Evaluation of the **ELOVL4** gene in patients with autosomal recessive retinitis pigmentosa and Leber congenital amaurosis

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**Purpose:** To determine whether pathogenic mutations exist in the **ELOVL4** gene in patients with inherited retinal degenerations other than Stargardt-like macular dystrophy or other hereditary macular degenerations.

**Methods:** All six exons comprising the open reading frame of the **ELOVL4** gene were evaluated by single-strand conformation analysis, direct nucleotide sequencing, or both methods.

**Results:** No pathogenic mutations were found among 84 patients with autosomal recessive retinitis pigmentosa or among 51 patients with Leber congenital amaurosis (congenital retinal blindness).

**Conclusions:** These data support the conclusion that recessive retinitis pigmentosa and Leber congenital amaurosis are rarely if ever associated with changes in the **ELOVL4** gene.

Inherited retinopathies are a group of retinal diseases that are clinically and genetically very heterogeneous. Most are characterized by the progressive degeneration of rod and cone photoreceptors, a phenomenon that ultimately leads to severe visual impairment and blindness. For the most common form of retinal dystrophy, retinitis pigmentosa (RP), rod loss exceeds cone loss in the early stages, and there is a progressive constriction of the visual field from the periphery [1]. Conversely, in cone-rod dystrophy, cone loss far exceeds rod loss, leading to impaired color vision and progressive deterioration of central visual acuity, followed by an eventual loss of peripheral visual field as well [2]. In hereditary macular degenerations, cone and rod photoreceptors are lost in the macula, resulting in loss of central visual field and visual acuity [3]. Another example of an inherited retinopathy is Leber congenital amaurosis (LCA), in which the loss of both rods and cones is particularly severe across the entire retina, leading to blindness or markedly reduced vision within the first few months of life [4].

Different forms of retinal degeneration are typically caused by mutations in different genes. However, there are some genes (e.g., **CRX** [5], **RPE65** [6], **CRB1** [7-9], **USH2A** [10,11], etc.) for which different mutations result in different phenotypes.

Mutations in **ELOVL4**, a gene possibly involved in the elongation of very long chain fatty acids, have been recently associated with various forms of dominant macular dystrophy, including autosomal dominant Stargardt disease-3 (**STGD3**, OMIM 600110), autosomal dominant macular dystrophy linked to chromosome 6 (adMD; OMIM 600110) [12,13], and a form of macular dystrophy with a wide range of clinical expression [14]. So far, only two mutations have been identified, both of which are frameshifts in the terminal exon [12-14]. Both alleles cause a premature termination of the reading frame and, since they occur within the last exon, likely lead to the production of a truncated protein having a dominant-negative effect, rather than leading to an unstable, and thus untranslated, mRNA [15].

Because of the extremely narrow range of mutations so far detected and in particular the absence of null alleles, we investigated the **ELOVL4** open reading frame and splice donor and acceptor sites for the presence of other mutations in patients affected with LCA and autosomal recessive RP in search of a possible correlation between different mutations and retinal phenotypes other than macular degeneration.

**METHODS**

This study involved human subjects and conformed to the Declaration of Helsinki. Leukocyte DNA was extracted from peripheral blood of 84 unrelated patients with autosomal recessive retinitis pigmentosa and 51 unrelated patients with Leber congenital amaurosis. Patients with autosomal recessive RP (arRP) included in this study were from families with multiple affected siblings and no previous generations had RP or LCA by history or were the offspring of a consanguineous mating. Patients with LCA came from families exhibiting a recessive inheritance pattern. All six exons of the **ELOVL4** gene and the immediate intronic sequences were amplified by the polymerase chain reaction (PCR) and evaluated by single strand conformation analysis, direct nucleotide sequencing, or both, by using either the primers reported in Table 1 or those previously reported [12].
**RESULTS**

The mutation analysis revealed the presence of the missense change Met299Val (ATG to GTG) in 16 patients with recessive RP (15 heterozygotes and 1 homozygote) and 12 patients with LCA (all heterozygotes). One patient with recessive RP carried heterozygously the missense change Ile267Thr (ATT to ACT), and one patient with LCA was found to carry the intron change IVS3 -18 C to T. All of these changes were previously reported and are interpreted as benign polymorphisms [16], since their allelic frequency does not differ significantly between patients and unaffected controls (Table 2).

**DISCUSSION**

ELOVL4 lies in a region of chromosome 6 that contains at least two other loci that have been associated with retinal degeneration: RP25 (recessive RP) [17,18], and LCA5 (recessive Leber congenital amaurosis) [19]. In a recent report, ELOVL4 was ruled out as the RP25 locus [20]. In the present paper, we could not provide any evidence that ELOVL4 is responsible for any of the inherited retinal conditions present in our set of patients. Our results make it unlikely that ELOVL4 corresponds to the actual LCA5 locus or that it accounts for large proportions of cases of arRP or LCA. To be precise, we used the binomial distribution to calculate the likelihood of our results (finding no cases with ELOVL4 mutations) if ELOVL4 caused a small percentage of arRP or LCA. Assuming that the frequency of ELOVL4 cases is \( f \), then the chance of finding no ELOVL4 cases among a set of \( N \) patients is \( (1-f)^N \). Based on the numbers of patients screened, we would have had more than a 95% chance of finding at least one ELOVL4 case if ELOVL4 caused 4% or more of arRP or 6% or more of LCA.

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**REFERENCES**


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