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Evaluation of the *ELOVL4* gene in families with retinitis pigmentosa linked to the *RP25* locus

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EDITOR—Retinitis pigmentosa (RP) is the most common form of retinal dystrophy. Patients present with night blindness and progressive narrowing of the visual field, eventually leading to central vision loss. Fundus examination usually shows bone spicula pigmentation, attenuation of blood vessels in the retina, and waxy pallor of the optic disc. Typically, the electroretinogram is notably diminished or even abolished.¹

RP shows important allelic and non-allelic genetic heterogeneity (RET-GEN-NET) with different modes of inheritance, including autosomal dominant (AD), autosomal recessive (AR), X linked, and digenic.

ARRP is the most common form of RP. A locus for ARRP, *RP25*, was mapped in 1998 to the long arm of chromosome 6 between microsatellite markers D6S257 and D6S1644 (MIM 602772).² Recently, we have excluded two candidates, *GABRR1* and *GABRR2*, as the disease causing gene.³

Several loci with retinal dystrophy phenotypes have been mapped to the pericentromeric region of chromosome 6. They include autosomal dominant Stargardt-like disease (*STGD3*),⁴ autosomal dominant macular atrophy (*ADMD*),⁵ autosomal dominant cone-rod dystrophy (*CORD7*),⁶ and Leber congenital amaurosis (*LCA5*).⁷

Recently, the gene responsible for *STGD3* and *ADMD* has been identified.⁸ All affected members in four independent families with *STGD3* and one family with *ADMD* shared a common founder haplotype, indicating a single

ancestral disease specific mutation. A single 5 bp deletion of a novel gene called *ELOVL4* was identified, which segregates with all affected members of the *STGD3* and *ADMD* families. *ELOVL4* shows cone and rod photoreceptor expression in the eye, is composed of six exons, and encodes a putative transmembrane protein of 314aa with similarities to the ELO family of proteins involved in elongation of very long chain fatty acids. Based on its similarity, it has been suggested that this protein is involved in synthesis of polyunsaturated fatty acids (PUFA) in the retina, such as DHA (docosahexaenoic acid). DHA represents 50% of PUFA in the outer segment of the photoreceptor cells and plays a crucial role in photoreceptor cell functions.

It is well known that one gene can cause distinct disease phenotypes with retinal degeneration.⁹⁻¹⁹ For example, *ABCR* (also called *ABCA4*), the gene responsible for recessive Stargardt macular dystrophy (*STGD1*), can cause either ARRP or autosomal recessive cone-rod dystrophy.¹⁷⁻²⁰⁻²³

It has been known for a long time that the retina possesses unique properties in lipid metabolism. The high level of PUFA, such as DHA, in the photoreceptors is thought to form an essential lipid environment for the phototransduction function. In addition, DHA is known to be lower in the serum of patients with retinitis pigmentosa. This evidence suggests that lipid metabolism may play a role in the pathogenesis of RP.²⁴⁻²⁸

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Table 1 PCR amplification of individual exons of the *ELOVL4* gene

Exon	Forward primers 5'→3'	Reverse primers 5'→3'	bp	°C
1	*tctctctgggtctccgctt	†gagggaggccttaacattc	347	58
2	*agccacttgaggagtcagt	†tgatggtttacattctcatttt	452	64
3	*agcaatcggaatcatgataa	†ttttcacagactggggccta	306	58
4	*tccatgccttgatcattttgtg	†tccactgaacatatatgcaat	348	60
5	*acatctcagtggtctactgcc	†gaaaattgtctaaaatgacattgcac	291	66
6	*gaagatgccgatgtgttaaaag	†gtcaacaacagttaaggccca	376	62

All the primers had the universal M13 primers attached 5'.

*M13F: cgccagggtttccagtcacgac and †M13R: tttcacacaggaacagctatgac.

ELOVL4 maps within the critical *RP25* region and has a potential role in DHA synthesis. We therefore considered *ELOVL4* to be a good candidate for the *RP25* gene and performed mutational analysis of *ELOVL4* in RP families linked to this locus. Eight families with 18 patients were included in these studies.

Each exon of the *ELOVL4* gene was amplified from genomic DNA derived from the index patients of the eight families using intronic primers (table 1). These PCR products were purified, analysed by EMD (enzymatic mutation detection),²⁹ and automatically sequenced as previously described.³

Only two heterozygous variations were identified, both of which (IVS2-99T→C and M299V) have been previously described by Zhang *et al*⁸ as non-pathogenic polymorphisms. These variants have been found in one family linked to *RP25*. In this particular family, there are two patients, both heterozygous for IVS2-99T→C and M299V. However, we did not detect any pathogenic variation in the *ELOVL4* gene in the RP patients. Therefore, we excluded *ELOVL4* as the gene responsible for *RP25*. Thus, our data indicate that at least two different genes involved in retinal degeneration are located in this region of the long arm of chromosome 6.

