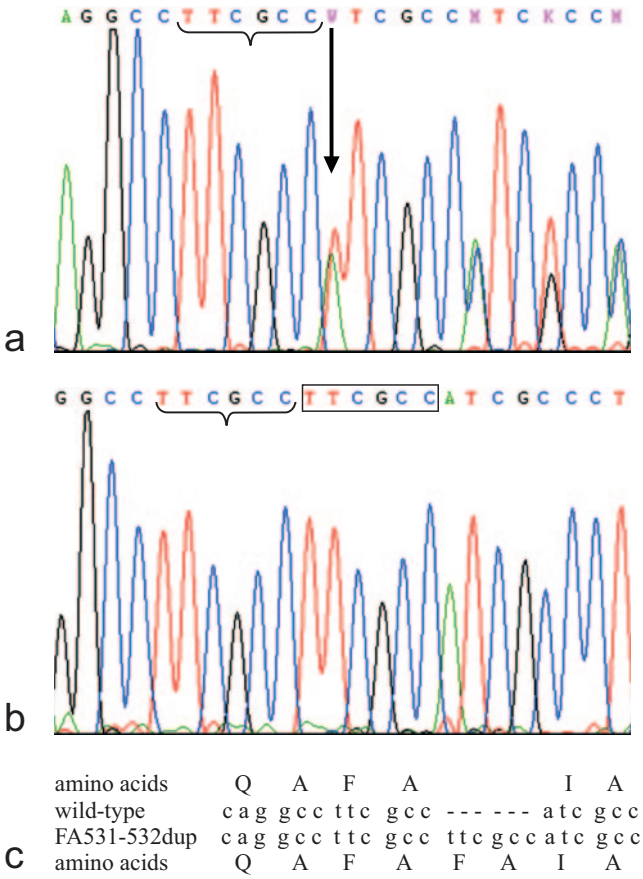

FIGURE 5. (**a**) Sequencing chromatogram from a subject heterozygous for the mutation, with two different series of bases superimposed from the point of the insertion/duplication (*arrow*) to the end. (**b**) Sequencing chromatogram from a subject homozygous for the duplication of TTCGCC. (**c**) Graphic depiction of the wild-type and mutated DNA sequences and the corresponding amino-acid sequences.

A body of evidence supports a causative role of the FA531-532dup mutation in this family. The mutation segregates in all affected members, is absent in the control population, and compromises the structure of the highly conserved carboxyl terminal end of the protein.

The heterozygous change A496T has previously been described as a polymorphism of uncertain pathogenicity,[20,22] since it was only found heterozygously in patients in whom an undetected compound heterozygosity could not be excluded. The coexistence of A496T with FA531-532dup in a normal subject (VI17) provides evidence that A496T is not a disease-causing mutation.

The prevalence of *TULP1* mutations has not been specifically investigated in patients with RP. Gu et al.[22] found *TULP1* double mutations in 2 (1.17%) of 171 patients with arRP. In 1.75% of the same cohort, single heterozygous, potentially pathogenic mutations were observed.[22] In their study of 536 patients with RP, Hagstrom et al.[20] found pathogenic *TULP1* mutations in <0.5% of the cases (2/536 or 0.37%). Even if these statistics underestimate the actual prevalence, since the mutation detection with current techniques is <100%, it is reasonable to say that *TULP1* mutations are a rare cause of RP or EORD.

Visual function in *TULP1*-related retinal degeneration has been described as severely reduced when compared with that of other patients with arRP,[20,28] or much better than in other groups of patients with LCA.[29] Lack of severe central visual dysfunction and absence of nystagmus was described in two 8-year-old Phe382Ser homozygotes, who presented acuities of 0.3 and 0.5, suggesting a less severe phenotype specific to this *TULP1* mutation.[27]

When comparing our patients to the numerous IVS14+1G>A homozygotes from the large Dominican kindred,[29] a similar course of severity and progression of visual handicap was noted. Relative preservation of visual acuity (20/100) and visual field (near-normal V4e isopter, undetectable I4e target) in the first decade of life, although nystagmus appeared as early as in the first year. Common clinical findings were a myopic refractive error in most of the patients, minimal peripheral pigmentary retinopathy, and vessel attenuation (early aspect of RP) present from the first decade, initial normal disc and macular appearance, eventual development of ophthalmoscopically visible maculopathy, and optic disc pallor. An annulus of yellow deposits in the central 10° of the macula was present in many patients in the second and third decades.[29] This finding was also reported in one of two Phe382Ser homozygotes.[27] Macular geographic atrophy, present in our eldest patient, was described in a 42-year-old IVS14+1G>A homozygote.[29]

Only nuclear sclerotic cataract was reported in a 42-year-old IVS14+1G>A homozygote,[29] whereas bilateral subcapsular opacities, similar to the ones in VI24, VI25 and VI26, have been described in a 26-year-old patient carrying the IVS4-2delAGA and c.937delC mutations.[28] Pigmentary retinopathy is referred to as typical RP or bone-spicule–like pigment changes mainly in the peripheral retina, in most patients with *TULP1*-related disease.[3,28,29] Apart from the vascular attenuation that is usually regarded as a typical feature of EORD or RP, we observed multiple severely sclerosed and occluded or ghost vessels in subjects VI25 and VI9. This feature, as well as peripapillary RPE atrophy, has not been reported in patients with *TULP1*-related LCA or EORD.

