



# HHS Public Access

Author manuscript

*Ophthalmic Genet.* Author manuscript; available in PMC 2018 January 17.

Published in final edited form as:

*Ophthalmic Genet.* 2017 ; 38(1): 7–15. doi:10.1080/13816810.2016.1275021.

## Leber congenital amaurosis, from darkness to light: An ode to Irene Maumenee

Razek Georges Coussa<sup>1,2</sup>, Irma Lopez Solache<sup>1</sup>, and Robert K. Koenekoop<sup>1,2</sup>

<sup>1</sup>Department of Paediatric Ophthalmology, Montreal Children's Hospital, McGill University Health Centre, Montreal, Quebec, Canada

<sup>2</sup>The McGill Ocular Genetics Laboratory, Paediatric Ophthalmology Division, Montreal Children's Hospital, McGill University Health Centre, Montreal, Quebec, Canada

### Abstract

This article is dedicated to Irene Hussels-Maumenee, Professor of Human Genetics and Ophthalmology, Johns Hopkins' Wilmer Eye Institute, Ocular Genetics Fellowship director in 1994–1995. Leber congenital amaurosis (LCA) has almost come full circle, from a profound and molecularly uncharacterized form of congenital retinal blindness to one in which a large number of causative genes and disease pathways are known; and the world's first human retinal disease to be treated by gene therapy. Dr. Maumenee's insights, efforts and leadership have contributed significantly to this remarkable scientific journey. In this manuscript, we present a short summary of the known LCA genes, LCA disease subtypes and emerging treatment options. Our manuscript consolidates previous knowledge with current findings in an attempt to provide a more comprehensive understanding of LCA.

### Keywords

Causal genes; disease subtypes; emerging treatment options; Leber congenital amaurosis (LCA)

---

In Ophthalmology and the study of visual sciences, there is nothing more profound and challenging than to diagnose and manage a baby with blindness and nystagmus. The process is filled with raw emotion and complexity due to the long differential diagnosis and the stakes involved. It is eventually extremely satisfying, as a clinically and molecular diagnosis are reached; allowing school, career, family and treatment planning.

In the summer of 1994 in Baltimore, a four-month old toddler sits proudly on his mother's lap, chin down, eyes slowly beating, with sunken globes. Every now and then he pushes his index finger or hand into the eye, hard. We have no eye contact, but obviously the baby is aware of me and his surroundings. The room is silent, the expectations and anxiety of the family are high. They have already seen five or six other doctors, specialists, neurologists

---

CONTACT Robert K. Koenekoop, MD, PhD robert.koenekoop@mcgill.ca The McGill Ocular Genetics Laboratory, Paediatric Ophthalmology Division, Montreal Children's Hospital Glen site, McGill University Health Centre, Room A02.3013, 1001 Boulevard Décarie, Montreal, QC, H4A 3J1, Canada.

### Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

and ophthalmologists. They have undergone heart-wrenching, time-consuming, expensive tests, brain scan, MRI, CT scan, ERG and EEG, to rule out brain disease, tumors, seizures. Thus far the diagnosis is retinal dystrophy “not yet diagnosed”, rule out blindness, rule out brain disease.

The baby’s eye examination reveals absence of fixation, wandering nystagmus, +7.00 D cycloplegic refraction and a normal retinal appearance.

Dr. Maumenee walks into the room and introduces herself to the parents, and quickly assesses the child, listens to my assessment and asks a few questions to the parents. She says “Rob, this is Leber Congenital Amaurosis. Do you see the sunken eyes, the oculodigital sign of Franceschetti, the amaurotic pupils. No more testing is necessary to make the diagnosis.”

Four months of parental anxiety, guilt, and fear of brain pathology dissipate. No more testing, a spot-on diagnosis guided by a wealth of clinical experience and knowledge of inherited retinal degenerations, especially LCA. The parents and I realize and appreciate Dr. Maumenee’s experience. Dr. Maumenee continues; “We need to provide your boy with glasses, stimulate him visually, and prepare him for Braille and an otherwise as normal a life as possible. He will be very smart. Many LCA patients excel and develop an intense sense of touch, hearing and smell, she says. We need to be careful because if you have another child there is a 25% chance that they have the same condition. Unfortunately, nothing else is known about this disease, she laments. “For other retinal degenerations we have identified some genes recently; for LCA we have no known genes, no candidate abnormal proteins and no idea what goes wrong with the retina, because we don’t know the disease pathways yet. We will take a blood sample from him and you both, to prepare for the future, but we don’t have any genetic leads right now.”

Happily, things have changed. It is now 2016, and LCA has almost come full circle, from a profound and molecularly uncharacterized form of congenital retinal blindness to one in which a large number of causative genes and disease pathways are known; and the world’s first human retinal disease to be treated by gene therapy. Dr. Maumenee’s insight, efforts and leadership have contributed significantly to this remarkable scientific journey. We present a short summary of the genes, disease subtypes and treatment options for LCA. Dr. Maumenee’s fingerprints are apparent on many of them.

## Gene discoveries

“In Leber congenital amaurosis, which probably comprises a number of genetically heterogeneous conditions, visual acuity remains stable despite progressive retinal pigmentary changes. The subgroup of patients with macular colobomas, however, may develop progressive decrease in vision.” (Heher, Traboulsi and Maumenee, 1992).

Leber congenital amaurosis (LCA) is the second most common inherited retinal dystrophy after retinitis pigmentosa (RP). The prevalence of LCA is estimated to be 2–3 per 100,000. LCA accounts for about 5% of all retinal dystrophies and about 20% of blindness in legally blind children<sup>1</sup>.

LCA manifest itself in the first 6 months of life with poor visual acuity (20/200 to NLP), sensory nystagmus, high hyperopia (3D or more) and later keratoconus. Fundus characteristics are variable and in some cases the retina may be normal in appearance. In other cases, pigmentary retinal changes, vascular attenuation and optic pallor develop over time, accompanied in some macular changes.

Currently 20 genes have been identified to cause the phenotype of LCA, representing about 70% of the causal genetic variants. These genes, which are all needed for the normal functioning of the retina are: *GUCY2D* (LCA1), *RPE65* (LCA2), *SPATA7* (LCA3), *AIP1* (LCA4), *LCA5* (LCA5), *RPGRIP1* (LCA6), *CRX* (LCA7), *CRB1* (LCA8), *NMNAT1* (LCA9), *CEP290* (LCA10), *IMPDH1* (LCA11), *RD3* (LCA12), *RDH12* (LCA13), *LRAT* (LCA14), *TULP1* (LCA15), *IQCB1*, *CLUAP1*, *PRPH2*, *KCNJ13* and *IFT140*. Herein we provide a description of the major LCA genes. After discussing how each gene was discovered and the function of the protein, we will discuss how LCA disease subtypes can be classified and the progress of treatment studies.

