

ONLINE MUTATION REPORT

Evidence of autosomal dominant Leber congenital amaurosis (LCA) underlain by a *CRX* heterozygous null allele

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J Med Genet 2003;40:e90(<http://www.jmedgenet.com/cgi/content/full/40/7/e90>)

Originally described by Theodore Leber in 1869, Leber congenital amaurosis (LCA, MIM 204000) is the most early and severe form of all hereditary retinal dystrophies, responsible for congenital blindness.¹ The diagnosis is usually made at birth or during the first months of life in an infant with total blindness or greatly impaired vision, normal fundus, and unrecordable electroretinogram (ERG).² It is usually accepted that LCA accounts for 5% of all inherited retinal dystrophies.³ However, this frequency is an underestimate since it is now agreed that in some cases LCA could represent the extreme end of a spectrum of severity of retinal dystrophies.⁴⁻⁶ Hitherto, LCA was considered as an autosomal recessive, genetically heterogeneous condition. Eight LCA genes have been identified or mapped so far, namely (1) the retinal specific guanylate cyclase gene (*retGCL1*) at the LCA1 locus (17p13.1),⁷ (2) the gene encoding the 65 kDa protein specific to the retinal pigment epithelium (*RPE65*) at the LCA2 locus (1p31),⁸ (3) the cone-rod homeobox containing gene (*CRX*, 19q13.3),⁹⁻¹¹ (4) the gene encoding the arylhydrocarbon receptor interacting protein-like 1 at the LCA4 locus (17p13.1),¹² (5) the gene encoding the retinitis pigmentosa GTPase regulator-interacting protein 1 (*RPGRIP1*) at the LCA6 locus (14q11),^{13,14} (6) the human homologue of the *Drosophila melanogaster* crumbs gene (*CRB1*, 1q31),^{15,16} (7) LCA3 on chromosome 14q24,¹⁷ and (8) LCA5 on chromosome 6q.¹⁸ The two last loci respectively account for the disease in a consanguineous Saudi Arabian LCA family and a multigenerational kindred of Old Order River Brethren, an isolate originating from Swiss immigrants to America in the 1750s.^{17,18} Altogether, the six identified genes account for about 48% of LCA

cases in our series¹⁹ and are consistent with autosomal recessive inheritance.

However, in 1960 and 1968 respectively, Sorsby and Williams²⁰ and François²¹ described a few families with a clearly dominant mode of transmission. These observations were largely dismissed or overlooked until some de novo *CRX* mutations associated with LCA shed new light on these old reports.^{8,9,22-24} In 1998, Sohocki *et al*²² reported different phenotypes associated with *CRX* mutations. Among these phenotypes, they described a large pedigree of autosomal dominant retinal dystrophy with intrafamilial variability ranging from typical retinitis pigmentosa to a very severe visual disturbance in some members who "share many of the characteristics of Leber congenital amaurosis (LCA)".²² However, the authors were cautious in diagnosing LCA in this family because of the existence of several members with a mild phenotype and particularly a male whose visual acuity was 20/80 at the age of 27. These diagnostic reservations were also discussed by Rivolta *et al* in 2001.²³

Here, we confirm autosomal dominant inheritance in an unambiguous LCA family underlain by a heterozygous null allele of *CRX*.

PATIENTS AND METHODS

Patients

A three generation family with unambiguous LCA in three members was ascertained through the genetic counselling service of Necker Hospital in Paris, France (fig 1). In this family, the first proband, II.2, was affected from birth with a severe congenital retinal dystrophy characterised by a searching nystagmus, no ocular pursuit, hypermetropia, marble aspect of the retina, and unrecordable electroretinogram (ERG) since

Key points

- Leber congenital amaurosis (LCA) is characterised by congenital blindness or greatly impaired vision from the first months of life.
- Hitherto, LCA was considered as an autosomal recessive, genetically heterogeneous condition. However, the identification of de novo mutations in one LCA gene, *CRX*, opened the debate of possible dominant inheritance in some LCA cases.
- Here, we report the transmission through two generations of an unambiguous LCA phenotype accounted for by a heterozygous 1 bp deletion of the *CRX* gene, suggesting dominant inheritance of LCA in this pedigree.
- Considering the parental consanguinity of the index case in this family, the hypotheses of digenism or a modifying effect of another gene were very unlikely, supporting the view that in rare cases LCA could be inherited as an autosomal dominant trait.

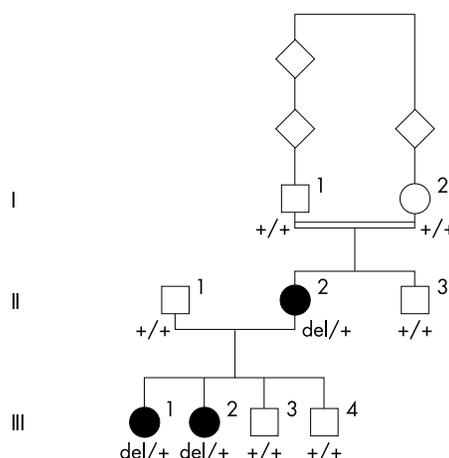


Figure 1 Pedigree of autosomal dominant LCA family segregating a heterozygous *CRX* null allele. +: wild type, del: *CRX* 509 del T.

the age of 8 months. At the age of 13, her fundus showed a large retinal reorganisation including macular atrophy, whitish spots, and very thin retinal vessels. Her visual acuity was reduced to counting fingers for one eye and light perception for the other eye. This female was born to consanguineous parents and the autosomal recessive inheritance of the disease appeared to be unquestionable.

