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ARTICLE

Spectrum of *retGC1* mutations in Leber's congenital amaurosis

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Leber's congenital amaurosis (LCA) is the earliest and most severe form of all inherited retinal dystrophies responsible for congenital blindness. Genetic heterogeneity of LCA has been suspected since the report by Waardenburg of normal children born to affected parents. In 1995 we localised the first disease causing gene, LCA1, to chromosome 17p13 and confirmed the genetic heterogeneity. In 1996 we ascribed LCA1 to mutations in the photoreceptor-specific guanylate cyclase gene (*retGC1*). Here, we report on the screening of the whole coding sequence of the *retGC1* gene in 118 patients affected with LCA. We found 22 different mutations in 24 unrelated families originating from various countries of the world. It is worth noting that all *retGC1* mutations consistently caused congenital cone-rod dystrophy in our series, confirming the previous genotype–phenotype correlations we were able to establish. RetGC1 is an essential protein implicated in the phototransduction cascade, especially in the recovery of the dark state after the excitation process of photoreceptor cells by light stimulation. We postulate that the *retGC1* mutations hinder the restoration of the basal level of cGMP of cone and rod photoreceptor cells, leading to a situation equivalent to consistent light exposure during photoreceptor development, explaining the severity of the visual disorder at birth. *European Journal of Human Genetics* (2000) **8**, 578–582.

Keywords: Leber's congenital amaurosis; genetic heterogeneity; mutations in the retinal–specific guanylate cyclase gene; dysfunction of the phototransduction cascade; genotype–phenotype correlations

Introduction

Leber's congenital amaurosis (LCA) is the most early and severe form of all inherited retinal dystrophies. Originally described by Leber in 1869, LCA (MIM 204000, 204100) is an autosomal recessive condition distinct from other retinal dystrophies and responsible for congenital blindness.[1] The diagnosis is generally made 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).[2] It is generally accepted that LCA accounts for 5% of all retinal dystrophies but considering the high rate of consanguinity in LCA families, we assume that this frequency is underestimated and that this condition is not uncommon in countries with a high rate of consanguineous unions.[3,4] A certain degree of clinical heterogeneity has long been recognised in LCA but these clinical differences have been largely ignored. Conversely, genetic heterogeneity has been accepted for a long time.[5]

In 1995, we localised the first disease-causing gene, *LCA1*, to chromosome 17p13 and confirmed the genetic heterogeneity.[6,7] In 1996, we ascribed LCA1 to mutations in the photoreceptor-specific guanylate cyclase gene (*retGC1*).[8] RetGC1 is an essential protein implicated in the phototransduction cascade, especially in the recovery of the dark state after the excitation process of photoreceptor cells by light stimulation.

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In 1997 LCA-causing mutations were reported in a second gene, *RPE65* which is the first retinal pigment epithelium specific gene.[9,10] The RPE65 protein is implicated in the metabolism of vitamin A, the precursor of the photoexcitable retinal pigment (rhodopsin).

Finally, a third gene, *CRX*, implicated in photoreceptors development has been suspected to cause a few cases of LCA.[11–13] Taken together, these three genes account for only 27% of LCA cases in our series.[14]

The search for genotype–phenotype correlations allowed us to demonstrate that *retGC1* gene mutations are responsible for a congenital cone-rod dystrophy with dramatic and invariable cone dysfunction, while *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.[15]

Here we report the study of *retGC1* gene in 118 LCA patients. A total of 21 different mutations were identified in 24 unrelated families. All patients in these families display the specific LCA1 phenotype.[15]

Patients and methods

Patients

A total of 118 unrelated patients were ascertained from various genetic and ophthalmologic sources. Our inclusion criteria were:

1) severe impairment of visual function detected at birth or during the first months of life, with pendular nystagmus, roving eye movements, eye poking, inability to follow lights or objects and normal fundus;
2) extinguished ERG;
3) exclusion of ophthalmological or systemic diseases sharing features with LCA.

Ophthalmological data were available for each affected individual. The time course of the disease was obtained by interviewing the patients or their parents and a pedigree was established. Among the 118 families, 40 were multiplex, 41 were consanguineous and 17 were both multiplex and consanguineous.