## 1. GUCY2D (LCA1)

Using PCR and fluorescence in situ hybridization, the *GUCY2D* gene was mapped to 17p13.1 in 1994<sup>2</sup>. Guanylate cyclase 2D, aka *GUCY2D*, accounts for about 6–21% of all known mutations. It was the first gene to be discovered in LCA, by the Josselyn Kaplan group in Paris, in 1994. The gene is better known for its related protein: retinal guanylyl cyclase 1, (GC1) which inherently controls the levels of cyclic guanosine monophosphate (cGMP), which is the intracellular second messenger regulating phototransduction in mammals. GC1 is believed to be located in the nuclei and inner segments of the rod and cone photoreceptor cells. In particular, in 1999, Duda et al. reported that GC1 is a calcium-regulated module linked to retinal synaptic activity and thus phototransduction<sup>3</sup>. In the dark, cGMP levels are normally high and the cGMP-gated sodium channels are open. In this dark current, the photoreceptor cells are depolarized. In the light, a photon of light sets off the phototransduction cascade, which eventually activates phosphodiesterase, which lowers cGMP and closes the sodium channels, leading to hyperpolarization of the photoreceptor cells. Normally GC1 then replenishes cGMP, but not in LCA, with mutant *GUCY2D*, this does not occur. In *GUCY2D*-related LCA, the cells are low on cGMP, the sodium channels are closed and thus in a constant state of hyperpolarization. Currently, there are 132 different *GUCY2D* mutations<sup>4</sup>. The majority of causal mutations result in either protein truncation or complete functional loss<sup>5</sup>. Phenotypic manifestations of *GUCY2D* pathologic mutations in LCA include a visual acuity ranging from 20/200 to LP, an initial normal fundus appearance in the majority of cases<sup>6</sup>.

## 2. RPE65 (LCA2)

*RPE65*, stands for Retinal pigment epithelium-specific 65, is a common LCA gene with a mutation rate of about 3–16%<sup>7</sup>. This gene codes for the retinoid isomerohydrolase enzyme, which when it complexes with lecithin retinol acyltransferase (LRAT), isomerizes and hydrolyzes all-trans retinyl ester to 11-cis retinol each time a photon hits 11-cis retinal<sup>8</sup>. *RPE65* is thus one of the crucial mediators of the retinoid (aka the visual or vitamin A) cycle. This cycle replenishes 11-cis retinal. Patients with *RPE65* mutations cannot make 11-

cis retinal, and therefore cannot see. Eventually the photoreceptor dies. In 1997, Marlhens et al. were the first to report that *RPE65* mutations are causally linked to LCA<sup>9</sup>. Currently, 132 *RPE65* mutations have been reported worldwide<sup>3</sup>. Fundus exam is characteristic for a blond translucent appearance and whitish drusen-like deposits in about 90% and for later developing chorioretinal atrophy peripheral patches in 64% of *RPE65* LCA patients<sup>6</sup>. Mutations in *RPE65* were also reported in RP patients having normal-looking fundi.<sup>10</sup>

### 3. SPATA7 (LCA3)

*SPATA7* codes for the spermatogenesis-associated protein 7, aka spermatogenesis-associated protein HSD3 and localizes to the connecting cilium of the photoreceptors, but is ubiquitously expressed with high levels in brain and testis. *SPATA7* is responsible for protein trafficking across the photoreceptor connecting cilium and for recruiting and localizing retinitis pigmentosa GTPase regulator interacting protein 1 (RPGRIP1) to the photoreceptor connecting cilium. In particular, Eblimit *et al.* reported not only a reduction in RPGRIP1 levels in the photoreceptor connecting cilium but also mislocalization to the inner segment in *SPATA7* knockdown mutant mice<sup>11</sup>. Its disease-causing rate is about 1.7%<sup>12</sup> and currently 14 *SPATA7* mutations have been reported<sup>8</sup>. LCA patients with *SPATA7* mutations have severe progressive rod cone degeneration with preserved foveal architecture. One case of CRD was described that converted to RP over time<sup>13</sup>. Most patients have isolated LCA, but rarely hearing loss and low sperm count have been documented.

### 4. AIPL1 (LCA4)

Aryl-hydrocarbon-interacting protein-like 1, aka *AIPL1*, is a major gene for LCA causing at least 7% of LCA cases<sup>14</sup>. Currently, there are 50 *AIPL1* mutations in *AIPL1*<sup>4</sup>. Both *GUCY2D* and *AIPL1* have different locus on the same chromosome. According to van der Spuy *et al.*, *AIPL1* is specific to rod photoreceptors in adult life only, showing a specific staining pattern extending from the inner segment to the synaptic junction in the outer plexiform layer<sup>15</sup>. *AIPL1* specifically target processing of farnesylated proteins causing destabilization of phosphodiesterase 6 (PDE6), which is believed to be compromised in LCA leading to early-onset retinal degeneration<sup>16</sup>. Most patients have a significant decrease in their visual acuity which ranges from LP to NLP in 43% of cases during their first decade<sup>6</sup>. Fundus is characterized by drusen like deposits and a variable pigmentary atrophic changes with patches of hypopigmentation and pigmentary clumping<sup>6</sup>. A pathognomonic electroretinogram pattern characterized by slow insensitive scotopic responses<sup>16</sup>. Interestingly, mutations in *AIPL1* also cause CRD and juvenile RP.

### 5. Lebercilin (LCA 5)

Irene always taught the fellows and residents that gene identification was much easier on islands. She had spent considerable time visiting and researching the Pingelap islands where severe colorblindness due to Achromatopsia is prevalent. The late Oliver Sachs worked in the Pingelap islands as well and visited Irene to prepare for his trips.

Religious groups represent genetic islands. Dr Maumenee's group studied a religious isolate named the Old Order River Brethren in the farmlands of Pennsylvania and identified a new LCA locus on chromosome 6q11 and named it LCA5<sup>18</sup>. LCA5 is a 5 exon gene and

accounts for about 1–2% of LCA mutations. Currently 12 LCA5 mutations have been documented<sup>2</sup>. *Lebercillin* is located at the cilium between the inner and outer segments of photoreceptors<sup>19</sup> and is known to interact with intraflagellar transport (IFT) machinery. Inactivation of *Lebercillin* results in photoreceptor degeneration due to failure in outer segment formation. This is believed to be due to the involvement of *lebercillin* in minus end-directed microtubule transport. *Lebercillin* pathologic mutations typically result in classic LCA findings including nystagmus, oculodigital sign, severe visual dysfunction despite a normal fundus. Atypically, macular colobomas were also reported<sup>20</sup>.

## 6. *RPGRIP1* (LCA6)

Retinitis pigmentosa GTPase regulator-interacting protein 1, aka *RPGRIP1*, accounts for about 5–6% of all LCA cases and 71 *RPGRIP1* mutations are currently known<sup>4,21</sup>. Most mutations in *RPGRIP1* result in protein truncation and complete functional loss. Retinitis pigmentosa GTPase regulator (RPGR) and RPGRIP are co-localized to outer segments of human rod and cone photoreceptors as well as neuronal cells such as amacrine cells<sup>22</sup>. Both RPGR and RPGRIP proteins co-localize specifically to the photoreceptor connecting cilium where it is essential for disk morphogenesis and regulation of actin cytoskeleton dynamic<sup>23</sup>. Similar to mutations in *AIPL1*, mutations in *RPGRIP1* result in LP or NLP vision within the first decade of life and drusen-like retinal deposits in 60% and 80% of cases, respectively<sup>6</sup>.

