Letters to the Editor

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A nonsense mutation in the retinal specific guanylate cyclase gene is the cause of Leber congenital amaurosis in a large inbred kindred from Jordan

EDITOR—Leber congenital amaurosis (LCA) (MIM 204000) has the earliest onset and is the most severe form of retinal dystrophy.[1–3] It is an autosomal recessive condition that is recognised within the first few months of life because of impaired vision and an extinguished electroretinogram.[4] Nystagmus, specifically pendular, and eye poking are frequently observed early on,[5] while hypermetropia and keratoconus may develop later during the course of the disease.[6 7] Genetic heterogeneity was confirmed when the first gene of LCA was mapped to chromosome 17p13.1 (*LCA1*) by homozygosity mapping in consanguineous Arab families.[8 9] Four different mutations in the retinal specific guanylate cyclase gene (*RETGC*) were found in four unrelated probands and thus *LCA1* was assumed to result from homozygous alterations in this gene.[10]

We report here a nonsense mutation in the *RETGC* gene, which in the homozygous state is responsible for LCA in a large inbred tribe from Jordan. We had already identified a large, highly inbred family from the Jordan valley consisting of about 2000 living subjects, in which affected members have LCA.[11] A 31 member subset of this family was investigated (fig 1). All members were examined by an ophthalmologist and a paediatrician. Four patients had ERG performed (Nos 3, 9, 13, 14). Blood samples were collected from 28 family members after obtaining informed consent from them or their legal guardian.

DNA was extracted from peripheral blood samples by standard procedures.[12] Seventeen different dinucleotide repeat markers reported to be linked to *LCA1* on chromo-

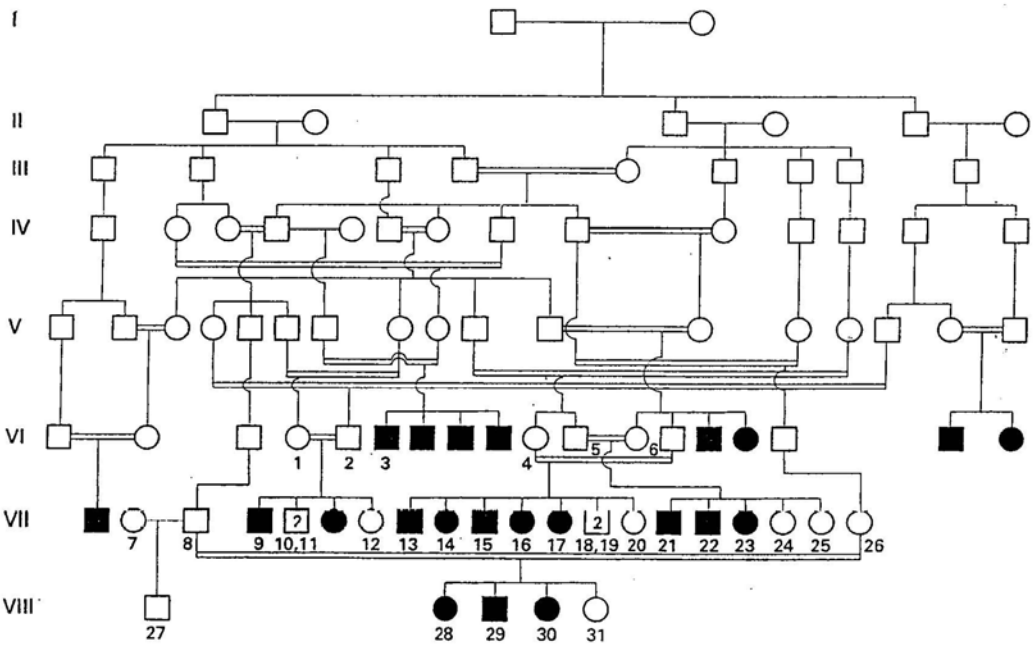

Figure 1 Extended partial pedigree of the clan. Note the extensive number of inbreeding loops in every generation. The 31 participating family members are marked in Arabic numerals.

