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Different Functional Outcome of RetGC1 and RPE65 Gene Mutations in Leber Congenital Amaurosis

To the Editor:

Leber congenital amaurosis (LCA) has the earliest age at onset and is the most severe of the inherited retinal dystrophies (Leber 1869). It accounts for ⩾5% of all retinal dystrophies and probably accounts for a higher percentage in countries with a high rate of consanguinity (Foxman et al. 1985; Kaplan et al. 1990). LCA is an autosomal recessive condition, distinct from all other retinopathies in that the visual disorder is diagnosed at birth or during the first months of life in an infant with total blindness or greatly impaired vision, normal fundus, and extinguished electroretinogram (ERG; Franceschetti and Dieterle 1954). Although major visual impairment is easily recognizable at birth, LCA was largely underdiagnosed until ERG performed on infants showed that the condition is not uncommon. A certain degree of clinical heterogeneity has long been recognized in LCA (Merin 1991; Trabousli et al. 1995); however, genetic heterogeneity of LCA has been suspected since the report by Waardenburg et al. (1963) of normal-sighted children born to parents who were both affected with LCA.

We mapped the first LCA gene (LCA1) on the short arm of chromosome 17p13.1 and confirmed the genetic heterogeneity of the disease (Camuzat et al. 1995, 1996). Perrault et al. (1996) ascribed LCA1 to mutations in the photoreceptor-specific guanylate cyclase gene, RetGC1, which catalyses the conversion of guanine triphosphate to cyclic guanine monophosphate (cGMP) in the retina. To date, a total of 18 different mutations have been found in 20 unrelated families originating from various countries across the world, especially the Mediterranean region. The observation of missense and frameshift RetGC1 mutations suggests that the cGMP production in photoreceptor cells is markedly reduced or abolished in LCA (Perrault et al. 1996). As a consequence, the excitation process of rod and cone photoreceptors would be markedly impaired because of constant closure of cGMP-gated cation channels, with hyperpolarization of

Table 1

***RPE65* Gene Mutations in Patients in the LCA2 Group**

Family Number and Location of Mutation	Base Change	Amino Acid Change	Comment	Conservation across Mouse and Human
1:				
Exon 9	G1043A	C330Y	Homozygous	+
2:				
Intron 1	G/A	65+5G/A	Homozygous	−
3:				
Exon 3	C244T	Q64X	Homozygous	+
4:				
Intron 8	G/A	912+1G/A		−
5:				
Exon 12	C1355T	A434V		+
6:				
Exon 10	DelA	1114DelA		+
Exon 7	C754T	R234X		+
7:				
Exon 10	C1141A	P363T		+
Exon 13	T1472A	V473N		+