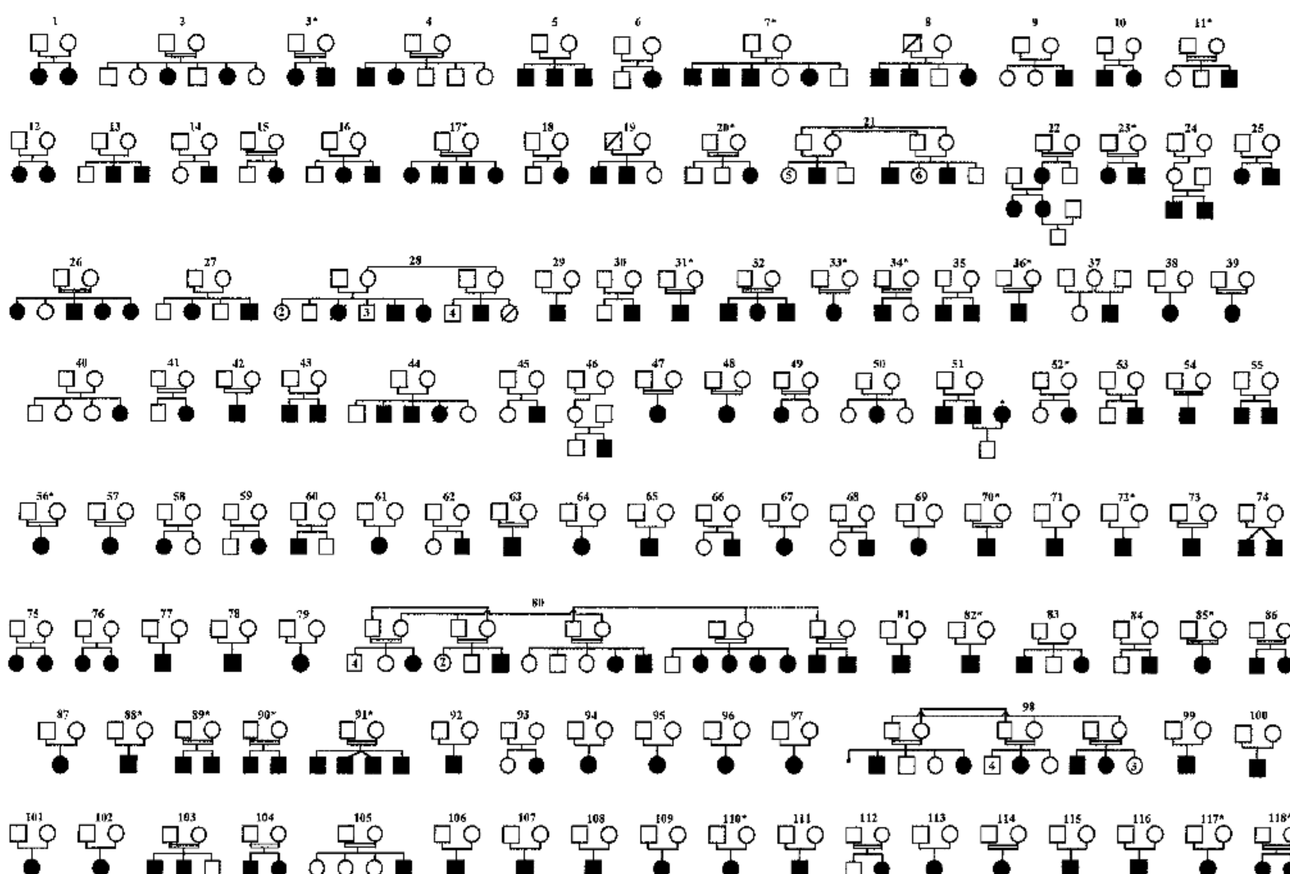

Figure 1 Pedigrees of the 118 LCA families studied. Asterisks refer to families harbouring *retGC1* mutations.

Methods

For single strand conformation polymorphism analysis and direct sequencing of the 20 exons of the *retGC1* gene, genomic DNA (200 ng) was submitted to PCR amplification using 1 μM of intronic primers[8] and alpha 33P dCTP (0.1 μl, 10 mCi/ml, Amersham Pharmacia Biotech Europe GmbH, Orsay, France) in an amplification mixture (20 μl) containing 200 mM dNTPs and 0.5 U Taq DNA polymerase (Life Technologies, Cergy Pontoise, France). Amplified DNA (2 μl) was mixed with an equal volume of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), denatured for 10 min at 95°C, loaded on to a polyacrylamide gel (MDE) and electrophoresed at room temperature for 14 h at 3 W. Gels were transferred on to 3 mm Whatman paper, dried and autoradiographed with Kodak X-OMAT films for 16–48 h.