Table 1 The main clinical manifestations of Leber congenital amaurosis in the 13 affected subjects

ID	Age/sex	Visual acuity: Right	Visual acuity: Left	Keratoconus	Retinal vessels	Optic discs	Remarks
3	45 y/M	No LP	No LP	Yes	Attenuated	Pale	Corneal hydrops
9	22 y/M	HM	HM	Yes	Attenuated	Pale	
13	14 y/M	No LP	No LP	Yes	Attenuated	Normal	Corneal hydrops
14	15 y/F	HM	HM	Yes	Attenuated	Normal	
15	19 y/M	6/60	5/60	Yes	Attenuated	Normal	
16	20 y/F	LP	LP		Microphthalmia	Not seen	Iris atrophy
17	23 y/F	HM	HM	Yes	Attenuated	Pale	Macular lesions
21	15 y/M	HM	HM	No	Attenuated	Normal	
22	13 y/M	HM	HM	Yes	Attenuated	Normal	
23	10 y/F	CF	CF	No	Attenuated	Normal	
28	11 y/F	No LP	LP	No	Not seen	Not seen	Cataracts
29	13 y/M	LP	LP	Yes	Not seen	Not seen	Cataracts
30	16 y/F	LP	LP	No	Attenuated	Normal	

M: male; F: female; LP: light perception; HM: hand movement; CF: counting fingers.

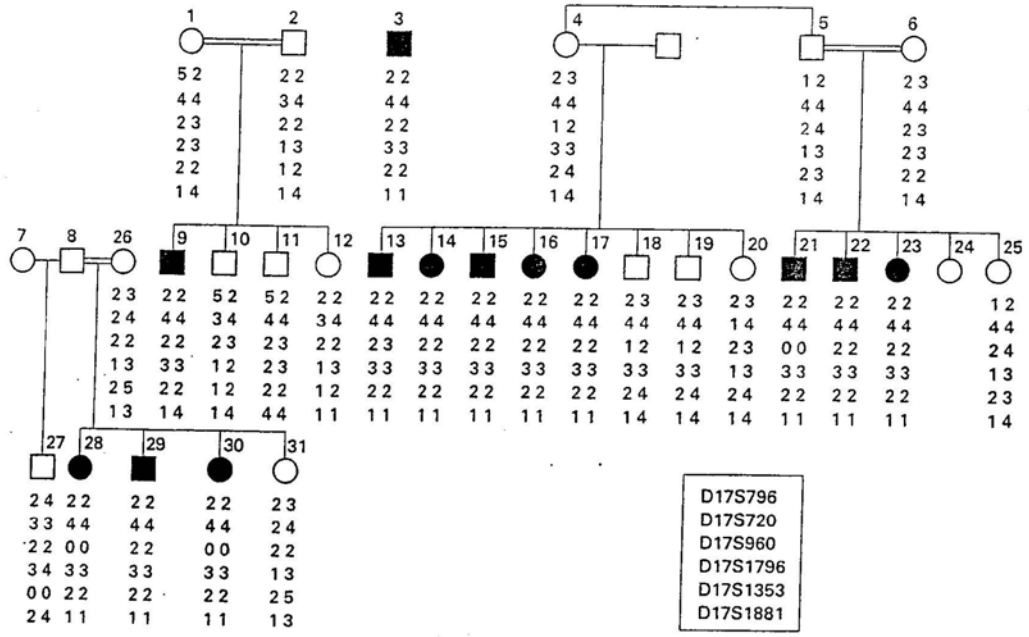

Figure 2 Haplotypes of six selected dinucleotide markers within the region of linkage on 17p13.1. The markers are arranged from telomere to centromere. The marker names are shown in the closed box. The affected subjects are homozygous for the extent of the haplotype.

some 17 were used to test for linkage.[8 9] Amplification of these markers was performed according to the manufacturer's conditions (Research Genetics). Products were analysed on 6% denaturing polyacrylamide gels (7.7 mol/l urea). The polyacrylamide gels were silver stained using the protocol of Bassam *et al.*[13] Haplotype analysis was performed and the obligatory cross over events were noted. Since the family was highly inbred, identity by descent was enough to establish linkage.

The 20 exons of the *RETGC* gene were amplified using intronic primers flanking exon sequences using the previously reported conditions.[10] When necessary, the fragments were digested by one or more restriction endonucleases to yield fragments suitable for SSCP analysis. Amplified fragments were run on a fan cooled MDE gel for SSCP analysis at 6 W for 14 hours and then silver stained.