In conclusion, we have conducted a mutational screen in *ELOVL4*, the gene responsible for STGD3 and ADMD in ARRP families linked to *RP25*. After direct molecular analysis of the coding sequence and the intron-exon boundaries of *ELOVL4*, we did not find any pathogenic variant. Our results indicate that the *ELOVL4* gene seems not to be involved in the pathogenesis of *RP25* and STGD3/ADMD and *RP25* are not allelic variants of the same gene. Nevertheless, a role for *ELOVL4* in other inherited forms of RP needs to be elucidated.

Data access: RET-GEN-NET, <http://www.sph.uth.tn.edu/Retnet/home.html> Online Mendelian Inherited in Man (OMIM), <http://www.ncbi.nlm.nih.gov/htbin-post/OMIM>

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PTEN mutations are uncommon in Proteus syndrome

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EDITOR—Proteus syndrome (MIM 176920) is a rare, congenital, hamartomatous disorder, which is a member of a group of local overgrowth diseases. Happle¹ proposed that some of these disorders are the result of the action of a lethal gene that can only survive in the mosaic state, which arises from an early somatic mutation or from a half chromatid mutation. Such a mechanism has been shown to be the underlying basis of McCune-Albright syndrome (MIM 174800).² One of the mandatory diagnostic criteria for Proteus syndrome is a mosaic distribution of lesions and sporadic occurrence, entirely consistent with Happle's hypothesis.

Currently, little is known about the molecular causes of Proteus syndrome. It is, however, likely that the overgrowth of tissue involves all germ layers. This may be because of hyperproliferation, an absence of appropriate apoptosis, or alternatively cellular hypertrophy. There have been few investigations into the molecular basis of Proteus syndrome. Zhou *et al*³ recently identified *PTEN* mutations in a patient with a Proteus-like syndrome. Germline *PTEN* mutations are found in a high proportion of patients with Cowden (MIM 158350) and Bannayan-Riley-Ruvalcaba (BRR) syndromes (MIM 153480),⁴⁻⁷ which share many features of Proteus syndrome. These observations make *PTEN* a strong candidate for a gene mutated in Proteus syndrome. To investigate this possibility, we examined eight patients with Proteus syndrome for *PTEN* mutations. All were unrelated and had classical Proteus syndrome using published diagnostic criteria.⁸ Samples were obtained with informed consent and local ethical review board approval. Fibroblasts were cultured from skin biopsies obtained from normal tissue and from regions of overgrowth. Genomic DNA was extracted from cultured cells using a standard sucrose lysis technique. *PTEN* mutational analysis was performed by PCR based conformational specific gel electrophoresis using published oligonucleotides⁹ and semi-automated sequencing using an ABI 377 Prism sequencer. A common exon 4 polymorphism was observed in three of the patients, but no missense or truncating mutations in any of the eight samples were detected, suggesting

that mutation in *PTEN* is unlikely to be a common cause of Proteus syndrome.

We evaluated *PTEN* as a candidate gene because of its role in the overgrowth syndrome Cowden disease and the recent report of a *PTEN* mutation in a boy with Proteus-like syndrome.³ *PTEN* plays a role in the regulation of PI3 kinase signalling, which is involved in the control of apoptosis and cell cycle progression.¹⁰ Hence, by removing the regulatory effects of *PTEN* on PI3 kinase signalling, deregulated cellular growth could occur. *PTEN* also appears to play a role in the regulation of cell size and a role for the PI3 kinase signalling pathway in the determination of organ size in mammals has been reported.¹¹ The boy reported by Zhou *et al*³ with Proteus-like syndrome had a germline single base transversion resulting in an Arg 335 to Ter substitution in *PTEN*. A second *PTEN* mutation resulting in Arg 130 to Ter was found in DNA from a naevus, lipoma, and an arteriovenous mass. The authors postulated that the first germline mutation gave rise to many of the features of BRR and that the second hit occurred early in embryogenesis causing mosaicism. In our study we did not detect *PTEN* mutations in any of the Proteus syndrome patients we examined. Zhou *et al*³ similarly failed to detect any *PTEN* mutations in five patients with classical Proteus syndrome; their patient with *PTEN* mutations did not fulfil the stringent diagnostic criteria for Proteus syndrome.

Mutations in the coding region of *PTEN* do not appear to be implicated in classical Proteus syndrome. *PTEN* may still be involved, as our finding does not preclude the possibility that it may be aberrantly imprinted in Proteus syndrome, for example by promoter methylation,¹² leading to reduced *PTEN* expression. Given the innumerable possibilities for a molecular basis of Proteus syndrome, the identification of which genes are disrupted will prove difficult. One strategy for dissecting the molecular pathways of Proteus and other overgrowth syndromes is through examining the expression patterns of genes in affected and unaffected tissues, which is becoming feasible with the advent of microarray technology.¹³

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