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

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**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

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**Leber congenital amaurosis (LCA)** is an autosomal recessive retinal degeneration first described by Theodore Leber in 1869 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 nyctagmus, 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. 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 keratocous 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)5 have been reported to be associated with LCA. LRET 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.3,11 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 VII5) and two other affected members (subjects VII2 and VII6). 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) were available only for 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) were available only for 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) were available only for the propositus (subject VII3) and two other affected members (subjects VI25 and VI26).

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/cgi-bin/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).
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 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 in most of the patients. None presented signs of keratoconus.

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 LRP 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-dependent manner. The youngest patient had no detectable maculopathy. Patients VI3, 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 (VI4). 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](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933442/ on 06/22/2017)
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at Visit (y)</th>
<th>Nystagmus</th>
<th>VA (OD, OS)</th>
<th>Goldman VF (Vic Stimulus) OD, OS</th>
<th>Refraction OD, OS (D)</th>
<th>Optic Disc</th>
<th>Anterior Segment</th>
<th>Macula</th>
<th>Vessels</th>
<th>Periphery</th>
</tr>
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<tbody>
<tr>
<td>VII4</td>
<td>3</td>
<td>Intermittent F + F</td>
<td>Not measurable</td>
<td>C0 50 × 180° C2 0 × 180°</td>
<td>Tilted optic discs + atrophy of temporal sector</td>
<td>Normal</td>
<td>Unremarkable</td>
<td>Normal</td>
<td>No lesions detected</td>
<td></td>
</tr>
<tr>
<td>VII3</td>
<td>8</td>
<td>Horizontal-rotational 20/500 20/250</td>
<td>Approximately 60° H, 40° V in both eyes</td>
<td>S + 1.50 C 2.50 × 10° S + 1.50 C 3.0 × 160°</td>
<td>Healthy-colored disc</td>
<td>Normal</td>
<td>Indistinct foveal reflex; no deposits</td>
<td>Slightly attenuated</td>
<td>Few pigment clumps, shiny white spots at mid periphery; discrete diffuse RPE mottling, salt-and-pepper appearance</td>
<td></td>
</tr>
<tr>
<td>VII10</td>
<td>20</td>
<td>Rotational CF CF</td>
<td>Not measurable</td>
<td>S + 2.50 S + 3.00</td>
<td>Healthy-colored disc</td>
<td>Normal</td>
<td>Indistinct foveal reflex; no deposits</td>
<td>Attenuated</td>
<td>Multiple prominent osteoblasts; pronounced pigment migration around vessels</td>
<td></td>
</tr>
<tr>
<td>VII9</td>
<td>26</td>
<td>Horizontal HM PL</td>
<td>Not measurable</td>
<td>Not measurable Healthy-colored disc</td>
<td>Normal</td>
<td>Indistinct foveal reflex; no deposits</td>
<td>Very attenuated, few ghost vessels</td>
<td>Multiple pigment clumps, shiny white spots at mid periphery; pronounced pigment migration around vessels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI126</td>
<td>33</td>
<td>Rotational 20/1000 HM</td>
<td>15° H, 15° V 10° H, 10° V</td>
<td>S5 C 2 × 180° S6 C 2 × 180°</td>
<td>Waxy pallor; peripapillary RPE atrophy</td>
<td>Posterior subcapsular cataract</td>
<td>Annulus of yellow pinpoint perifoveal deposits</td>
<td>Severely attenuated; Multiple completely fibrosed arterioles</td>
<td>Discrete diffuse RPE mottling</td>
<td></td>
</tr>
<tr>
<td>VI125</td>
<td>37</td>
<td>Vertical 20/1000 20/1000</td>
<td>25° H, 20° V 40° H, 20° V</td>
<td>S3 C 2 × 180° S2 C 3 × 180°</td>
<td>Pale optic disc with peripapillary RPE atrophy</td>
<td>Posterior subcapsular cataract</td>
<td>Annulus of yellow pinpoint perifoveal deposits</td>
<td>Very attenuated</td>
<td>Few pigment clumps, shiny white spots at mid periphery</td>
<td></td>
</tr>
<tr>
<td>VI124</td>
<td>44</td>
<td>Horizontal CF HM</td>
<td>Not measurable</td>
<td>S3 C 1 × 180°</td>
<td>Mild disc pallor; pronounced peripapillary RPE atrophy</td>
<td>Posterior subcapsular cataract, dense nuclear sclerosis OD</td>
<td>Well-defined geographic atrophy (diameter 300 μm); no deposits</td>
<td>Very attenuated; Sclerosis of large choroidal vessels</td>
<td>Areas of accentuated retinal mottling; pronounced chorioretinal atrophy along the temporal arcades</td>
<td></td>
</tr>
</tbody>
</table>

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.
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 1594dupT-TGCC (HSTULPS01-5, Retina International Mutation Database www.retina-international.org), was identified in exon 15 (Figs. 5a, 5b). 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.
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.

A previous linkage analysis in a large Dominican pedigree 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.

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

TULP1 appears to be a rare cause of LCA, as mutations of this gene are reported in only 1.7% of patients with LCA. 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), five splice-site mutations (c.99-1G>A, IVS2DS G-A, IVS14-6C-A, IVS4-2delAGA, and IVS14DS G-A+1), one nonsense mutation (Glu402X), and one frame-shift deletion (c.957delC). 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.
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 is compatible with the findings in a reported Dominican kindred, in which such carriers were also found to have normal ERGs.29 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 vision30 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. 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— for example, in differentiating from RPE65 mutations—as they have been found to
lead to lack of FAF.

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.

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References