## 7. *CRX* (LCA7)

*CRX* is most similar to the human OTX1 and OTX2 homeodomain proteins. *CRX* is expressed as an abundant 4.5-kb transcript in retina. *CRX* is transcription factor essential for both photoreceptor outer segment elongation and phototransduction cascade as it activates a specific sequence related to upstream processing of photoreceptor-specific genes<sup>24</sup>. Furthermore, some components of the phototransduction cascade such as recoverin, were expressed by *CRX*<sup>25</sup>. Currently, 51 *CRX* mutations have been reported to cause stable but poor visual acuity<sup>6,25</sup> with diffuse pigmentary retinal changes and colobomatous-like atrophic macular changes<sup>26</sup>. Mutations in *CRX* are mostly associated with autosomal dominant LCA.<sup>27</sup>

## 8. *CRB1* (LCA8)

*CRB1*, also known as Protein crumbs homolog 1, is an essential protein for retinal morphogenesis and localizes to the inner segment of mammalian photoreceptors. *CRB1* controls the photoreceptor outer segment orientation and its adherens junctions with Muller cells by stabilizing the membrane-associated spectrin cytoskeleton<sup>28</sup>. *CRB1* was identified as the gene for PPRPE and RP12, but also as a major gene for LCA with 166 mutations reported<sup>4,29</sup>. The phenotypic particularity of *CRB1* pathologic LCA mutations is an abnormally “unlaminated” thickened retina, almost resembling that of an immature state; this is thought to result from the interruption of the naturally occurring apoptotic retinal processes<sup>30</sup>.

### 9. *NMNAT1* (LCA9)

In another “island population” in 2003, the research groups of Drs Maumenee and Inglehearn mapped a new LCA locus (LCA9 locus) to chromosome 1p36.22 using whole genome linkage analysis in a consanguineous Pakistani family<sup>31</sup>. Nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1, aka *NMNAT1*, was initially not considered a good retinal and LCA candidate gene as it is ubiquitously and highly conserved protein that catalyzes the conversion of nicotinamide mononucleotide (NMN) and ATP into NAD<sup>+</sup> as well as the cleavage of the latter<sup>32</sup>. *NMNAT1* is essential for cell survival. *NMNAT1* affects protein folding of the resultant NAD molecule<sup>33</sup>. Recently *NMNAT1* mutations were found in LCA patients and this is the LCA9 gene. Phenotypic manifestations of pathologic mutations in *NMNAT1* result in a severe form of LCA characterized by progressive central macular “colobomatous” atrophic lesions with outer hyperpigmented borders as well as typical peripheral pigmentary changes, optic nerve pallor and retinal blood vessels attenuation<sup>33</sup>.

### 10. *CEP290* (LCA10)

Centrosomal protein Cep290, aka *CEP290* or alternatively also called nephrocystin-6 (*NPHP6*), is involved in ciliogenesis. It is localized to centromer and cilia and is heavily concentrated in retinal photoreceptors cells<sup>34</sup>. *CEP290* involvement in cyclogenesis is based on its interaction with other building blocks, including pericentriolar material 1 (PCM1), to properly ensure centromer localization<sup>35</sup>. *CEP290* is likely the most common gene mutated in LCA with 153 causal mutations accounting for 21% of all LCA mutations<sup>4,36</sup>. The c. 2991+1655A→G mutation, which creates a splice-donor site, is the most common *CEP290* mutation<sup>36</sup>. Renal failure, hypotonia, ataxia and even Joubert syndrome were all reported as extraretinal findings in patients with mutations in *CEP290*<sup>37</sup>. Despite the *CEP290* related LCA severity, surprising foveal cones have been shown to be preserved by OCT<sup>13</sup>.

### 11. *IMPDH1* (LCA11)

Inosine-5-prime-monophosphate dehydrogenase, aka *IMPDH1*, catalyzes the conversion of Inosine-5-prime-monophosphate into xanthine monophosphate. In fact, *IMPDH1* affects the *de novo* synthesis of guanine nucleotides by being the rate-limiting enzyme<sup>38</sup>. Currently, 13 *IMPDH1* mutations are known and cause typical LCA phenotypic manifestations<sup>4</sup>.

### 12. *RD3* (LCA12)

*RD3* aka Retinal degeneration 3, is only expressed in the retina and functions as a chaperone protein required for the exit of guanylate cyclase from the endoplasmic reticulum of photoreceptors as part of its trafficking to photoreceptor outer segments<sup>39</sup>. *RD3* is necessary for vesicular trafficking of photoreceptor guanylate cyclases from the inner to outer segments<sup>39</sup>. The disease burden of *RD3* in LCA is low, with less than 1% of patients affected with *RD3* mutations. Eight mutations are currently known<sup>4</sup>. Phenotypically, *RD3* mutations results in characteristic retinal layers disorganization at a young age and thinning of the plexiform layers as well as the inner nuclear, ganglion cell and nerve fiber layers<sup>40</sup>.



### 13. *RDH12* (LCA13)

Retinol dehydrogenase 12, *RDH12*, is a dual specificity NADPH-dependent retinal reductase with catalytic activity towards retinoids. In the retina, *RDH12* localizes to the inner segment base of photoreceptors<sup>41</sup>. *RDH12* leads the transformation of 11-*cis*-retinol into 11-*cis*-retinal, which is the initial key step in phototransduction and vision sensation<sup>42</sup>. Currently there are 65 known *RDH12* mutations<sup>4</sup>. Atrophic macular lesions and disorganization of the retinal architecture are often reported in LCA due to *RDH12* mutations<sup>43</sup>.

### 14. *LRAT* (LCA14)

*LRAT*, aka Lecithin retinol acyltransferase, catalyzes the esterification of all-trans-retinol in the RPE layer into all-trans-retinyl ester, which is a crucial step in vitamin A metabolism. It acts as a molecular switch necessary for the conversion of RPE65 into its membrane-associated form (mRPE65), which then enters the visual cycle for 11-*cis*-retinal processing<sup>44</sup>. Currently 10 *LRAT* mutations are known<sup>4</sup>. Phenotypic manifestations of *LRAT* mutations in LCA included normal appearing posterior poles with sharp peripheral hypopigmentation outside the arcades<sup>45</sup>.

### 15. *TULP1* (LCA15)

Tubby like protein 1, aka *TULP1*, is exclusively expressed in the retinal photoreceptors. It contributes to protein transport between the inner and outer segments of photoreceptor cells, especially rhodopsin<sup>46</sup>. It also stimulates the phagocytosis of RPE cells, which is necessary for RPE maintenance and regeneration. There are currently 31 *TULP1* known mutations<sup>4</sup>. Funduscopy findings of patients with *TULP1* LCA mutations vary significantly and include a normal fundus at early age progressing to indistinct foveolar reflex and pronounced maculopathy later on<sup>47</sup>. The retinal normal layering was reported to be thinned and disrupted with absent outer retina structures<sup>47</sup>.

### 16. *IQCB1* (NPHP5)

IQ motif containing B1, aka *IQCB1* or *NPHP5*, localizes to the connecting cilia of photoreceptors. *NPHP5* plays a key role in ciliary function through its interaction with calmodulin<sup>48</sup>. Currently 24 *NPHP5* mutations are identified<sup>3</sup>. Pathologic mutations in *NPHP5* traditionally cause Senior-Loken syndrome. LCA causal mutations are characterized by “lobular pattern of hypo- and hyperpigmentation around the vascular arcades” as well as retinal vessels straightening<sup>49</sup>. Due to *NPHP5* mutations high association with renal failure, it is recommended that all *NPHP5* LCA patients undergo renal function monitoring.