We found a totally normal clinical appearance in most of the FA531-532dup mutation carriers (15/17) of all ages. The presence of macular drusenlike deposits in two heterozygotes is in accordance with the findings in a reported Dominican kindred, in which such carriers were also found to have normal ERGs.[29] So far, no clinical feature has been identified to raise the suspicion of a *TULP1* mutation carrier state that would guide genotyping in families affected by LCA/EORD.

Quantitative analysis of retinal layers in the macular area in the proband (VII3) and in the age-matched control subject (Fig. 3) reveals comparable distances between the nerve fiber layer and the inner plexiform layer. On the contrary, VII3 exhibited missing reflective layers corresponding to the outer retinal structures, and the reduced signal intensity suggested an already affected outer plexiform layer. This in vivo biomicroscopic characterization of the diseased retina suggests a retrograde process of degeneration as a result of the primary photoreceptor decay.

Of interest is the simultaneous presence of a perifoveal ring of increased AF in the same patient. This finding appears not to be uncommon in patients with RP with preserved central vision[30] and has also been described in some patients with LCA, although not in correlation with a specific genotype.[31] FAF is an index of lipofuscin accumulation in the RPE, its preservation reflects the level of metabolic activity and thus indicates at least a partial presence of structurally intact photoreceptors and photoreceptor/RPE complex.[31] In our patient, the AF image revealed that the homozygous FA531-532dup mutation in *TULP1* does not halt the formation of lipofuscin fluorophores and causes macular photoreceptor cell dysfunction as opposed to cell death, at the corresponding stage of the disease. This finding can be helpful in directing mutation screening in patients with LCA/EORD[3]—for example, in differentiating from *RPE65* mutations—as they have been found to

lead to lack of FAF.[32] FAF can also be useful in monitoring possible photoreceptor cell rescue therapies in the future.[31]

Evaluation of autofluorescence by HRA and retinal thickness by OCT may help to discriminate between *RPE65* (and possibly LRAT), *TULP1*, and *CRMB1*-related rod-cone LCAs. Further characterization of patients with *TULP1* mutations with the help of these devices may shed some light on the pathogenesis of this rare retinopathy.

Acknowledgments

The authors thank the members of the family for their cooperation and Celine Agosti, Tatiana Favez, Isabelle Favre, Sylviane Métrailler, and Marc Curchod for technical assistance.