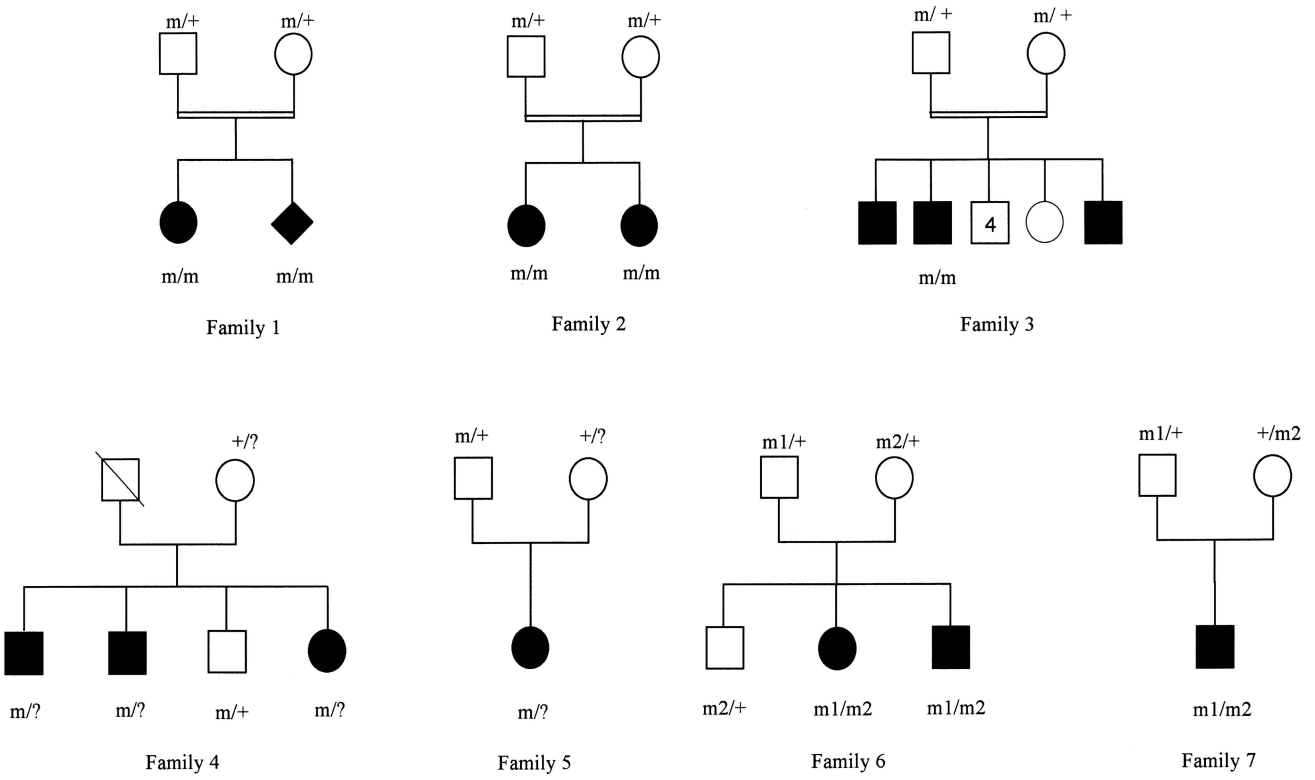

Figure 1 Segregation of RPE65 mutations in seven families in group LCA2. An "m" denotes a mutant allele; a plus sign (+) denotes a wild-type allele; and a question mark (?) denotes a second mutation not yet identified.

Table 2

Phenotypic Differences in the LCA1 and LCA2 Groups

Characteristic	LCA1	LCA2
Disease gene	*RetGC1*	*RPE65*
Age at onset	Birth	Birth
Mode of onset	Inability to follow light or items	Inability to follow light or items
	Roving eye movements	Roving eye movements
	Pendular nystagmus	Pendular nystagmus
	Normal fundus at birth followed by salt-and-pepper aspect of the retina and typical aspect of RP[a]	Normal fundus at birth followed by salt-and-pepper aspect of the retina and typical aspect of RP[a]
Ophthalmologic examination	Nonrecordable ERG (never done before 3 mo)	Nonrecordable ERG (never done before 3 mo)
	Severe hyperopia	Moderate or no hyperopia, sometimes low myopia
	Nonrecordable visual field	Concentric reduction of visual field
Outcome	Nonevolutive congenital blindness	Transient improvement: ability to follow light or large items, especially during daytime
	Severe photophobia	Night blindness
Visual acuity	Light perception or finger counting	6/60–6/30

[a] RP = retinitis pigmentosa.

the plasma membrane. Indeed, the cGMP concentration in photoreceptor cells could not be restored to the dark level, leading to a situation equivalent to constant light exposure during photoreceptor development. This hypothesis is consistent with the markedly impaired vision noted in the first days of life.

Another gene, the retinal pigment epithelium (RPE)–specific gene (RPE65), mapped to chromosome 1p31, has been found to carry deleterious mutations in patients with LCA (Marlhens et al. 1997; Morimura et al. 1998) and autosomal recessive childhood-onset severe retinal dystrophies (CSRD; Gu et al. 1997). RPE65 is believed to act as the isomerase catalyzing the conversion of all-*trans*-retinyl-ester to 11-*cis*-retinol in the RPE, an essential step in the metabolism of vitamin A, the precursor of rhodopsin (Hamel et al. 1998). Thus, in contrast with RetGC1 mutations, RPE65 mutations would decrease the rhodopsin production, leading to a situation equivalent to a retina kept in a constant dark state. Hence, these cases of Leber amaurosis would represent the extreme end of a spectrum of diseases classified as retinal dystrophies (Morimura et al. 1998).

We have studied a series of 15 multiplex families with LCA unlinked to chromosome 17p13.1, using polymorphic markers flanking the RPE65 locus (Gu et al. 1997). Combination of all findings for the 15 families gave no significant LOD scores (data not shown, available on request), but 8 of 15 families were consistent with linkage to 1p31. For this reason, we did a search for RPE65 gene mutations by SSCP analysis and direct sequencing in the eight families with linkage to 1p31, as well as in sporadic cases (Marlhens et al. 1997). RPE65 gene mutations were identified in five of eight multiplex families with linkage to 1p31 and in two families with sporadic cases (fig. 1). All missense mutations involved conserved amino acids and were absent in 50 healthy controls. Three patients born to consanguineous parents carried homozygous RPE65 gene mutations (table 1).