### 17. *CLUAP1*

Clusterin associated protein 1, aka *CLUAP1*, is specifically implicated in intraflagellar transport due to its bidirectional movement along the axoneme<sup>50</sup>. In fact, *CLUAP1* is found to at the connecting cilium between the inner and outer segments of retinal photoreceptors<sup>51</sup>. Currently, there is only 1 mutation in *CLUAP1* has been described in LCA<sup>4</sup>.

## 18. IFT140

Intraflagellar transport 140, abbreviated *IFT140*, is involved in retrograde ciliary transport. Currently, 11 *IFT140* mutations have been documented<sup>6</sup>. Phenotypic manifestations include RPE atrophy, hyperfluorescent macular ring, IS/OS disruption and an increased foveal thickness on OCT<sup>52</sup>. Interestingly, biallelic mutations in *IFT140* were found in Saldino Mainzer Syndrome (SMS) which represents a syndrome with retinal dystrophy (like LCA), severe renal disease, cerebellar ataxia and skeletal dysplasia<sup>53</sup>.

## 19. *PRPH2* (previously RDS/peripherin)

Retinal degeneration slow, abbreviated *RDS*, is a tetraspanning membrane protein involved in disc morphogenesis and/or maintenance<sup>54</sup>. All subsequent retinal degeneration phenotypes ranging from macular dystrophies to full-blown RP were found to harbor dominant (heterozygous) mutations in *RDS*, aka RDS/peripherin. *PRPH2* is a major retinal gene with an unusual large range of retinal diseases and phenotypes. The gene and protein were renamed *PRPH2*. Currently there are 112 mutations reported in *PRPH2* with only 2 reported to cause LCA; the other are associated with adRP, digenic RP as well as autosomal dominant macular degeneration<sup>4,55</sup>.

## 20. *KCNJ13* is a rare cause of LCA

*KCNJ13* is a potassium channel and are members of inwardly rectifying potassium (Kir) channels which are expressed in a wide variety of cells involved in maintaining the resting membrane potential near the potassium (K) equilibrium potential. *KCNJ13* is expressed in the apical microvilli of retinal pigment epithelia, but not in neural retina or in choroid. Mutations in *KCNJ13* are found in Snowflake vitreoretinal degeneration<sup>1</sup>. *KCNJ13* is a very rare cause of LCA, with only 3 mutations known<sup>3</sup>. Sergouniotis *et al.* in 2011 and Pattnaik *et al.* found biallelic *KCNJ13* mutations in 3 LCA patients<sup>56,57</sup>. The phenotype was severe with nummular pigment and retinal degeneration and thinning, without lenticular and vitreal involvements.

*CAPB4* and *GDF6* are likely not LCA genes. *CAPB4*, aka Ca<sup>2+</sup>-binding protein 4, is a Cav1.4 channel regulator that plays an essential role in photoreceptor synaptic function<sup>58</sup>. *CAPB4* is localized to cone and rod photoreceptor synaptic vesicles. Littink *et al.* in 2009 found novel homozygous nonsense mutations in *CAPB4* in patients with an unusual ERG without night blindness and suggested a likely congenital cone-rod synaptic disorder<sup>59</sup>. Aldahmeah *et al.*, in 2010, then found *CAPB4* mutations in a consanguineous Bedouin family and reported an LCA type phenotype characterized by normal appearing retinas even in early adulthood<sup>60</sup>. LCA can have normal appearing retinas, but eventually subtle vessel narrowing and retinal pigmentation is almost always seen and usually LCA is progressive. One of the sibs had a decreased cone ERG and an almost normal rod ERG, which is not compatible with the diagnosis of LCA. Also *CAPB4* is expressed in the synapses, not the photoreceptor cell body, outer or inner segment where all the other LCA gene and proteins are found. Taken together, these are five strikes against LCA, and more likely a cone-rod

<sup>1</sup>Snowflake vitreoretinal degeneration is a developmental and progressive hereditary multilayer eye disorder including fibrillar degeneration of the vitreous humor, early-onset cataract, minute crystalline deposits in the neurosensory retina, and retinal detachment.



synaptic disorder. *GDF6* aka Growth/differentiation factor-6 is a member of the transforming growth factor-beta. In immunohistochemical analyses of mouse retina, Zhang *et al.* detected *GDF6* in ganglion cell layer, inner plexiform layer, and retinal pigment epithelia, not the photoreceptors<sup>61</sup>. Duplication and deletions of *GDF6* cause microphthalmia and colobomas (clefts of the iris and retina). Asai-Coakwell *et al.* found two LCA/juvenile RP patients with biallelic *GDF6* mutations<sup>62</sup>. *GDF6* was listed as a gene for LCA. Subsequently, these two patients were found to have *PRPH2* mutations that co-segregated in the family and are more likely the cause of disease. Extensive WES studies did not document further *GDF6* mutations in LCA in large cohorts. In conjunction with the fact that *GDF6* is expressed in retina but not in photoreceptor, the LCA link is likely not tenable. *GDF6* mutations are likely very important for retinal function, but unlikely to be causal for LCA by themselves.

## LCA disease subtypes

“Photoaversion can be a prominent clinical feature in some patients with Leber congenital amaurosis. The ERG clinches the diagnosis. These patients may constitute a distinct genetic subtype of the disease and molecular genetic studies will help resolve this issue.” (Traboulsi and Maumenee 1995). Also, the ateliotic fovea defined as an unfinished primordial fovea, may represent a subtype of retinal disease (Maumenee, 2001).

Initially, at the time of first description by Theodor Leber in 1869, LCA was defined as congenital retinal blindness with nystagmus and poorly reactive pupils (“*tapetoretinal degeneration mit amblyopie*”) and thought to represent a homogenous form of congenital blindness<sup>63</sup>. Subtypes were not considered. Histological studies (pre-genome) suggested several different morphological subtypes of LCA<sup>64</sup>. The genetic and genomic era solidified that perception, as currently 20 genetic types of LCA are known<sup>4</sup>. The currently known 20 LCA genes participate in seven disparate retinal cycles/processes. These retinal cycles/processes are likely to harbour the still unknown LCA genes.

These seven retinal cycles/processes are interrupted resulting in one of the seven **LCA disease pathways**, and result in similar phenotypes and likely require similar treatments. **LCA disease pathway 1** is in the phototransduction cascade, the retinal enzymatic process of capturing a photon of light, resulting in photoreceptor ion channel closure and hyperpolarization (*GYCY2D*, *AIPL1*, and *RD3*). **Disease pathway 2** is in the retinoid cycle, to replenish 11 cis retinal after it is converted to all trans-retinal (*RPE65*, *LRAT* and *RDH12*). **Disease pathway 3** is in retinal transcription factors (*CRX*). **Disease pathway 4** is in the Intra Flagellar Transport (IFT), the anterograde and retrograde protein transport (conveyor belt) from inner to outer segment and back again (*SPATA7*, *LCA5*, *RPGRI1*, *CEP290*, *TULP1*, *NPHP5*, *CLUAP1* and *IFT140*). **Disease pathway 5** is in photoreceptor structure, both external morphogenesis and internal cell structure (*CRB1* and *PRPH2*). **Disease pathway 6** is in metabolic enzymes for cellular survival (*IMPDH1* and *NMNAT1*). **Disease pathway 7** is in photoreceptor ion channels, such as Potassium ion channels (*KCNJ13*).