References

1. Leber T. Uber Retinitis pigmentosa und Angeborene Amaurose. *Graefes Arch Klin Exp Ophthalmol.* 1869;15:13–20.
2. Franceschetti A, Dieterlé P. Die Differentaldiagnostische Bedeutung des ERG's bei tapeto-retinalen Degenerationen: Electroretinographie. *Bibl Ophthalmol.* 1956;48:61.
3. Hanein S, Perrault I, Gerber S, et al. Leber congenital amaurosis: comprehensive survey of the genetic heterogeneity, refinement of the clinical definition, and genotype-phenotype correlations as a strategy for molecular diagnosis. *Hum Mutat*. 2004;23:306–317.
4. Cremers FPM, van der Hurk JAJM, den Hollander AI. Molecular genetics of Leber congenital amaurosis. *Hum Mol Genet*. 2002;11: 1169–1176.
5. Weleber RG. Infantile and childhood retinal blindness: a molecular perspective. *Ophthalmic Genet*. 2002;23:71–97.
6. Foxman SG, Heckenlively JR, Bateman JB, Wirtschafter JD. Classification of congenital and early onset retinitis pigmentosa. *Arch Ophthalmol*. 1985;103:1502–1506.
7. Janecke AR, Thompson DA, Uterman G, et al. Mutations in RDH12 encoding a photoreceptor cell retinol dehydrogenase cause childhood-onset severe retinal dystrophy. *Nat Genet*. 2004;36:850–854.
8. Bowne SJ, Sullivan LS, Mortimer SE, et al. Spectrum and frequency of mutations in IMPDH1 associated with autosomal dominant retinitis pigmentosa and Leber congenital amaurosis. *Invest Ophthalmol Vis Sci*. 2006;47:34–42.
9. den Hollander AI, Koenekoop RK, Yzer S, et al. Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber Congenital Amaurosis. *Am J Hum Gen*. 2006;79:556–561.
10. Zernant J, Maigi K, Dharmaraj S, et al. Genotyping microarray (disease chip) for Leber congenital amaurosis: detection of modifier alleles. *Invest Ophthalmol Vis Sci*. 2005;46:3052–3059.
11. Koenekoop RK. An overview of Leber congenital amaurosis: a model to understand human retinal development. *Surv Ophthalmol*. 2004;49:379–398.
12. Galvin JA, Fishman GA, Stone EM, Koenekoop RK. Evaluation of genotype-phenotype associations in Leber congenital amaurosis. *Retina*. 2005;25:919–929.
13. Galvin JA, Fishman GA, Stone EM, Koenekoop RK. Clinical phenotypes in carriers of Leber congenital amaurosis mutations. *Ophthalmology*. 2005;112:349–356.
14. Marmor MF, Zrenner E. Standard for clinical electroretinography (1999 update). International Society for Clinical Electrophysiology of Vision. *Doc Ophthalmol.* 1998–99;97:143–156.
15. Barthelmes D, Sutter FK, Kurz-Levin MM, et al. Quantitative analysis of OCT characteristics in patients with achromatopsia and blue-cone monochromatism. *Invest Ophthalmol Vis Sci*. 2006;47: 1161–1166.
16. Jacobson SG, Cideciyan AV, Aleman TS, et al. Crumbs homolog 1 (CRB1) mutations result in a thick human retina with abnormal lamination. *Hum Mol Genet*. 2003;12:1073–1078.
17. Jacobson SG, Cideciyan AV, Aleman TS, et al. RDH12 and RPE65, visual cycle genes causing Leber congenital amaurosis, differ in disease expression. *Invest Ophthalmol Vis Sci*. 2007;48:332–338.
18. Antonarakis SE and the Nomenclature Working Group. Recommendations for a nomenclature system for human gene mutations. *Hum Mutat.* 1998;11:1–3.
19. Knowles JA, Shugart Y, Banerjee P, Gilliam TC, Lewis CA, Jacobson SG, Ott J. Identification of a locus, distinct from RDS-peripherin, for autosomal recessive retinitis pigmentosa on chromosome 6p. *Hum Mol Genet*. 1994;3:1401–1403.
20. Hagstrom SA, North MA, Nishina PM, Berson EL, Dryja TP. Recessive mutations in the gene encoding the tubby-like protein TULP1 in patients with retinitis pigmentosa. *Nat Genet*. 1998;18:174–176.
21. Banerjee P, Kleyn PW, Knowles JA, et al. TULP1 mutation in two extended Dominican kindreds with autosomal recessive retinitis pigmentosa. *Nature Genet*. 1998;18:177–179.
22. Gu S, Lennon A, Li Y, et al. Tubby-like protein-1 mutations in autosomal recessive retinitis pigmentosa. *Lancet*. 1998;351:1103–1104.
23. Ikeda S, He W, Ikeda A, Naggert JK, North MA, Nishina PM. Cell-specific expression of tubby gene family members (tub, Tulp1,2, and 3) in the retina. *Invest Ophthalmol Vis Sci*. 1999;40: 2706–2712.
24. Boggon TJ, Shan WS, Santagata S, Myers SC, Shapiro L. Implication of tubby proteins as transcription factors by structure-based functional analysis. *Science*. 1999;286:2119–2125.
25. Xi Q, Pauer GJ, Marmorstein AD, Crabb JW, Hagstrom SA. Tubby-like protein 1 (TULP1) interacts with F-actin in photoreceptor cells. *Invest Ophthalmol Vis Sci*. 2005;46:4754–4761.
26. Hagstrom SA, Adamian M, Scimeca M, Pawlyk BS, Yue G, Li T. A role for the Tubby-like protein 1 in rhodopsin transport. *Invest Ophthalmol Vis Sci*. 2001;42:1955–1962.
27. Kondo H, Qin M, Mizota A, et al. A homozygosity-based search for mutations in patients with autosomal recessive retinitis pigmentosa, using microsatellite markers. *Invest Ophthalmol Vis Sci*. 2004;45:4433–4439.
28. Paloma E, Hjelmqvist L, Bayés M, et al. *Invest Ophthalmol Vis Sci*. 2000;41:656–659.
29. Lewis CA, Batlle IR, Batlle KG, et al. Tubby-like protein 1 homozygous splice-site mutation causes early-onset severe retinal degeneration. *Invest Ophthalmol Vis Sci*. 1999;40:2106–2114.
30. Robson AG, El-Amir A, Bailey C, et al. Pattern ERG correlates of abnormal fundus autofluorescence in patients with retinitis pigmentosa and normal visual acuity. *Invest Ophthalmol Vis Sci*. 2003;44:3544–3550.
31. Scholl HPN, Chong NHV, Robson AG, Holder GE, Moore AT, Bird AC. Fundus autofluorescence in patients with Leber congenital amaurosis. *Invest Ophthalmol Vis Sci*. 2004;45:2747–2752.
32. Lorenz B, Wabbels B, Wegscheider E, Hamel CP, Drexler W, Preising MN. Lack of fundus autofluorescence to 488 nanometers from childhood on in patients with early-onset sever retinal dystrophy associated with mutations in RPE65. *Ophthalmology*. 2004; 111:1585–1594.