

## CONGENITAL BLINDNESS: REPORT OF LEBER CONGENITAL AMAUROSIS IN A LARGE IRANIAN KINDRED

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### Abstract

**Background-**Leber congenital amaurosis (LCA) is a hereditary neonatal blindness. Congenital blindness is common among a specific branch of the Lore tribes in Kerman province, central Iran. This study was designed to identify all affected patients, construct a pedigree for obtaining the transmission pattern, establish definite diagnosis, and finally determine the genetic origin of the blindness among this tribe.

**Methods-**Using several field studies, over a period of 2 years, and conducting interviews with senior members of the tribe, a total of 25 patients were identified. Electrophysiological tests and karyotyping were undertaken for appropriate cases. DNA samples collected from a group of affected individuals and their first-degree relatives were used to evaluate genetic linkage to a number of known loci on different chromosomes.

**Results-**Autosomal recessive pattern of inheritance and neonatal visual impairment without any noticeable eye lesions was documented. Infantile nystagmus, keratoconus, narrowing of retinal vessels, retinal degeneration, mild pigmentary retinopathy and electrophysiological investigations were consistent with LCA. Only one locus on 17p13.1 was consistent with linkage in this kindred.

**Conclusion-**We made a large pedigree of LCA for the first time in Iran. Mutation screening of the responsible gene is currently in progress.

**Keywords** • Congenital blindness • Leber congenital amaurosis • retinopathy  
• 17p13.1 • GUCY2D mutation

### Introduction

Theodor Leber (1840-1917), a German ophthalmologist, described a familial neuro-ophthalmologic disease in the late 1860s<sup>1</sup>, which is now commonly referred to as Leber hereditary optic neuropathy (LHON). LHON presents in mid-life as an acute or subacute central vision loss leading to central scotoma and blindness. This is known to be a mitochondrial disorder caused by at least 18 different allelic mutations. In 1869, Leber also described another

condition as pigmentary retinopathy with congenital amaurosis.<sup>2</sup> He noted (1871) the familial nature of this disease and speculated on the role of consanguinity in the development of this particular eye condition.<sup>3</sup> This clinical entity is now known as Leber congenital amaurosis (LCA).<sup>4</sup> The LCA both clinically and genetically is a heterogeneous disease and transmitted as an autosomal recessive trait.<sup>3-6</sup> LCA is one of the most common (16-18%) causes of congenital blindness and visual impairment without any noticeable lesions that are recognized within the first few months after birth. Infantile nystagmus, sluggish pupillary responses, occasionally a paradoxical

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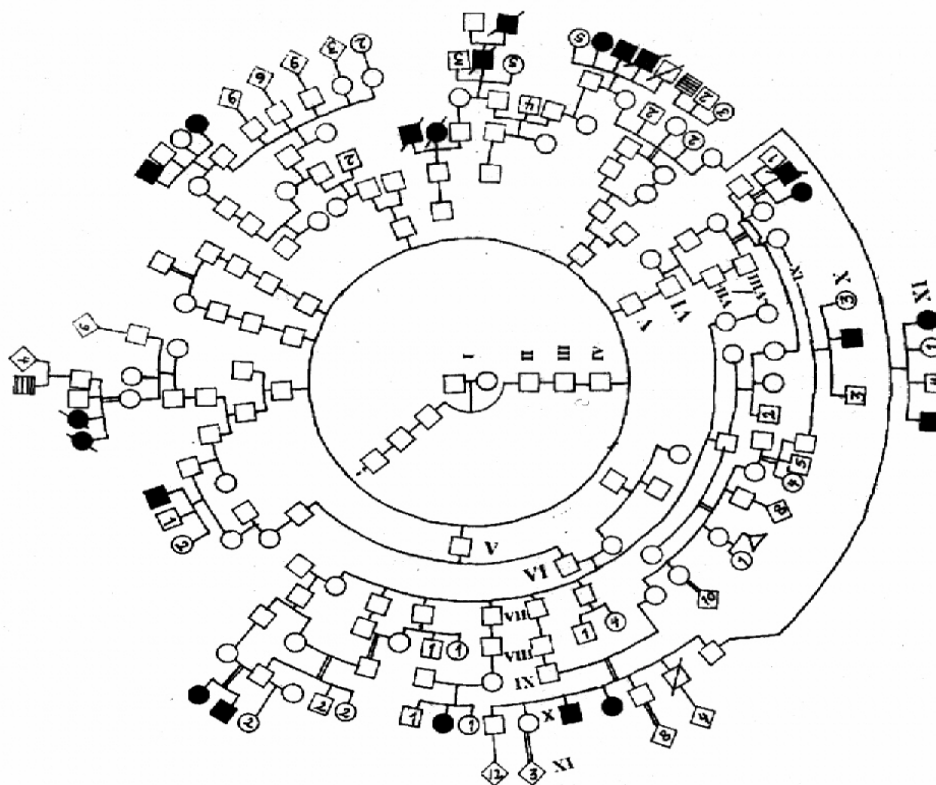
pupil response, and absent or poorly detectable electroretinographic response early in life are the cardinal manifestations of this condition.<sup>5-6</sup> Additional clinical presentations including symmetrical and mid-facial hypoplasia with enophthalmos and hypermetropic refractive errors have also been reported.<sup>4-6</sup> While there is a substantial interfamilial variation, intra-familial similarities are also evident.<sup>5-6</sup> Association of this condition with mental retardation and various neuropsychiatric disorders has also been reported.<sup>7-13</sup>