For direct sequencing, amplification products were loaded on to a 0.7% agarose gel, purified by phenol–chloroform extraction and recovered by ethanol precipitation. Purified fragments were directly sequenced using the same specific intronic primers and the PRISM™ Ready Reaction Sequencing Kit (Perkin Elmer Cetus, Foster City, CA, USA), on an automatic fluorometric DNA sequencer (Applied Biosystems, Perkin Elmer Cetus, Foster City, USA).

Results

A total of 21 different mutations were identified in 24 unrelated patients. Among them, 17 were consanguineous families of which six were multiplex; all these 17 families were homozygous for the mutation. On the other hand, four families were compound heterozygotes of which one was multiplex and three families (one multiplex) were single heterozygotes, the second mutation being so far undetected. These 21 mutations are made of five frameshift mutations leading to a premature stop codon, one large inframe duplication of 48 nucleotides, two nonsense mutations, two splice mutations and 11 missense mutations (Figure 2). It is worth noting that three of the frameshift mutations as well as one nonsense mutation lie in the first part of the gene encoding the extracellular domain of the protein. These mutations are expected to lead to markedly truncated products. On the other hand, one frameshift and the three splice site mutations lie in the kinase-like domain and are expected to have the same consequences. Finally, one frameshift and one nonsense mutation are expected to alter the retGC1 enzymatic activity as they lie in the last part of the gene encoding the catalytic domain (Figure 2).

Three homozygous mutations were identified in the original study: 387delC, 620delC in two unrelated families and F565S in two others.[8] Furthermore, the 387delC and the 620delC mutations were found in three and one unrelated consanguineous families, respectively. The F565S was also found in one other family. It is worth noting that the four families harbouring the 387delC were Jewish families originating from North Africa, whilst 620delC was found in a Jewish family and a Moslem family both originating from Tunisia. In addition, all three families harbouring the F565S mutation were Moslem families originating from Algeria. The 19 remaining mutations were identified in 17 unrelated families originating from various countries across the world (Table 1). All these mutations are scattered throughout the entire coding sequence except in exons 11 to 13 encoding the putative dimerisation domain (Figure 1). All missense mutations affect amino-acids conserved in the *retGC1* mouse ortholog, GC-E, and are absent in 100 healthy controls. All patients belonging to the 24 families display the same phenotype with onset at birth, inability to follow light or objects, roving eye movements, pendular nystagmus, normal

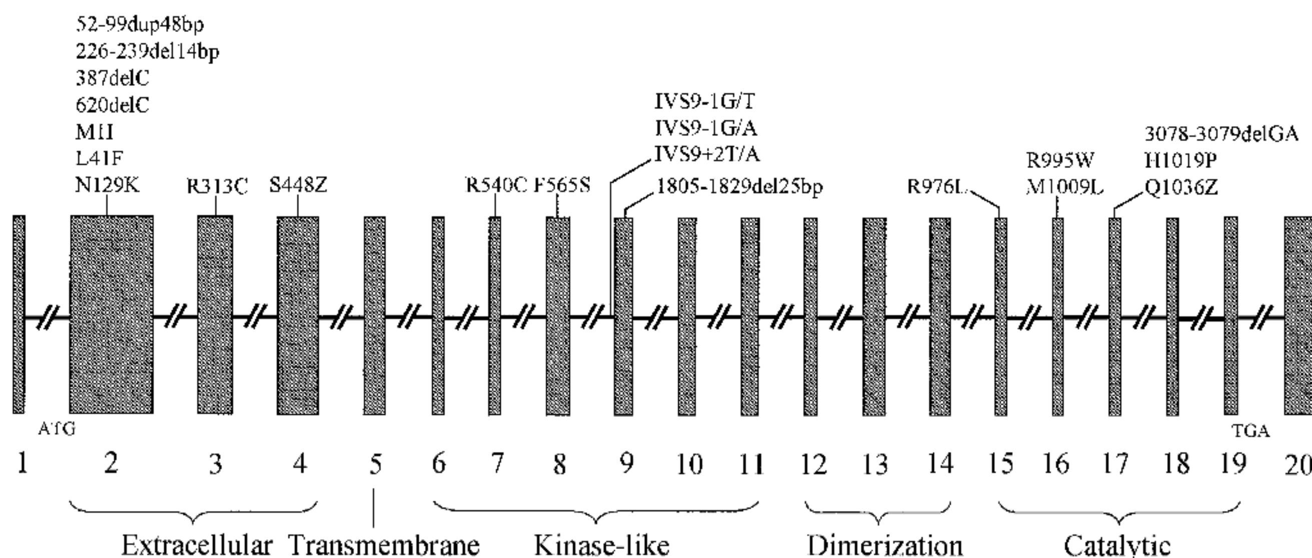

Figure 2 Structure of the human *retGC1* gene and position of mutations identified in LCA patients.