By clinical definition, LCA patients are phenotypically relatively homogeneous and invariably show severe visual loss early in life with nystagmus, sluggish pupils, oculodigital

sign, and relatively normal appearing retinas (with later changes), but the retinal morphological basis of these visual malfunctions differs among genotypes<sup>64,65</sup>.

Jacobson *et al.*, in 2016, categorized the retinal morphological structural changes with *in-vivo* retinal architectural studies by optical coherence tomography (OCT) and concluded that some are associated with a specific genotype while others are indistinguishable<sup>13</sup>. Based on their classification, we can identify four major retinal structural categories (OCT types of LCA):

#### **OCT type 1: Normal *in vivo* retinal architecture**

Some OCT scans of LCA patients are remarkable for normal retinal layers thickness. Other young LCA patients may have subnormal retinal and ONL thicknesses within the central few degrees and normal thickness across the rest of the scan. This pattern was typical of LCA patients with *GUCY2D* mutations.

#### **OCT type 2: Thickened retina**

This pattern is specific for a reduced foveal ONL, limited extra-central ONL and thickened dysplastic-appearing retina across the remainder of the retina. Retained central ONL and better acuity in some patients may lead to a diagnosis of CRB1-RP<sup>65</sup>. Whether LCA or RP, patients show extra-central coarse and abnormal lamination with thickening.

#### **OCT type 3: Normal foveal architecture**

Five genotypes showed a preserved central island of ONL that decreases in thickness eccentrically. The foveal ONL peak could be normal or reduced. A common mechanism resulting in this pattern is a ciliopathy, which includes *Lebercilin* (LCA5), *RPGRIP1* (LCA6), *CEP290* (LCA10), and *TULP1* (LCA15), although the latter disorder may have a more complex mechanism<sup>66</sup>. *RPE65* (LCA2) could show a similar pattern. Retinal thickness was at the lower limit of normal or actually subnormal, in contrast, for example, to *CRB1* (LCA8).

#### **OCT type 4: Severe macular atrophy or foveal mal-development**

This group includes two with macular disease (*AIPL1* (LCA4) and *RDH12* (LCA13)) and one that appears to be a developmental abnormality with lack of foveal formation and inner retinal laminae crossing the central retina (*NMNATI* (LCA9)). ONL thickness in all three is detectable, but reduced centrally. Retinal thickness varies in *AIPL1* (LCA4) and *RDH12* (LCA13), while the *NMNATI* (LCA15) patient had a thinned retina.

This last retinal subtype was predicted and suggested in 2001 by Irene Maumenee. “The ateliotic macula” coined by Irene Maumenee was defined and characterized by an unfinished or primordial appearance.

Pregenome histological studies of LCA eyes from cadavers or blind painful enucleated eyes revealed a wide diversity of morphological retinal subtypes. Koenekoop in 2004 summarized 13 retinal studies of these LCA patients eyes<sup>67</sup>. These studies have led to a view that LCA may not be one disorder, but multiple disorders, which may be classified into three types of

histological categories: **Histological type 1**: an aplasia (or dysplasia), with abnormal embryological formation of photoreceptors; **Histological type 2**: a retinal degeneration, with early, progressive photoreceptor cell death; and **Histological type 3**; a retinal dysfunction, in which the retinal anatomy remains essentially normal, but a key biochemical message is missing<sup>67</sup>. These three histological types correlate with the OCT categories proposed by Jacobson *et al.* in 2016<sup>13</sup>. We may also predict that the retinal aplasia (or dysplasia) found by histology and by OCT may be represented by *CRB1*. Retinal degenerations are found in most LCA patients and are represented by *RPGRIP1*, *LCA5*, *RPE65* and other genes. Retinal dysfunction is likely represented by *GUCY2D*.

Thus despite the relatively homogeneous and severe clinical phenotype of LCA, there appear to be at least three major retinal morphological groups, based on OCT and histological studies<sup>13,64</sup>. Visual loss can be due to severe outer retinal degeneration (**Group 1** called retinal degeneration) or retinal disorganization with loss of photoreceptors in early life (**Group 2** called retinal dysplasia). A few molecular forms of LCA, however, have unexpectedly shown retained outer retinal structure and appear to be dysfunctions (**Group 3** called retinal dysfunctions)<sup>13,64</sup>.

## Treatment options

In 1994, before the explosion of molecular, genetic and genomic discoveries of the disease pathways of LCA, no-one would have predicted 20 LCA genes (this review), seven LCA disease pathways (this review), three LCA histological types<sup>68</sup>, four LCA OCT types<sup>13</sup> and six potential treatments. Today, LCA remains officially untreatable, but at least six treatments for LCA are in human clinical trials. It is likely that at least for some types of LCA a treatment will be available to our patients soon.

The LCA patient and his family I saw with Dr Maumenee in 1994 may now be eligible for a clinical trial or may be authorized to an approved treatment soon. The exciting LCA treatments being tested in humans right now are gene replacement, stem cell replacement, retinal camera, CNTF, an oral drug and gene editing.

Excellent reviews of human RPE65 **gene replacement** in LCA are available<sup>69,70</sup>. RPE65 gene replacement works to increase vision and is safe, in repeated trials, one or both eyes. The consensus on the durability of RPE65 gene replacement therapy is still debatable. On the one hand, Brainbridge *et al.* (the London group), in their trial, found that the effect wears off with subsequent follow-ups and the degeneration may not be halted, perhaps calling for earlier therapy<sup>69-72</sup>. On the other hand, Maguire *et al.* and Testa *et al.* (the Philadelphia group) reported a three years maintained and stable improvement in visual acuity, area of the visual field enlargement and nystagmus<sup>73,74</sup>. It is worth mentioning that both studies used different adeno-associated virus vectors, delivery techniques as well as different patients' age ranges.

Other LCA gene replacement trials are likely to start soon (*GUCY2D*, *AIPL1*, *RPGRIP1*, *LCA5*, *CEP290* and others)<sup>75,76</sup>. **Stem cell therapy** to introduce retinal progenitor cells into the atrophic LCA retinas is now in phase I clinical trials<sup>77</sup>. LCA patients with severe retinal

cell loss and patients with maculopathies such as NMNAT1, AIPL1 and RDH12 are potential candidates. The **Argus II camera** captures visual information through the inner retina and has been approved for adults with retinal degenerations. This device will likely be available to children in the near future. **CNTF** is a neurotrophic factor that has been shown to rescue photoreceptors in animal models of retinal degeneration. In human trials CNTF treatments show variable responses, from maintaining viable cone photoreceptors<sup>78</sup> to no response on visual fields<sup>79</sup>. So far, CNTF studies were conducted only in adults. In the future, it is likely that CNTF will be tested in children but its effects could be less appreciated compared to adults as the age of patient markedly changes its predicted pathophysiological effect. An **oral drug QLT 091001** was shown to be safe and efficacious in children with RPE65 and LRAT mutations<sup>80</sup> and in older adults with RP and RPE65 and LRAT mutations<sup>81</sup>, and this drug is now entering a phase III trial. An example of **genetic editing** is anti-sense oligonucleotide (ASO) treatment, targeting the very common LCA CEP290 mutation (c.2991 + 1655A > G). This splice mutation creates a cryptic splice donor site resulting in the insertion of a pseudo exon into the CEP290 mRNA. ASO delivery fully restored CEP290 pre-mRNA splicing, significantly increased CEP290 protein levels and rescued a ciliary phenotype present in LCA patient-derived fibroblast cells<sup>82</sup>. A human clinical trial is being planned.