Nickel and Hoyt (1982) examined 31 patients with limited light perception. CT scan revealed cerebellar vermis hypoplasia in 3 cases.<sup>9</sup> Differentiation of the cerebellar vermis and the photoreceptor layer of the retina occur concurrently at 12 weeks of gestation.<sup>9</sup>

Congenital blindness is frequently observed among a specific branch of the Lore tribe in the rural areas (Kheyr Abad) of the city of Sirjan in Kerman. This tribe is often alluded to as the blind clan (*tayefeh-e-koorha*). Initial investigation

revealed that blindness in this tribe is hereditary and that the condition was transmitted as an autosomal recessive trait. We therefore designed a project with the following goals:

1. to track down all the affected patients within this tribe, and perform a complete clinical and ophthalmological examination to reach a definite clinical diagnosis,
2. to determine the hereditary pattern of inheritance,
3. to promote a molecular study to map and clone the defective gene,
4. to identify the exact nature of the causative mutation and study the biochemical structure of the normal and defective gene products within the eye as well as other target tissues,
5. to develop an accurate molecular detection and prenatal diagnostic test to prevent further recurrence of the disease among in families with a high probability of being a gene carrier,
6. to determine the genetic origin of this tribe and extend this study to other families in this tribe.



**Figure 1.** Pedigree of Leber congenital amaurosis showing 11 generations, 22 affected persons, 14 of whom are alive.

## Materials and Methods

Several field studies were undertaken over a period of 24 months. Senior members of the tribe were interviewed and a comprehensive pedigree was constructed which covered 11 generations. A total of 25 patients were identified in different branches of this kindred, 8 of whom had died before the beginning of the study (see Figure 1 for the pedigree). Fourteen out of 17 living patients aged between 8 and 48 years (mean 21.5 years) were carefully investigated by a clinician and ophthalmologist. Electrophysiological tests including visual evoked potentials (VEP) and electroretinogram (ERG) were carried out for two patients. Twelve affected cases were karyotyped for detection of chromosomal abnormalities. A total of 47 blood samples were collected and DNA was extracted using standard procedures. DNA samples of all affected patients and their first-degree relatives are currently being analyzed for determination of the defective gene sequence. Three patients did not participate in this study.



**Figure 2.** Facies and retinal view of case R.S. (ten-year-old male with visual impairment since neonatal period.)

## Results

Blindness was evident in early neonatal period (14/14), without any noticeable lesions. Infantile nystagmus (10/14), 4 bilateral and 2 unilateral keratoconus (6/14), 3 bilateral, and one right-sided leukoma (4/14), 3 bilateral, and one right-sided cataract (4/14) which appeared later in life were documented. Fundus visualization, which was, conducted in 12 cases, revealed narrowing of the retinal vessels (12/12) dispersed pigment deposition (4/12), white spot, and retinal degeneration (1/12) (Figure 2). Blindness was profound, ranging from 0.2 (one case), finger counting (4/14), hand movement (3/14), light perception (5/14), to complete blindness with no light perception (NLP) in one case. Intra-ocular pressure was normal in five out of six cases. Photophobia with better night vision was a common complaint. No history of seizure, mental retardation or myotonic weakness was noted. One patient and his sister showed moderate obesity and another patient had a mild atrial septal defect. These findings are summarized in Table 1.

Electrophysiological tests of the two patients revealed delay in P100 peak of visual-evoked potential (VEP) and fall in voltage of the b wave in electroretinogram (ERG), which are indicative of visual pathway and photoreceptor degeneration, respectively. Karyotyping of 12 affected subjects did not show any apparent chromosomal abnormalities.