Because there are different pathophysiologic pathways involved in the disease, the patients with LCA were split into two groups, LCA1 and LCA2, on the basis of their underlying mutations, and their clinical histories were revisited. No difference in age or mode of onset was observed between the RetGC1 (LCA1) and the RPE65 (LCA2) groups. Indeed, symptoms of LCA always began at birth or in the first few weeks of life and were characterized by roving eye movements and inability to follow light or objects. The results of fundus examination in the two groups were quite similar as well. There was an initially normal fundus, which gradually displayed a salt-and-pepper appearance with a reduction in blood-vessel diameter and often the typical appearance of retinitis pigmentosa. Moreover, the ERG response was totally extinguished, even at age 3 mo.

Despite the similarities listed above, outcome of the disease was different for each of the groups. Indeed, in the patients harboring RetGC1 mutations, no visual improvement was observed, the pendular nystagmus remained unchanged, and visual acuity was reduced to light perception or ability to count fingers held in the visual field. In addition, the patients complained of severe photophobia and usually preferred half light. A significant hyperopia was observed consistently, and the visual field was not recordable because of profound loss of visual acuity (table 2). However, in the patients harboring RPE65 mutations, transient improvement was regularly noted by the parents. Young children became able to follow light or objects, especially during daytime. They complained of night blindness and usually preferred bright light. Visual acuity reached 6/60–6/30, mild or no hyperopia was observed, and mild myopia occured occasionally. Finally, the visual field in this group was usually recordable and frequently displayed a peripheric concentric reduction (table 2).

In conclusion, the present study supports variable functional outcome in LCA, depending on the disease-causing gene. RetGC1 gene mutations are responsible for a congenital cone-rod dystrophy with dramatic and invariable cone dysfunction. RPE65 gene mutations are responsible for a severe yet progressive rod-cone dystrophy, still different from the congenital stationary blindness caused by RetGC1 gene mutations. These discrepancies are of particular importance for anticipating the final outcome in a blind infant and for directing further genetic studies in older patients.

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The *APC* I1307K Allele and *BRCA*-Associated Ovarian Cancer Risk

To the Editor:

Most ovarian cancers attributable to autosomal dominant genetic predisposition (~10% of all cases) are associated with germ-line mutations in the *BRCA1* or *BRCA2* genes (reviewed in Boyd 1998). Estimates of the lifetime probability of developing ovarian cancer in association with a *BRCA* mutation have a range of 16%–63% (Easton et al. 1995; Struewing et al. 1997; Ford et al. 1998). This large variation in penetrance is widely presumed to reflect the effects of various hormonal, environmental, and genetic modifiers, but few such modifying factors have been identified. The use of oral contraceptives was recently shown to substantially reduce the risk of ovarian cancer in women with *BRCA* mutations (Narod et al. 1998), yet it has been suggested that bearing more offspring increases ovarian cancer risk in *BRCA1* carriers (Narod et al. 1995). The only genetic modifier of *BRCA* penetrance yet shown is the *HRAS1* locus, rare alleles of which are associated with an increased risk of ovarian cancer in *BRCA1* carriers (Phelan et al. 1996).

The *APC* I1307K allele is a plausible candidate modifier of *BRCA* penetrance. First identified as a founder mutation occurring in ~6% of the Ashkenazi Jewish population, the allele is present in a significantly higher proportion of Jewish colorectal cancer patients and in those with a family history of colorectal cancer (Laken et al. 1997). The mechanism through which this allele contributes to the development of colorectal cancer appears to involve the creation of a small hypermutable region that undergoes somatic frameshift alterations leading to *APC* inactivation and the initiation of tumorigenesis (Laken et al. 1997; Gryfe et al. 1998). Consistent with this molecular genetic scenario are the well-established roles of somatic *APC* mutations in the initiation of sporadic colorectal cancer and germ-line *APC* mutations in predisposition to familial adenomatous polyposis (Kinzler and Vogelstein 1996).

Attempts to confirm and extend the original observation of *APC* I1307K-associated cancer risk in Ashkenazi Jews have produced inconsistent findings. Results from one follow-up study implied that the *APC* I1307K mutation alone does not significantly increase the risk of colorectal cancer (Petrukhin et al. 1997). Recent data from a large community-based study of Ashkenazi Jews indicated that *APC* I1307K confers a modest but significant risk of cancer in general but that odds ratios for any particular cancer are not increased to statistically significant levels (Woodage et al. 1998). Remarkably, however, there is an apparent synergy between *APC* I1307K and a mutant *BRCA* allele in relation to breast cancer risk (Redston et al. 1998). Taken together, these data suggest that *APC* I1307K may function as a low-penetrance modifier of cancer risk in association with high-penetrance cancer-predisposition alleles such as *BRCA1* or *BRCA2*. Thus, even though *APC* I1307K alone does not appear to confer a substantial risk of