It is very likely that matching the treatment with the LCA disease types; the LCA OCT types and/or the LCA histological types will increase treatment success. Gene replacement and gene editing must be performed in LCA retinas that harbour living cells, thus in LCA OCT type 1 and 3, but not likely in LCA OCT type 2 and 4. Success with gene replacement and editing is more likely for LCA histological type 2 (before the degeneration is too far advanced) and in type 3, but not likely in type 1. Stem cell replacement is likely reserved for LCA OCT type 4 and for advanced degeneration in all types. Oral drugs and CNTF is more likely to succeed in early degeneration and in the retinal dysfunctions. Argus II is reserved for advanced LCA with some intact inner retinal architecture. Combination therapies are likely to be tested soon.

The future for LCA is very bright. From nothing to 20 genes and six treatments in 20 years. We can now genetically diagnose LCA in at least 70% of patients, guide their careers, aid their schooling and help plan their families. Some patients have entered gene specific treatment trials; others will soon follow. Due to Irene Maumenee's visions and mentorships, she has stimulated, guided and participated in this remarkable evolution.

## Acknowledgments

This article is dedicated to Irene Hussels-Maumenee, Professor of Human Genetics and Ophthalmology, Johns Hopkins' Wilmer Eye Institute, Ocular Genetics Fellowship director in 1994–1995.

### **Funding**

FFB–Canada, CIHR, FRSQ, and NIH to RKK.

## References

1. [accessed on Nov. 3rd 2016] GeneReviews® [Internet]. <https://www.ncbi.nlm.nih.gov/books/NBK1298/>, which was
2. Oliveira L, Miniou P, Viegas-Pequignot E, et al. Human retinal guanylate cyclase (GUC2D) maps to chromosome 17p13.1. *Genomics*. 1994; 22:478–481. [PubMed: 7806240]
3. Duda T, Venkataraman V, Goracznik R, et al. Functional consequences of a rod outer segment membrane guanylate cyclase (ROS-GC1) gene mutation linked with Leber's congenital amaurosis. *Biochemistry*. 1999; 38:509–515. [PubMed: 9888789]
4. The Human Gene Mutation Database (HGMD®). <http://www.hgmd.cf.ac.uk>
5. Rozet JM, Perrault I, Gerber S, et al. Complete abolition of the retinal-specific guanylyl cyclase (retGC-1) catalytic ability consistently leads to leber congenital amaurosis (LCA). *Invest Ophthalmol Vis Sci*. 2001; 42:1190–2. [PubMed: 11328726]
6. Galvin JA, Fishman GA, Stone EM, et al. Evaluation of genotype-phenotype associations in leber congenital amaurosis. *Retina*. 2005; 25:919–29. [PubMed: 16205573]
7. Morimura H, Fishman GA, Grover SA, et al. Mutations in the RPE65 gene in patients with autosomal recessive retinitis pigmentosa or Leber congenital amaurosis. *Proc Nat Acad Sci*. 1998; 95:3088–3093. [PubMed: 9501220]
8. Moiseyev G, Chen Y, Takahashi Y, et al. RPE65 is the isomerohydrolase in the retinoid visual cycle. *Proc Nat Acad Sci*. 2005; 102:12413–12418. [PubMed: 16116091]
9. Marlhens F, Bareil C, Griffoin J-M, et al. Mutations in RPE65 cause Leber's congenital amaurosis. (Letter) *Nature Genet*. 1997; 17:139–141. [PubMed: 9326927]
10. [Accessed on Nov. 1 2016] OMIM database. <http://www.omim.org/entry/180069>
11. Eblimit A, Nguyen TM, Chen Y, et al. Spata7 is a retinal ciliopathy gene critical for correct RPGRIP1 localization and protein trafficking in the retina. *Hum Mol Genet*. 2015; 24:1584–601. [PubMed: 25398945]
12. Mackay DS, Ocaka LA, Borman AD, et al. Screening of SPATA7 in patients with Leber congenital amaurosis and severe childhood-onset retinal dystrophy reveals disease-causing mutations. *Invest Ophthalmol Vis Sci*. 2011; 52:3032–3038. [PubMed: 21310915]
13. Jacobson SG, Cideciyan AV, Huang WC, et al. Leber Congenital Amaurosis: Genotypes and Retinal Structure Phenotypes. *Adv Exp Med Biol*. 2016; 854:169–75. [PubMed: 26427408]
14. Sohocki MM, Perrault I, Leroy BP, et al. Prevalence of AIPL1 mutations in inherited retinal degenerative disease. *Molec Genet Metab*. 2000; 70:142–150. [PubMed: 10873396]
15. van der Spuy J, Chapple JP, Clark BJ, et al. The Leber congenital amaurosis gene product AIPL1 is localized exclusively in rod photoreceptors of the adult human retina. *Hum Molec Genet*. 2002; 11:823–831. [PubMed: 11929855]
16. Ramamurthy V, Roberts M, van den Akker F, et al. AIPL1, a protein implicated in Leber's congenital amaurosis, interacts with and aids in processing of farnesylated proteins. *Proc Nat Acad Sci*. 2003; 100:12630–12635. [PubMed: 14555765]
17. Pennesi ME, Stover NB, Stone EM, et al. Residual electroretinograms in young Leber congenital amaurosis patients with mutations of AIPL1. *Invest Ophthalmol Vis Sci*. 2011; 52:8166–73. [PubMed: 21900377]
18. Dharmaraj S, Li Y, Robitaille JM, IH, et al. A novel locus for Leber congenital amaurosis maps to chromosome 6q. (Letter) *Am J Hum Genet*. 2000; 66:319–326. [PubMed: 10631161]
19. Boldt K, Mans DA, Won J, et al. Disruption of intraflagellar protein transport in photoreceptor cilia causes Leber congenital amaurosis in humans and mice. *J Clin Invest*. 2011; 121:2169–80. [PubMed: 21606596]
20. Mohamed MD, Topping NC, Jafri H, et al. Progression of phenotype in Leber's congenital amaurosis with a mutation at the LCA5 locus. *Br J Ophthalmol*. 2003; 87:473–5. [PubMed: 12642313]
21. Dryja TP, Adams SM, Grimsby JL, et al. Null RPGRIP1 alleles in patients with Leber congenital amaurosis. *Am J Hum Genet*. 2001; 68:1295–1298. [PubMed: 11283794]