DNA from regions of the *RETGC* gene that showed unusual mobility of one allele in carriers and of the two alleles in affected subjects was sequenced using an automated ABI sequencer with dye terminator chemistry.

The reported extended family is a 2000 member tribe inhabiting a village in the Jordan valley, mostly depending on agricultural resources. The successive consanguineous marriages led to an extreme example of inbreeding and is reflected in the high prevalence of blindness which turned out to be LCA. The inbreeding coefficient in this tribe ranged from 0.037 to 0.09374 with an average of 0.0687.

The subset of the family included 13 affected subjects, their ages ranging from 10 to 45 years at the time of examination. All patients had poor vision noted at birth or shortly afterwards, as well as wandering eyes or pendular nystagmus. The visual acuity ranged from no light perception to 6/60. The majority had attenuated retinal blood vessels on fundus examination, some had pale optic discs, and about two thirds had keratoconus. Two patients had congenital cataracts (Nos 28 and 29) and one patient had bilateral microphthalmia and iris atrophy (No 16). One patient had bilateral macular abnormality similar to target macular lesions (no 17). Extinguished ERG was present in the four patients who underwent the test. The details of the clinical picture in the 13 affected subjects are summarised in table 1.

By analysing the haplotypes, it is quite obvious that the LCA in this family is linked to the *LCA1* locus previously described. All affected members were identical by descent for the disease haplotype (fig 2).

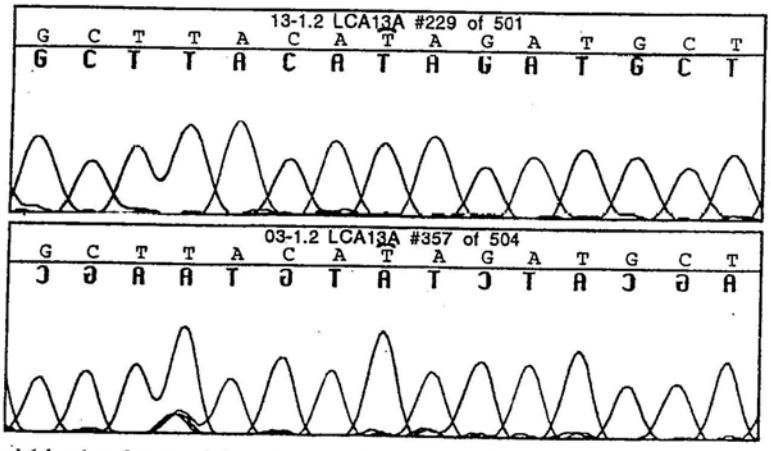

Figure 3 Sequence from forward (above) and reverse (below) directions. The mutation is homozygous and lies within exon 13. The change is a T (TAG=stop) in place of C (CAG=Gln). The T is flanked by As and is marked with a cap.

The SSCP assay of exon 13 showed a homozygous pattern in affected subjects and a heterozygous pattern in obligate carriers. This pattern was not present in 10 unrelated controls. The DNA sequencing in all affected members showed a homozygous Gln to stop mutation in exon 13 at nucleotide position 2646 (cDNA) (CAG→TAG) (fig 3). Obligate carriers were heterozygous for this nonsense mutation.

Despite being homozygous for the same mutation, affected family members showed clinical heterogeneity for symptoms and signs other than the impaired visual acuity and nystagmus. This suggests that other factors, possibly environmental, as well as genetic play a role in the variability in clinical expression of this monogenic disorder.

Since the mutations detected so far denote either profound instability of the protein or premature translation termination, it strongly suggested that LCA is the result of abolished production in cGMP in photoreceptor cells.[10] The mutation in our family produces premature termination in translation, which strengthens this suggestion. The presence of congenital cataracts and congenital microphthalmia in this family suggest that the *RETGC* gene may play a role in eye development in utero as well.

Linkage analysis in this family followed by the detection of the mutation provides us with a potent set of tools for carrier identification. This can be applied to premarital testing and counselling, which provides a socially acceptable solution to this problem in a large family with widely practised intermarriage. Prenatal diagnosis can also be provided to married couples who are known carriers.

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