Table 1 RetGC1 mutations in patients affected with LCA. The family number refers to Figure 1

Family	Exon	Base change	Amino acid change	Homozygous+/+ Heterozygous+/−	Conservation across mouse and human	Ethnic origin
85	2	52-99Dup48bp	52-99Dup48bp	+/+	+	Tunisia
110	2	226-239Del14bp	226-239Del14bp	+/+	+	Antillas
3	2	387DelC	387DelC	+/+	+	Morocco
90	2	387DelC	387DelC	+/+	+	Tunisia
91	2	387DelC	387DelC	+/+	+	Tunisia
118	2	387DelC	387DelC	+/+	+	Israel
23	2	620DelC	620DelC	+/+	+	Tunisia
34	2	620DelC	620DelC	+/+	+	Tunisia
70	Intron 9	T(1956+2)A	IVS9+2T/A	+/+		Morocco
20	9	1805-1829Del25bp	1805-1829Del25bp	+/+	+	Mali
33	17	3201-3202DelGA	3201-3202DelGA	+/+	+	Algeria
11	2	G3C	M1I	+/+	+	Benin
7	8	T1694C	F565S	+/+	+	Algeria
17	8	T1694C	F565S	+/+	+	Algeria
36	8	T1694C	F565S	+/+	+	Algeria
31	15	G2927T	R976L	+/+	+	Turkey
56	17	A3956C	H1019P	+/+	+	Pakistan
51	2	G3A	M1I	+/−	+	France
	Intron 9	G(1957-1)T	IVS9-1G/T	+/−		France
82	2	C387A	N129K	+/−	+	Italy
	16	C2983T	R995W	+/−	+	Italy
52	3	C937T	R313C	+/−	+	Italy
	4	C1343A	S448Z	+/−	+	Italy
88	16	A3025C	M1009L	+/−	+	Belgium
	Intron 9	G(1957-1)A	IVS9-1G/A	+/−		Algeria
89	2	C121T	L41F	+/−	+	Tunisia
117	7	C1618T	R540C	+/−	+	Netherlands
72	17	C3106T	Q1036Z	+/−	+	France

fundus at birth followed by salt-and-pepper aspect of the retina and typical aspect of RP, non-recordable ERG, severe hyperopia and severe photophobia, non-recordable visual field and non-evolutive congenital blindness.

Discussion

Here, we report a total of 21 different mutations in the *retGC1* gene in 24 unrelated patients affected with the LCA1 specific phenotype, ie congenital stationary cone-rod dystrophy with profound visual deficiency, photoaversion and severe hypermetropia.[15] Indeed, photoreceptor cells normally convert light energy into an electrical signal through a transduction process that consists of an enzymatic cascade, resulting in the hydrolysis of cGMP and the closure of cGMP-gated cation channels with hyperpolarisation of the plasma membrane.[16–18]

The discovery of missense and frameshift retGC-1 mutations suggests that cGMP production in photoreceptor cells is abolished in LCA. Consequently, the excitation process of rod and cone photoreceptors is markedly impaired, due to consistent closure of cGMP-gated cation channels, with hyperpolarisation of the plasma membrane. The cGMP concentration in photoreceptor cells cannot be restored to the dark level, leading to a situation equivalent to consistent light exposure during photoreceptor development, explaining the severity of the visual disorder at birth.

It is worth noting that the knock-out of the murine homolog of *retGC1*: GC-E,[19] emphasises the very severe alterations of photoreceptors.[20] Indeed, in GCE -/- mice, the most striking abnormality was the 98% reduction in cone ERG activity at 3–5 weeks of age and the complete absence of detectable cone ERG activity in mice over 12 weeks. An early loss or a failure of cones to develop was confirmed by histological analyses. The maximum rod photoresponse amplitude was 70% lower than normal at 3–5 weeks, despite normal histological appearance of rods, and was 92% lower by 26 weeks with mild histological alterations.[20] These findings suggest that loss of *GCE* activity leads to an early and profound loss of cone function in *GCE* -/- mouse, as it is observed in LCA1 patients.

In conclusion, the mutations in the *retGC1* gene account for 24/118 patients with LCA in our series (20%). It is worth noting that 17/24 families originated from Mediterranean countries. This geographic prevalence as well as the specific LCA1 clinical features are of particular help for genetic studies especially as it is now accepted that LCA is clinically heterogeneous.[15]

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