22. Mavlyutov TA, Zhao H, Ferreira PA. Species-specific subcellular localization of RPGR and RPGRIP isoforms: implications for the phenotypic variability of congenital retinopathies among species. *Hum Molec Genet.* 2002; 11:1899–1907. [PubMed: 12140192]
23. Roepman R, Bernoud-Hubac N, Schick DE, et al. The retinitis pigmentosa GTPase regulator (RPGR) interacts with novel transport-like proteins in the outer segments of rod photoreceptors. *Hum Molec Genet.* 2000; 9:2095–2105. [PubMed: 10958648]
24. Kimura A, Singh D, Wawrousek EF, et al. Both PCE-1/RX and OTX/CRX interactions are necessary for photoreceptor-specific gene expression. *J Biol Chem.* 2000; 275:1152–60. [PubMed: 10625658]
25. Akagi T, Mandai M, Ooto S, et al. Otx2 homeobox gene induces photoreceptor-specific phenotypes in cells derived from adult iris and ciliary tissue. *Invest Ophthal Vis Sci.* 2004; 45:4570–4575. [PubMed: 15557469]
26. Swaroop A, Wang Q, Wu W, et al. Leber congenital amaurosis caused by a homozygous mutation (R90W) in the homeodomain of the retinal transcription factor CRX: direct evidence for the involvement of CRX in the development of photoreceptor function. *Hum Mol Genet.* 1999; 8:299–305. [PubMed: 9931337]
27. Nichols LL 2nd, Alur RP, Boobalan E, et al. Two novel CRX mutant proteins causing autosomal dominant Leber congenital amaurosis interact differently with NRL. *Hum Mutat.* 2010; 31(6):E1472–83. [PubMed: 20513135]
28. Pellikka M, Tanentzapf G, Pinto M, et al. Crumbs, the *Drosophila* homologue of human CRB1/RP12, is essential for photoreceptor morphogenesis. *Nature.* 2002; 416:143–149. [PubMed: 11850625]
29. Lotery AJ, Malik A, Shami SA, et al. CRB1 mutations may result in retinitis pigmentosa without para-arteriolar RPE preservation. *Ophthalmic Genet.* 2001; 22:163–9. [PubMed: 11559858]
30. Jacobson SG, Cideciyan AV, Aleman TS, et al. Crumbs homolog 1 (CRB1) mutations result in a thick human retina with abnormal lamination. *Hum Molec Genet.* 2003; 12:1073–1078. [PubMed: 12700176]
31. Keen TJ, Mohamed MD, McKibbin M, et al. Identification of a locus (LCA9) for Leber's congenital amaurosis on chromosome 1p36. *Europ J Hum Genet.* 2003; 11:420–423. [PubMed: 12734549]
32. Emanuelli M, Carnevali F, Saccucci F, et al. Molecular cloning, chromosomal localization, tissue mRNA levels, bacterial expression, and enzymatic properties of human NMN adenylyltransferase. *J Biol Chem.* 2001; 276:406–12. [PubMed: 11027696]
33. Koenekoop RK, Wang H, Majewski J, et al. Mutations in NMNAT1 cause Leber congenital amaurosis and identify a new disease pathway for retinal degeneration. *Nat Genet.* 2012; 44:1035–9. [PubMed: 22842230]
34. Valente EM, Silhav JL, Brancati F, et al. Mutations in CEP290, which encodes a centrosomal protein, cause pleiotropic forms of Joubert syndrome. *Nature Genet.* 2006; 38:623–625. [PubMed: 16682970]
35. Stowe TR, Wilkinson CJ, Iqbal A, et al. The centriolar satellite proteins Cep72 and Cep290 interact and are required for recruitment of BBS proteins to the cilium. *Molec Biol Cell.* 2012; 23:3322–3335. [PubMed: 22767577]
36. den Hollander AI, Koenekoop RK, Yzer S, et al. Mutations in the *CEP290* (*NPHP6*) Gene Are a Frequent Cause of Leber Congenital Amaurosis. *Am J Hum Genet.* 2006; 79(3):556–61. [PubMed: 16909394]
37. Perrault I, Delphin N, Hanein S, et al. Spectrum of NPHP6/CEP290 mutations in Leber congenital amaurosis and delineation of the associated phenotype. *Hum Mutat.* 2007; 28:416.
38. Collart FR, Huberman E. Cloning and sequence analysis of the human and Chinese hamster inosine-5-prime-monophosphate dehydrogenase cDNAs. *J Biol Chem.* 1988; 263:15769–15772. [PubMed: 2902093]
39. Molday LL, Djajadi H, Yan P, et al. RD3 gene delivery restores guanylate cyclase localization and rescues photoreceptors in the Rd3 mouse model of Leber congenital amaurosis 12. *Hum Molec Genet.* 2013; 22:3894–3905. [PubMed: 23740938]



40. Preising MN, Hausotter-Will N, Solbach MC, et al. Mutations in RD3 are associated with an extremely rare and severe form of early onset retinal dystrophy. *Invest Ophthalmol Vis Sci.* 2012; 53:3463–3472. [PubMed: 22531706]
41. Haeseleer F, Jang G-F, Imanishi Y, et al. Dual-substrate specificity short chain retinol dehydrogenases from the vertebrate retina. *J Biol Chem.* 2002; 277:45537–45546. [PubMed: 12226107]
42. McBee JK, Palczewski K, Baehr W, et al. Confronting complexity: the interlink of phototransduction and retinoid metabolism in the vertebrate retina. *Prog Retin Eye Res.* 2001; 20:469–529. [PubMed: 11390257]
43. Jacobson SG, Cideciyan AV, Aleman TS, et al. RDH12 and RPE65, visual cycle genes causing Leber congenital amaurosis, differ in disease expression. *Invest Ophthalmol Vis Sci.* 2007; 48:332–338. [PubMed: 17197551]
44. Xue L, Gollapalli DR, Maiti P, et al. A palmitoylation switch mechanism in the regulation of the visual cycle. *Cell.* 2004; 117:761–771. [PubMed: 15186777]
45. den Hollander AI, Lopez I, Yzer S, et al. Identification of novel mutations in patients with Leber congenital amaurosis and juvenile RP by genome-wide homozygosity mapping with SNP microarrays. *Invest Ophthalmol Vis Sci.* 2007; 48:5690–5698. [PubMed: 18055821]
46. Xi Q, Pauer GJ, Marmorstein AD, et al. Tubby-like protein 1 (TULP1) interacts with F-actin in photoreceptor cells. *Invest Ophthalmol Vis Sci.* 2005; 46:4754–61. [PubMed: 16303976]
47. Mataftsi A, Schorderet DF, Chachoua L, et al. Novel TULP1 mutation causing Leber congenital amaurosis or early onset retinal degeneration. *Invest Ophthalmol Vis Sci.* 2007; 48:5160–5167. [PubMed: 17962469]
48. Otto EA, Loeys B, Khanna H, et al. Nephrocystin-5, a ciliary IQ domain protein, is mutated in Senior-Loken syndrome and interacts with RPGR and calmodulin. *Nature Genet.* 2005; 37:282–288. [PubMed: 15723066]
49. Estrada-Cuzcano A, Koenekoop RK, Coppieters F, et al. IQCB1 mutations in patients with leber congenital amaurosis. *Invest Ophthalmol Vis Sci.* 2011; 52:834–9. [PubMed: 20881296]
50. Lee C, Wallingford JB, Gross JM. Cluap1 is essential for ciliogenesis and photoreceptor maintenance in the vertebrate eye. *Invest Ophthalmol.* 2014; 55:4585–4592.
51. Botilde Y, Yoshida S, Shinohara K, et al. Cluap1 localizes preferentially to the base and tip of cilia and is required for ciliogenesis in the mouse embryo. *Dev Biol.* 2013; 381:203–212. [PubMed: 23742838]
52. Xu M, Yang L, Wang F, Li H, et al. Mutations in human IFT140 cause non-syndromic retinal degeneration. *Hum Genet.* 2015; 134:1069–78. [PubMed: 26216056]
53. Perrault I, Saunier S, Hanein S, et al. Mainzer-Saldino syndrome is a ciliopathy caused by IFT140 mutations. *Am J Hum Genet.* 2012; 90:864–870. [PubMed: 22503633]
54. Travis GH, Christerson L, Danielson PE, et al. The human retinal degeneration slow (RDS) gene: chromosome assignment and structure of the mRNA. *Genomics.* 1991; 10:733–739. [PubMed: 1679750]
55. [Accessed on Nov. 3rd 2016] OMIM database. <https://omim.org/entry/179605>
56. Sergouniotis PI, Davidson AE, Mackay DS, et al. Recessive mutations in KCNJ13, encoding an inwardly rectifying potassium channel subunit, cause Leber congenital amaurosis. *Am J Hum Genet.* 2011; 89:183–190. [PubMed: 21763485]
57. Pattnaik BR, Shahi PK, Marino MJ, et al. A novel KCNJ13 nonsense mutation and loss of Kir7.1 channel function causes Leber congenital amaurosis (LCA16). *Hum Mutat.* 2015; 36:720–727. [PubMed: 25921210]
58. Haeseleer F, Imanishi Y, Maeda T, et al. Essential role of Ca(2+)-binding protein 4, a Ca(V)1.4 channel regulator, in photoreceptor synaptic function. *Nature Neurosci.* 2004; 7:1079–1087. [PubMed: 15452577]
59. Littink KW, van Genderen MM, Collin RWJ, et al. A novel homozygous nonsense mutation in CABP4 causes congenital cone-rod synaptic disorder. *Invest Ophthalmol Vis Sci.* 2009; 50:2344–2350. [PubMed: 19074807]
60. Aldahmesh MA, Al-Owain M, Alqahtani F, et al. A null mutation in CABP4 causes Leber's congenital amaurosis-like phenotype. *Molec Vision.* 2010; 16:207–212.