Even though she married an unrelated healthy man, she gave birth to four children of whom two were affected with unambiguous Leber congenital amaurosis. III.1 was examined in the first weeks of life because of congenital nystagmus with exotropia and photophobia. Her fundus showed a typical salt and pepper aspect of the retina, and her ERG was flat at the age of 7 months. Her sister, III.2, displayed roving eye movements until the first month of life. Her fundus was identical to her sister's and rapidly progressed to a severe macular atrophy. Her ERG was unrecordable at the age of 6 months.

Screening of LCA genes

A search for mutations in *GUCY2D*, *RPE65*, *CRX*, *AIPL1*, *RPGRIP1*, and *CRB1* was carried out by denaturing high pressure liquid chromatography (DHPLC). Heteroduplex formation was induced by heat denaturation of PCR (primers and PCR conditions available on request) products at 94°C for 10 minutes, followed by gradual reannealing from 94°C to 25°C over 30 minutes. DHPLC analysis was performed with the WAVE DNA fragment analysis system (Transgenomic, Cheshire, UK). PCR products were eluted at a flow rate of 0.9 ml/minutes with a linear acetonitrile gradient. The values of the buffer gradients (buffer A, 0.1 mol/l triethylammoniumacetate; buffer B, 0.1 mol/l triethylammoniumacetate/25% acetonitrile), start and end points of the gradient, and melting temperature predictions were determined by the WAVE-MAKER software (Transgenomic, Cheshire, UK). Analysis per sample took six minutes, including regeneration and re-equilibration to the starting conditions. Optimal run temperatures were empirically determined. Mobile phase temperatures were assessed within a 5°C window above and below the suggested run temperature on the basis of each fragment's characteristic melting profile. PCR fragments displaying abnormal DHPLC profiles were further sequenced using the Big Dye Terminator Cycle Sequencing Kit v2 (ABI Prism, Applied Biosystems, Foster City, USA on a 3100 automated sequencer).

RESULTS

An abnormal DHPLC profile was identified in exon 3 of the *CRX* gene in patient II.2. Direct sequencing showed a heterozygous deletion of nucleotide 510 in this exon in the three affected members, while both consanguineous parents (I.1 and I.2) and all other healthy relatives (I.1, II.3, III.3, and III.4) did not harbour the mutation. This nt510 deletion leads to the appearance of a premature stop codon 47 nucleotides downstream. Therefore, the normal protein is 39% truncated especially with three important deleted domains, SYFSG, LSPM, and OTX tail. The whole coding sequence as well as the intron-exon boundaries of the *CRX* gene were screened by direct sequencing but no other sequence alteration was found. No mutation was found either in any of the five other *LCA* genes.

DISCUSSION

LCA is a relatively common disease with a high rate of consanguinity. It has long been assumed that this condition could be accounted for by recessive mutations in various genes.²⁵ However, in 1960 and 1968, Sorsby and Williams²⁰ and François²¹ respectively described a few families with a clearly dominant mode of transmission.²⁰⁻²¹ These observations were largely overlooked. Here, we report a multiplex family in which the first patient, born to consanguineous parents, gave

birth to two affected girls. The transmission of the disease through two generations in this family raised the questions of "false dominance" owing to an unrecognised relationship between II.1 and II.2 or autosomal dominant inheritance despite the consanguinity of I.1 and I.2.

Genealogical investigations failed to identify any relationship between II.1 and II.2. Moreover, the identification of a heterozygous frameshift mutation of *CRX* in the three affected subjects, which was absent in the healthy relatives, strongly suggested autosomal dominant inheritance.

These findings raised the question of possible digenism in this pedigree or a modifying effect of a variant sequence in another gene. However, the screening of all other LCA genes (*retG1*, *RPE65*, *AIPL1*, *RPGRIP1*, and *CRB1*) failed to detect any other mutation. Although taken together these genes only account for 48% of all LCA patients in our series,¹⁹ it is worth noting that the normal sequence of the *CRX* gene in both consanguineous parents (I.1 and I.2) favours autosomal dominant inheritance in this pedigree. In addition, the consanguinity of the first patient's parents makes the hypotheses of digenism or modifier polymorphisms very unlikely.

Our family is similar to that published by Sohocki *et al*²² in 1998, who reported a *CRX* heterozygous mutation in an autosomal dominant family with a variable retinal dystrophy resembling LCA in some members.²² Here, we confirm autosomal dominant inheritance in an unambiguous LCA family underlain by a heterozygous null allele of *CRX*. However, this mode of inheritance does remain an exception in this condition (1/200 families in our series).

ACKNOWLEDGEMENTS

We are grateful to the Association Retina France and the Association Valentin Haüy for their financial support.

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