## Discussion

Five different mutant genes have so far been identified for the LCA phenotype. The five corresponding genotypes are as follows<sup>6</sup>:

LCA type 1 (LCA1; online mendelian inheritance in men, OMIM#204000) maps to the 17p13<sup>1</sup> and is due to mutations in the retinal guanylate cyclase (GUCY2D; OMIM#600179) gene.<sup>14-16</sup> A second form of LCA (LCA2; OMIM#240100) maps to 1p31 and is caused by mutations in the retinal pigment epithelium-specific 65 KD protein (RPE65; OMIM#1800069).<sup>6</sup> The third locus for LCA (LCA3; OMIM#604232) maps to 14q24 but no mutation has been identified. Another form of this disorder is caused by mutation in the photoreceptor-specific homeobox gene CRX (OMIM#602225) on chromosome 19. Mutation in the AIPL1 gene (OMIM#604392), which, like

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Name	Sex	Age (year)	Nystag.		Kerato.		Vision		Cornea		Fundus		Lens		Intra-Ocular pressure		Retinal photography
			R	L	R	L	R	L	R	L	R	L	R	L	R	L	
R. Z.	F	8	-	-	+	+	NL										Mild degen.
R. S.	M	10	+	+	+	+	LP										RVN
M. S.	F	11	+	+	-	-	LP	LP	N	N	RVN	RVN	N	N	---	---	RVN
R. I.	M	12	+	+	-	-	CF 20 cm	CF 50 cm	N	N	RVN	RVN	N	N	---	---	RVN
P. F.	F	13	-	-	-	-	0.2	0.2	N	N	RVN, no reflex Mild hyper. pig.	RVN	N	N	N	N	RVN, mild hyper.pig.
R. M.	M	18	+	+	-	-	CF 2 m	CF 0.5 m	N	N	RVN	RVN	N	N	---	---	RVN
R. A.	M	20	+	+	-	-	CF	CF	N	N	RVN	RVN	N	N	---	---	RVN
P. A.	M	21	-	-	-	-	HM	HM	N	N	RVN, no reflex, hyper. pig.	RVN	N	N	N	N	RVN, hyper.pig.
S. M.	M	23	+	+	-	-	HM	HM	N	N	RVN	RVN	N	N	---	---	RVN
B. R.	F	23	+	+	-	-	CF	CF	Microcornea		RVN+hyper. pig.	RVN+hyper. pig.	N	N	High	High	RVN+hyper pig.
S. F.	M	24	+	+			LP	LP	N	N	Mild RVN Hyper. pig.	Hyper. pig.	Cat.	Cat.	High	N	RVN,hyper. Pig.
R. F.	F	25	+	+	+	+	LP	NLP	N	N	Reti. deg RPE, atrophy	Not seen	Cat. R.& L (operated)		---	---	---
R. M.	F	45	-	-	-	-	HM	HM	N	N	RVN No macular reflex	RVN	N	N	N	N	RVN
R. J.	M	48	+	+	+	+	LP	LP	Leuk. Staph.	N	Not seen	Not seen	Cataract		---	---	---

**Table 1. Ophthalmological findings.**

GUCY 2D, maps to 17q13.1 causes LCA4 (OMIM#604393).<sup>17</sup> A fifth Locus (LCA5; OMIM#604537) has been mapped to chromosome 6q11-q16 but no mutations have been reported.

We have already come to the conclusion that our patients are of LCA type 1. As mentioned previously, the LCA1 is caused by mutations in the retinal guanylate cyclase (GUCY2D) gene on chromosome 17p13.1.<sup>18</sup> Our preliminary molecular study indicated that this family is most probably linked to the LCA1 locus on 17p13.1. Therefore, it is anticipated that a mutation in the GUCY2D gene is responsible for the clinical entity that is herein being reported for this kindred. Mutation screening of the GUCY2D is currently in progress and we expect to identify a disease-causing mutation in this kindred in the near future.

We believe that for this kindred, we are dealing with a classical form of LCA without any associated functional or organic malformation such as those reported by other investigators.<sup>7-13</sup> The mode of inheritance is in favor of an autosomal recessive trait. The subjective clinical presentations, ophthalmological findings, as well as electrophysiological tests are all characteristic of the LCA type 1 (LCA1) in this very large family.

The rarity of the affected individuals in the ancestral generations of this kindred may be due to a sense of guilt and the attempt to conceal the affliction. To the best of our knowledge, this is the first report of such a large LCA pedigree from Iran.

Recently we found that a mutation in the LCA1/GUCY2D gene but not the LCA4/AIPL1, gene is more likely to be involved.<sup>19</sup> Therefore, this gene was amplified and fully sequenced in an affected subject. A homozygous missense mutation (ATC→AGC; 1816S) within the exon 13 of GUCY2D was identified. Sequencing of a 328-bp PCR fragment containing this mutation in 19 subjects fully segregated in 6 affected, 8 gene carrier parents and 5 normal members, including 2 heterozygote gene carrier parents and 5 normal members. SSCP screening of 1816S did not identify this mutation in 184 normal control chromosomes (70 Iranian and 114 other Caucasians). The 1816S is a novel mutation in the GUCY2D gene and, this is the first mutation report in an Iranian LCA kindred.

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