61. Zhang L, Lim SL, Du H, et al. High temperature requirement factor A1 (HTRA1) gene regulates angiogenesis through transforming growth factor-beta family member growth differentiation factor 6. *J Biol Chem.* 2012; 287:1520–1526. [PubMed: 22049084]
62. Asai-Coakwell M, French CR, Berry KM. GDF6, a novel locus for a spectrum of ocular developmental anomalies. *Am J Hum Genet.* 2007; 80:306–315. [PubMed: 17236135]
63. Leber T. Über Retinitis pigmentosa und angeborene Amaurose. *Archiv für Ophthalmologie* (in German). 1869; 15:1–25.
64. Koenekoop RK, Fishman GA, Iannaccone A, et al. Electroretinographic abnormalities in parents of patients with Leber congenital amaurosis who have heterozygous GUCY2D mutations. *Arch Ophthalmol.* 2002; 120:1325–30. [PubMed: 12365911]
65. Jacobson SG, Cideciyan AV, Aleman TS, et al. Crumbs homolog 1 (CRB1) mutations result in a thick human retina with abnormal lamination. *Hum Mol Genet.* 2003; 12:1073–8. [PubMed: 12700176]
66. Jacobson SG, Cideciyan AV, Huang WC, et al. TULP1 mutations causing early-onset retinal degeneration: preserved but insensitive macular cones. *Invest Ophthalmol Vis Sci.* 2014; 55:5354–64. [PubMed: 25074776]
67. Koenekoop RK. An overview of Leber congenital amaurosis: a model to understand human retinal development. *Surv Ophthalmol.* 2004; 49:379–98. [PubMed: 15231395]
68. Koenekoop, RK. Retinal Degenerations: Biology, Diagnostics, and Therapeutics. In: Tombran-Tink, Joyce, Barnstable, Colin J., editors. *Leber congenital amaurosis*. Publisher Humana Press; p. 61-90. Copyright 2007
69. Koenekoop RK. Successful RPE65 gene replacement and improved visual function in humans. *Ophthalmic Genet.* 2008; 29:89–91. [PubMed: 18766986]
70. den Hollander AI, Roepman R, Koenekoop RK, et al. Leber congenital amaurosis: genes, proteins and disease mechanisms. *Prog Retin Eye Res.* 2008; 27:391–419. [PubMed: 18632300]
71. Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med.* 2008; 358(21):2231–9. [PubMed: 18441371]
72. Bainbridge JW, Mehat MS, Sundaram V, et al. Long-term effect of gene therapy on Leber's congenital amaurosis. *N Engl J Med.* 2015; 372(20):1887–97. [PubMed: 25938638]
73. Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med.* 2008; 358(21):2240–8. [PubMed: 18441370]
74. Testa F, Maguire AM, Rossi S, et al. Three-year follow-up after unilateral subretinal delivery of adeno-associated virus in patients with Leber congenital Amaurosis type 2. *Ophthalmology.* 2013; 120(6):1283–91. [PubMed: 23474247]
75. Burnight ER, Wiley LA, Drack AV, et al. CEP290 gene transfer rescues Leber congenital amaurosis cellular phenotype. *Gene Ther.* 2014; 21(7):662–72. [PubMed: 24807808]
76. Pawlyk BS, Bulgakov OV, Liu X, et al. Replacement gene therapy with a human RPGRIP1 sequence slows photoreceptor degeneration in a murine model of Leber congenital amaurosis. *Hum Gene Ther.* 2010; 21(8):993–1004. [PubMed: 20384479]
77. [Accessed on Aug 5th 2016] [www.clinicaltrials.gov](http://www.clinicaltrials.gov)
78. Duncan, Jacque Lynne. Phase 2 Study of CNTF on Photoreceptor Structure in Retinitis Pigmentosa. National Institute of Health (NIH); Project #: 5R01FD004100-2. Project start: 2012-09-15. Project end: 2017-05-31
79. Birch DG, Bennett LD, Duncan JL, et al. Long-term follow-up of patients with retinitis pigmentosa (RP) receiving intraocular ciliary neurotrophic factor implants. *Am J Ophthalmol.* 2016 Jul 22;doi: 10.1016/j.ajo.2016.07.013
80. Koenekoop RK, Sui R, Sallum J, et al. Oral 9-cis retinoid for childhood blindness due to Leber congenital amaurosis caused by RPE65 or LRAT mutations: an open-label phase 1b trial. *Lancet.* 2014; 384(9953):1513–20. [PubMed: 25030840]
81. Scholl HP, Moore AT, Koenekoop RK, et al. Safety and Proof-of-Concept Study of Oral QLT091001 in Retinitis Pigmentosa Due to Inherited Deficiencies of Retinal Pigment Epithelial 65 Protein (RPE65) or Lecithin:Retinol Acyltransferase (LRAT). *PLoS One.* 2015; 10:e0143846. [PubMed: 26656277]

82. Collin RW, den Hollander AI, van der Velde-Visser SD. Antisense Oligonucleotide (AON)-based Therapy for Leber Congenital Amaurosis Caused by a Frequent Mutation in CEP290. *Mol Ther Nucleic Acids*. 2012; 1:e14. [PubMed: 23343883]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript