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Hypomorphic mutations identified in candidate Leber congenital amaurosis disease gene *CLUAP1*

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Abstract

Purpose—Leber congenital amaurosis (LCA) is an early-onset form of retinal degeneration and six of the 22 known LCA disease genes encode photoreceptor ciliary proteins. Despite the identification of 22 LCA disease genes, the genetic basis of approximately 30% of LCA patients remains unknown. We sought to investigate the cause of disease in the remaining 30% by examining cilia-associated genes.

Methods—Whole-exome sequencing was performed on an LCA cohort of 212 unsolved probands previously screened for mutations in known retinal disease genes.

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SUPPLEMENTARY MATERIAL

Supplementary information is available at the Genetics in Medicine website (<http://www.nature.com/gim>).

Immunohistochemistry using mouse retinas was used to confirm protein localization and zebrafish were used to perform rescue experiments.

Results—A homozygous nonsynonymous mutation was found in a single proband in *CLUAPI*, a gene required for ciliogenesis and cilia maintenance. *Cluap1* knockout zebrafish exhibit photoreceptor cell death as early as five days post fertilization and rescue experiments revealed that our proband's mutation is significantly hypomorphic.

Conclusion—Consistent with the knowledge that *CLUAPI* plays an important role in cilia function and that cilia are critical to photoreceptor function, our results indicate that hypomorphic mutations in *CLUAPI* can result in dysfunctional photoreceptors without systemic abnormalities. This represents the first report linking mutations in *CLUAPI* to human disease and establishes *CLUAPI* as a candidate LCA gene.

Keywords

Leber congenital amaurosis (LCA); *CLUAPI*; early-onset retinal disease; photoreceptor connecting cilium; ciliopathy

INTRODUCTION

Leber congenital amaurosis (LCA, <http://www.omim.org/phenotypicSeries/PS204000>) is the most severe form of nonsyndromic inherited retinal disease with severe visual impairment within the first year of life. LCA is characterized by nystagmus and an extinguished electroretinogram (ERG). LCA affects between 1/30,000 and 1/80,000 individuals differing by population and accounts for 5% of all inherited retinal diseases.¹ As of 2015, 22 genes have been linked to LCA; however, conventional techniques for molecular diagnosis find mutations in these genes in only ~70% of LCA patients, highlighting the genetic heterogeneity of LCA.¹⁻⁵ Gene therapy in the retina of human LCA patients has made significant progress in restoring sight, emphasizing the importance of understanding the genetic etiology of every patient's disease to allow access to such technologies.⁶ Genetic counseling based on a patient's molecular diagnosis also provides the opportunity for mutation screening in offspring and spouses. Discovering the cause of disease in the remaining ~30% of LCA patients is therefore critical to enable treatment for this devastating disease.

An absent ERG, a hallmark of LCA, is indicative of malfunctioning photoreceptor cells which initiate the recorded electrical signal and are responsible for the generation of the a-wave.⁷ Every identified LCA disease gene has a role relevant to the proper functioning of photoreceptor cells.^{1,8-14} Photoreceptors are remarkable cells with a highly specialized morphology designed to facilitate photon detection and transduction of visual information. The most striking feature of photoreceptors cells is the elongated outer segment, which contains stacks of membrane discs which house the transmembrane photosensitive opsin proteins that capture photons and activate the visual transduction cascade. The outer segment is linked to the inner segment of photoreceptor cells by a cilium called the connecting cilium from which the outer segment develops; the outer segment in conjunction with the connecting cilium is considered a large modified primary cilium. The inner segment contains

the cell's protein synthesis machinery and therefore every protein with a role in the outer segment must be transported through the connecting cilium for proper function. The outer segment undergoes a massive rate of protein turnover in order to accomplish phototransduction including 10% of the outer segment being shed every day. Shed discs are phagocytized by neighboring retinal pigmented epithelial cells and are then replaced through the voluminous protein transport needed for new disc formation.¹⁵ The connecting cilium is therefore homologous to the transition zone of primary cilia found in other cell types and operates as a gatekeeper of protein trafficking to the outer segment.¹⁶

Dysfunction of the connecting cilium results in varying degrees of photoreceptor degeneration depending on which component of the connecting cilium is perturbed. In contrast to LCA cilia genes, mutations in *MAK*, which localizes to the connecting cilium, cause a form of retinal degeneration which onsets at an average age of 20.¹⁷ Highlighting the importance of cilia in retinal disease, ~15% of known retinal disease genes are involved in connecting cilium function. Interestingly, this proportion increases to ~25% when only considering known LCA genes (*CEP290*, *IQCB1*, *LCA5*, *RPGRIP1*, *SPATA7*, *TULP1*), supporting the established notion that cilia are critical to early stages of photoreceptor cell development and maintenance.^{9,18} Combining the knowledge that cilia genes are good retinal disease gene candidates with the knowledge that cilia are important for early photoreceptor development leads to the conclusion that cilia genes are excellent candidate LCA genes.¹⁹

Clusterin-associated protein 1 or *CLUAPI*, is required for ciliogenesis and localizes to the base and tip of cilia *in vitro* in mouse embryonic fibroblasts.²⁰ *CLUAP1* associates with the intraflagellar transport (IFT) complex B group of proteins and undergoes IFT in both invertebrates and vertebrates.^{21,22} *Cluap1*^{-/-} mice exhibit mid-gestation lethality due to developmental complications attributed to their lack of primary cilia.²⁰ Interestingly zebrafish with abolished *cluap1* expression also display premature mortality due to nonexistent ciliogenesis but can survive until at least 11 days post fertilization (dpf) allowing the examination of retinal tissue. Photoreceptor defects are apparent as early as 3 dpf, the same time point when wildtype zebrafish photoreceptors are undergoing outer segment formation, and rhodopsin in the mutant fish is mislocalized in the photoreceptor layer, an indication of aberrant IFT.²² Aberrant IFT precedes cell death in animal models of LCA caused by mutations in all six known LCA cilia genes.^{9,23-27} The retinas of *cluap1*^{-/-} zebrafish lack photoreceptor cells by 5 dpf while the remaining cell layers are intact.²⁸ The *CLUAP1* protein contains two major domains, an N-terminal calponin homology-like domain and a C-terminal coiled-coil domain. Both domains are highly conserved from zebrafish to humans and homologous domains can be found in many microtubule binding proteins.²⁹ The two major isoforms of *CLUAPI* are expressed in the human retina at moderate levels similar to IFT protein transcripts.³⁰

Based on this evidence we concluded *CLUAPI* is a cilia gene important for photoreceptor outer segment formation in vertebrates and therefore an excellent candidate LCA disease gene. In this study of 212 unsettled LCA patients, we found a single proband homozygous for a nonsynonymous amino acid substitution in *CLUAPI*. Rescue experiments using zebrafish as a model system proved that our mutant allele is hypomorphic.

MATERIALS AND METHODS

Patient Recruitment, Clinical Diagnosis, and DNA Preparation

Proband MOGL3628 was recruited and diagnosed with LCA at the Montreal Children's Hospital at the McGill University Health Centre. This patient was diagnosed with LCA after we found nystagmus and very poor visual fixation at 6 weeks of age. An ERG was non-detectable and the retina appeared normal. There were no systemic abnormalities. This study was approved by the McGill University Health Center Research Institute Research Ethics Board and adhered to the tenets of the declaration of Helsinki. Blood samples were collected from the proband and both parents after obtaining informed consent; DNA was extracted using the Qiagen blood genomic DNA extraction kit.

WES and Next-Generation Sequencing (NGS) Data Processing

1µg of total patient DNA was sheared into 300–500bp fragments. Fragments were end-repaired and a 3' adenosine base added. Illumina Y-shaped adapters were ligated to DNA fragments and 10 cycles of PCR were used to amplify the library with a unique barcode. Library DNA concentration was quantified using Life Technologies picogreen assay and the library was pooled together with five other libraries. 3µg of pooled DNA was capture enriched using the NimbleGen SeqCap EZ Human Exome Library v2.0 Hybridization and Wash Kit. Exome captured libraries were quantified and multiplexed sequenced on an Illumina HiSeq 2000.

NGS reads were mapped to the hg19 human reference genome using BWA-MEM, duplicate reads were removed using Picard, local realignments were performed using GATK, and variants were called using Atlas2. A population frequency threshold of 0.5% was used to filter out common variants which occur too frequently to be the cause of a rare Mendelian disease. Four NGS cohort databases were used to determine allele frequencies. The functional consequence of remaining rare variants was annotated using ANNOVAR, and dbNSFP was used to compile *in silico* predictions about the deleteriousness of nonsynonymous variants. UGENE was used to perform the multiple sequence alignment using the MUSCLE alignment algorithm.

Please see the Supplementary Methods for references and details.

Sanger Sequencing

Sanger sequencing was used to confirm the authenticity of the variant identified by NGS, to confirm the variant properly segregated in the proband's parents, and to screen five additional LCA probands for mutations in *CLUAP1*. A PCR primer pair was designed for each exon of interest. After PCR amplification, the amplicons were sequenced on an Applied Biosystems 3730XL or 3500XL Genetic Analyzer.

Immunohistochemistry

In vivo immunohistochemistry was performed on adult mouse retinal sections. Anti-CLUAP1 and anti-acetylated α -tubulin were used for primary antibodies while DAPI was used as a counterstain. *In vitro* immunohistochemistry was performed on hTERT-RPE1 cells

transiently transfected to overexpress human FLAG-tagged *CLUAPI* under control of a CMV promoter. *CLUAPI* cDNA was mutagenized to recreate the proband's mutation. Anti-FLAG tag and anti-acetylated α -tubulin were used for primary antibodies while DAPI was used as a counterstain.

Please see the Supplementary Methods for experimental details, reagent sources, and reagent concentrations.

Zebrafish Functional Experiments

The proband's mutation was recreated at the homologous zebrafish cDNA residue. Capped mRNA was amplified, template DNA degraded, and mRNA purified. Embryos were lysed following a previously published protocol.³¹ Day 0 embryos were lysed at 8~9 hours post fertilization (hpf), and day 1 embryos were lysed at 24 hpf. Western blotting was performed using anti-GFP. Anti- β -tubulin was used as a loading control. Zebrafish rescue experiments were performed by injecting embryos from *cluap1*^{-/-} incrosses at the one cell stage with varying concentrations of zebrafish wildtype and mutant *cluap1* mRNA tagged with *GFP*. *GFP* mRNA was injected as a negative control. An average of 96.5 embryos were injected per allele per concentration as well as for controls. The percent of phenotypic zebrafish was quantified at 3 dpf. Rescue experiments were performed twice thus reported data is the average of two experiments. P-values were obtained using a student's t-test. Error bars represent the standard deviation of the two independent experiments.

RESULTS

WES Identifies Homozygous Variants in *CLUAP1* as Candidate Pathogenic Mutations in an LCA Proband

Saudi Arabian proband MOGL3628, currently age 5, exhibited severe visual function limited to light perception by 6 weeks of age accompanied by nystagmus, the oculo-digit sign, and an extinguished ERG (Table 1). The proband's fundi appeared relatively normal (Supplementary Figure S1). Whole exome sequencing identified 349 rare protein altering variants (Supplementary Table S1). No causal mutations were found among the 8 variants located in known retinal disease genes (Supplementary Table S2) so a list of all potentially biallelic variants was created assuming the recessive inheritance pattern usually seen in LCA patients (Supplementary Table S3). Due to the importance of cilia associated genes in retinal disease, a list of cilia genes (Supplementary Methods) was compared to the genes present in the biallelic variant list and the cilia gene *CLUAP1* was identified as containing a homozygous nonsynonymous mutation which was used as reasoning for further analysis of this proband. All 35 potentially biallelic variants in 21 genes were subsequently subjected to a systematic candidate disease gene prioritization strategy in order to exclude or prioritize each gene for further testing. Both gene-level and variant-level information were leveraged during the prioritization process and the primary reasons behind gene exclusion or prioritization are included in Supplementary Table S3. A description of the process is found in the Supplementary Methods. The mutations in *CLUAP1* were deemed the most likely candidates for the cause of disease and so their authenticity was validated and proper segregation of the mutations confirmed by Sanger sequencing (Figure 1A).

CLUAP1 has two isoforms, which are both expressed in the human retina, a long isoform (NM_015041, NP_055856) which encodes 413 amino acids and a short isoform (NM_024793, NP_079069) which encodes 247 amino acids. The mutation occurs in the eighth exon of *CLUAP1* and affects the cDNA sequence of both isoforms, c.817C>T long, c.319C>T short (Table 2). This mutation results in a leucine to phenylalanine substitution p.L273F long and p.L107F short (Table 2), which is predicted to be either damaging or have a functional impact by 11/12 of the *in silico* prediction algorithms queried in dbNSFP (Table 2). The mutation is extraordinarily rare and is found only in the Exome Aggregation Consortium database at a frequency of 1/121,086 chromosomes or 0.0008%, although Saudi Arabians are underrepresented in the examined databases and the single individual seen to be a carrier for the allele is of reported European descent (Table 2).

Our proband's mutation falls in *CLUAP1*'s coiled-coil domain, one of two characterized functional domains in the *CLUAP1* protein. Both the coiled-coil and calponin homology-like domains have been reported to be involved in microtubule binding activity as shown in IFT proteins with homologous domains. The coiled-coil domain is also associated with facilitating protein-protein interactions, and it is within this region that our proband's mutation lies (Figure 1B). A protein multiple sequence alignment of human *CLUAP1* and its orthologs reveals that the affected leucine residue is conserved in all sighted organisms, including the fruitfly *D. melanogaster* and the body louse *P. humanus corporis* whose protein sequences display much less overall conservation compared to analyzed vertebrates. Similar to *CLUAP1*, the worm ortholog DYF-3 has been shown to be required for neuronal sensory cilium formation in *C. elegans* yet the specific residue is not conserved in either worm aligned (Figure 1C).³²

To ascertain additional patients with *CLUAP1* mutations, we specifically corresponded with our LCA expert clinician-scientist collaborator (R.K.K) who was able to identify five additional unsolved LCA probands for whom homozygosity mapping had mapped a significant homozygous interval surrounding and containing *CLUAP1*. Direct Sanger sequencing of the *CLUAP1* gene in these individuals did not identify any candidate mutations. We additionally contacted members of the European Retinal Disease Consortium, of which R.K.K. is a member, as well as submitted our interest in *CLUAP1* to GeneMatcher, a component of the Matchmaker Exchange network, but were unable to identify potential collaborators having retinal disease patients with *CLUAP1* mutations.

CLUAP1 is Localized at the Connecting Cilium of Photoreceptor Cells

Although *CLUAP1* is proposed to be a ciliary protein based on functional studies, its precise localization in the retina is unknown. Given our proband's phenotype, we hypothesize that *CLUAP1* is likely to be expressed in photoreceptor cells. To test this, we performed immunohistochemistry on sections of mouse retinas using an anti-*CLUAP1* antibody as well as anti-acetylated α -tubulin and DAPI. *CLUAP1* localizes to the connecting cilium of photoreceptor cells located between the inner and outer segment layers of the retina (Figure 2). An inconsistent *CLUAP1* signal is also seen in certain areas of the outer nuclear layer but this may be the result of non-specific antibody binding.

The Proband's Missense Mutation Negatively Impacts CLUAP1 Function

To test if the proband's mutation affects CLUAP1 function by altering its localization we performed immunofluorescence on hTERT-RPE1 cells transiently overexpressing either FLAG-CLUAP1 or FLAG-CLUAP1^{Mut}. CLUAP1 harboring the proband's mutation was observed to localize to the base of cilia similarly to wildtype (Supplementary Figure S2). To test if the proband's mutation is detrimental to CLUAP1 function *in vivo*, mutagenesis of wild type zebrafish *cluap1* cDNA was performed to generate a cDNA construct containing a mutation at the zebrafish residue that is homologous to the human variant. Taking advantage of the *cluap1* knockout zebrafish model, which shows developmental defects typical for a ciliopathy, a functional analysis of the mutant *cluap1* was performed using rescue experiments. As described in the methods section, GFP-tagged wild type and mutant mRNA were injected into zebrafish embryos resulting from *cluap1*^{+/-} incrosses.

To test if the mutation affects *cluap1* expression, we first performed Western blots using embryo lysates. Mutant Cluap1 was expressed at similar levels to wildtype leading to the conclusion that the mutation does not affect protein expression or degradation (Figure 3A). To assess the dysfunction of our mutant Cluap1, we screened for the ventral spine curvature feature present in *cluap1*^{-/-} knockout zebrafish at an early age. The percent of phenotypic embryos was compared to the expected ~25% observed after control injections of *GFP*. To determine if mutant Cluap1 could rescue the phenotype of *cluap1*^{-/-} knockout zebrafish, low and high concentrations (1 and 130 pg/nl) of mutant mRNA were tested along with wildtype. At the lower concentration of 1 pg/nl, no significant rescue effect was observed from mutant *cluap1* mRNA injected embryos (25.7% phenotypic progeny, p=0.118, Figure 3B and Supplementary Table S4) while wildtype *cluap1* mRNA displayed a borderline significant rescue (9.1% phenotypic progeny, p=0.053, Figure 3B and Supplementary Table S4). At the higher concentration of 130 pg/nl, mutant *cluap1* performed similar to wildtype and exhibited a clearly significant rescue effect (4.5% phenotypic progeny, p=0.007, Figure 3B and Supplementary Table S4).

Since a high concentration of mutant *cluap1* rescued the spine curvature feature, we next quantified the functionality of the mutant protein by varying the concentration of *cluap1* mRNA injections from 0.25 – 130 pg/nl. Graphing the dose response curve of these rescue experiments revealed that the proband's mutation does indeed interfere with Cluap1 function. While wildtype *cluap1* mRNA can reach a borderline significant rescue effect at the previously mentioned concentration of 1 pg/nl, mutant *cluap1* mRNA requires a concentration of 20 pg/nl in order to achieve a similar level of rescue significance (9.6% phenotypic progeny, p=0.047, Figure 3C and Supplementary Table S4). To visualize this data in an alternate form, we compared the percent of progeny that were expected to be phenotypic based on control injections to the percent actually observed after mutant and wildtype *cluap1* mRNA injections to determine the percent of progeny that were rescued which highlights the hypomorphic nature of our proband's allele (Figure 3D). Based on these functional rescue assays Cluap1 containing our proband's mutation has as little as 5% of its remaining activity as judged from the 20 fold increase in mutant Cluap1 concentration required to achieve a rescue significance comparable to wildtype.

DISCUSSION

We conclude that since *CLUAPI* is required for photoreceptor function in other vertebrates, and our LCA proband's allele is an extremely hypomorphic *CLUAPI* allele, that *CLUAPI* is very likely to be necessary for photoreceptor function in humans. This therefore represents the first report linking hypomorphic mutations in *CLUAPI* to human disease and establishes *CLUAPI* as a candidate LCA gene. Identifying *CLUAPI* as a novel candidate LCA gene is important both for understanding human biology and for unlocking the future molecular diagnosis of patients affected by mutations in this gene. *CLUAPI* can now be studied in the context of being a human ciliopathy gene and establishing a new candidate disease gene allows for quicker and less expensive molecular diagnosis of future patients since geneticists can target the *CLUAPI* locus for examination. *CLUAPI* mutations appear to be a rare cause of LCA so further studies screening additional patient cohorts are needed to determine the true proportion of LCA patients with *CLUAPI* mutations.

The importance of cilia to many areas of the body and the importance of *CLUAPI* to cilia raises a concern about the clinical presentation of the presented proband and why syndromic features have not been observed. We considered addressing this question by sectioning the retinas of embryos rescued with mutant *cluap1* mRNA to verify that a retinal phenotype persists after the correction of spine curvature. Unfortunately due to the rapid decay of the injected mRNA and the exogenous protein being minimally detectable at 1 dpf (Figure 3A), it is unlikely that this approach would be informative as photoreceptor development isn't complete until around 5 dpf. However, three reasonable explanations exist for this observation. The first is that our proband does indeed have a syndromic disease, but additional features have yet to manifest due to the young age. This would not be surprising as multiple syndromic ciliopathies such as Joubert syndrome, Senior-Løken syndrome, and Alström syndrome are known to present a retinal degeneration feature preceding complications in other tissues. These syndromes can in fact be misdiagnosed as LCA because of the lack of additional symptoms at an affected individual's initial clinical presentation.¹

The second explanation is that the specific mutation that our proband harbors is only detrimental to a retinal specific function, and therefore only a retinal phenotype is observed. The connecting cilium in photoreceptors is a unique form of cilia containing proteins not found in most other ciliated cell types in the body. The presence of retinal specific proteins at the connecting cilium means that proteins like *CLUAP1* whose expression is not restricted to the retina conceivably have a purpose specialized to the connecting cilium involving interactions with retinal specific proteins. This explanation is supported by the fact that the proband's mutation causes a nonsynonymous amino acid substitution and that the mutation resides in *CLUAP1*'s coiled-coil domain known to accommodate protein-protein interactions. The residue that our proband's mutation affects is not conserved in *C. elegans* and *C. briggsae*, implying the residue is not essential for ciliogenesis but instead is important for some function specific to the organisms in which it is strictly conserved. The absence of eyes is one of the defining features separating these worms from the other species in the multiple sequence alignment and so a safe hypothesis appears to be that the conserved leucine is required for a function involved in vision.

The third explanation is that our proband's mutation results in a general hypomorphic form of *CLUAP1* and that the retina is more sensitive to this decrease in *CLUAP1* function than other tissue types possibly comparable to the ubiquitously expressed LCA gene *NMNAT1*.⁸ The variability in phenotype caused by mutations in the same gene can range drastically, especially in retinal disease, depending on the exact nature of the patients' alleles. It is possible that a mutation residing in *CLUAP1*'s calponin homology-like domain would confer a different phenotype impacting other tissues, and it is likely the complete loss of function of *CLUAP1* would not be compatible with human life as seen in other vertebrates. The function and expression of genes are also not always conserved across species and human *CLUAP1* is not detected in the human kidney by northern blotting which could explain the lack of kidney defects observed in the original zebrafish polycystic kidney disease screen.^{33,34}

In 2008 known LCA genes were reported to account for 70% of LCA cases, based on our experience in 2015 this percentage can vary up to 75% but for the most part has unappreciatively changed despite the identification of an additional eight genes. At least six of the reported LCA disease genes including the two most recently published genes account for a remarkably small portion (~1%) of all LCA cases.^{11-13,35-37} *CLUAP1* adds to this trend accounting for 1/212 LCA patients in our cohort (0.47%). The more than a quarter of all LCA patients waiting for a molecular diagnosis inspires two interesting hypotheses regarding future directions for studying the genetics of LCA. The first is the big fish hypothesis which can be summarized in that any one gene responsible for a significant proportion of patients' disease (a "big fish") will likely have already been discovered at this stage in human genetics research. This means that only "little fish" are left, which is why we see new LCA disease genes accounting for a minor fraction of patients. It is therefore possible that the source of disease representing the 30% of unsolved LCA cases will be composed of >25 novel disease genes each accounting for an ever smaller fraction of patients. The second alternative hypothesis is that the cause of disease resides in known LCA genes but modern genotyping techniques cannot identify the causal variation. For most genetic disorders whole exome sequencing is widely considered the standard method for molecular diagnosis but whole exome sequencing is currently unsuitable for identifying potentially causative variation in introns, promoters, enhancers, nor large structural variations. Even in regions captured by whole exome sequencing there is data we do not fully know how to interpret including variation in UTRs, synonymous variation and intronic variation close to an exon but outside of the 2bp splicing consensus sequence. Understanding variation in these atypical regions is imperative in the research of human disease because groups may focus efforts on finding novel disease genes when the disease-causing mutations they seek are "right under their noses". The truth is most likely a combination of these two hypotheses leaving numerous possibilities for the future of LCA research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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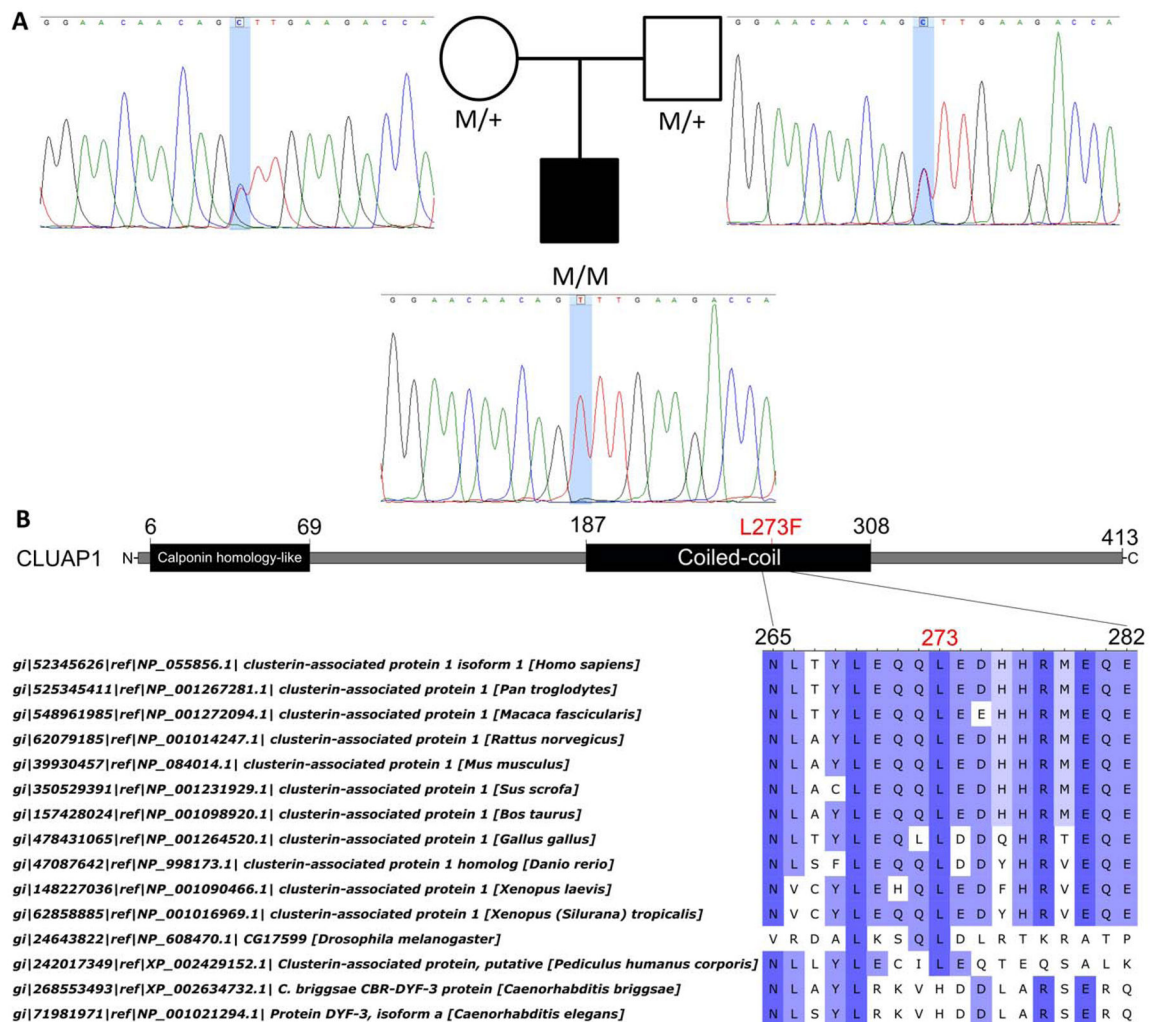


Figure 1.

Pedigree, Sanger sequencing traces, and 2D CLUAP1 protein structure with an MSA of the affected residue. (A) The proband's pedigree and Sanger sequencing traces showing proper segregation of the mutant allele. (B) A 2D graphical representation of the long isoform of CLUAP1 containing two characterized functional domains with the location of the proband's mutation displayed in red. A protein multiple sequence alignment surrounding the amino acid affected by the proband's mutation showing the leucine at position 273 is conserved in sighted organisms. Aligned columns are colored a darker blue with increasing conservation at that position.

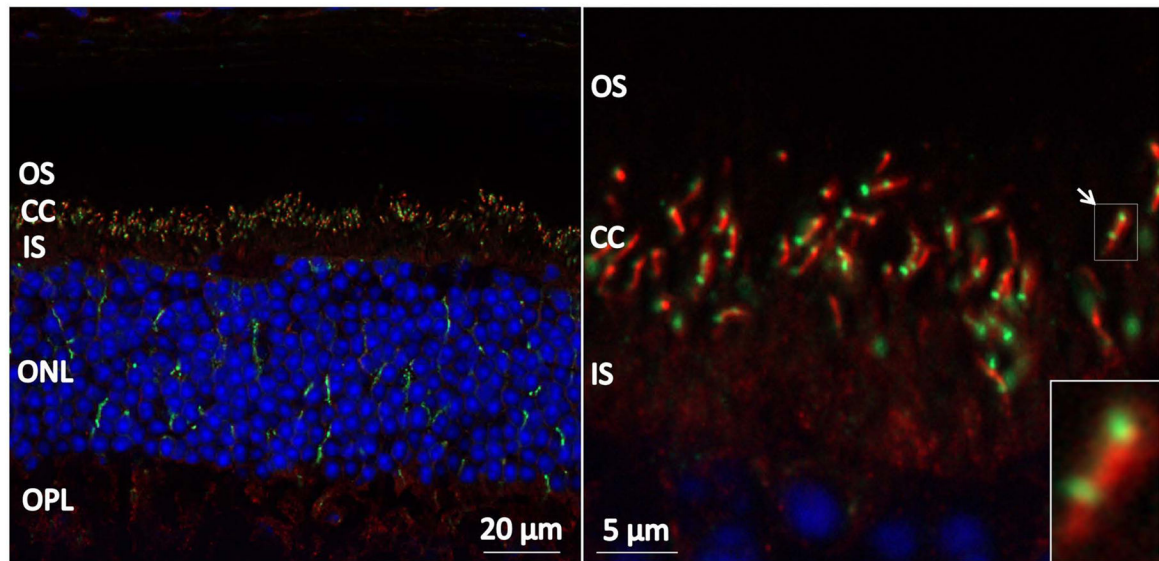


Figure 2. CLUAP1 localizes to the connecting cilium in adult mouse retinas. Mouse immunohistochemistry staining using anti-CLUAP1 (green), anti-acetylated α -tubulin (red), and DAPI (blue). CLUAP1 can be seen to localize to specific puncti between the inner segment (IS) and outer segment (OS) layers of photoreceptor cells. CLUAP1 localization overlaps with the tip and base of the acetylated α -tubulin staining corresponding to the tip and base of connecting cilia (CC). ONL = outer nuclear layer, OPL = outer plexiform layer.

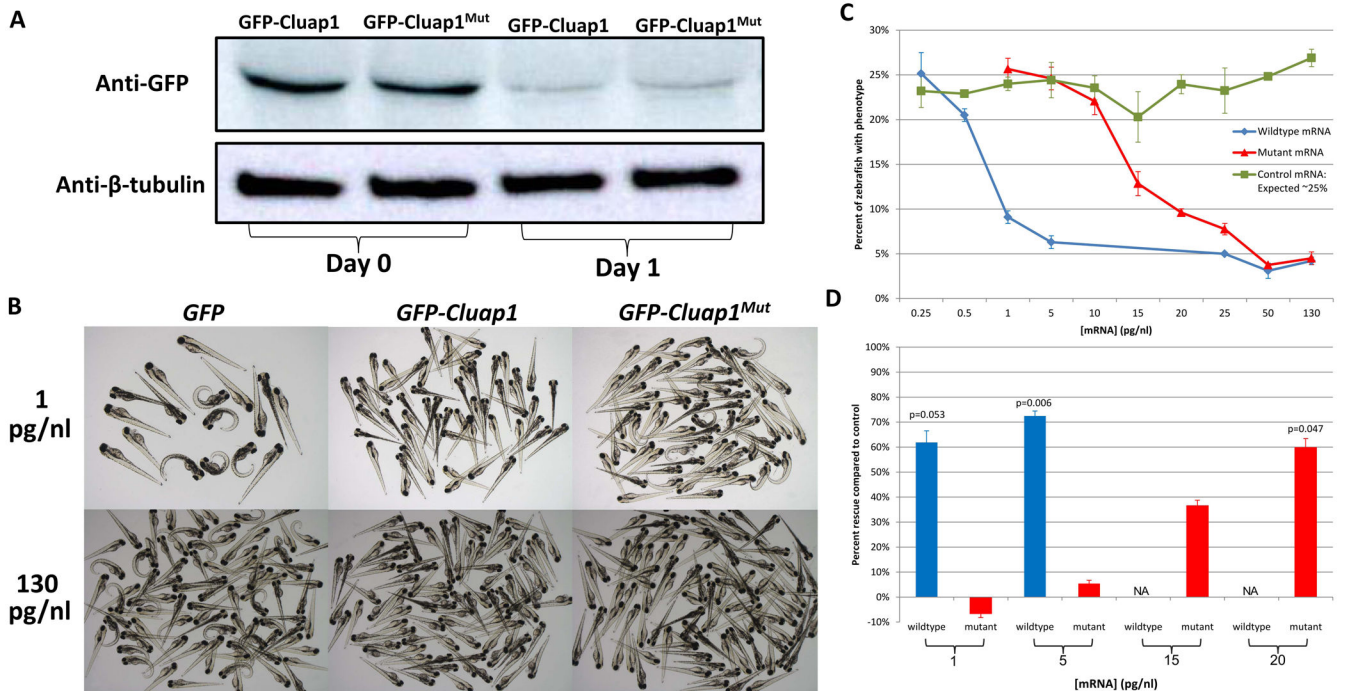


Figure 3.

Mutant *Cluap1* is expressed at levels similar to wildtype but requires 20 times the amount in order to achieve an equivalent rescue effect. (A) Zebrafish embryos injected with 25 pg/nl *GFP-cluap1* or *GFP-cluap1^{Mut}* mRNA express similar amounts of *Cluap1* as verified by western blotting. (B) Embryos resulting from *cluap1^{-/-}* incrosses injected with control *GFP* mRNA exhibit the expected 25% phenotypic proportion. At a low concentration (1 pg/nl), wildtype *cluap1* mRNA noticeably rescues the spine curvature feature of *cluap1^{-/-}* zebrafish embryos while mutant *cluap1* mRNA does not. At a high concentration (130 pg/nl), mutant *cluap1* mRNA rescues the spine curvature feature comparable to wildtype *cluap1* mRNA revealing a dose-dependent response. (C) Dose response curves of rescue experiments. Zebrafish injected with control *GFP* mRNA (green) showed approximately the expected 25% Mendelian ratio. Zebrafish injected with *GFP-cluap1* mRNA (blue) reached a borderline significant ($p=0.053$) rescue at 1 pg/nl. Zebrafish injected with *GFP-cluap1^{Mut}* mRNA (red) significantly ($p=0.047$) rescued the mutant phenotype at a concentration of 20 pg/nl. (D) The percent of progeny rescued as compared to control injections at the same concentration highlights the 20-fold increase in the amount of mutant *cluap1* mRNA required to achieve a level of rescue significance comparable to wildtype *cluap1*. Errors bars in (C) and (D) represent the standard deviation from two independent experiments.

Table 1

Proband MOGL3628 information

| Age | Age of onset | Best corrected visual acuity (both eyes) | Nystagmus | Oculodigit sign | Electroretinogram |
|-----|---------------|--|-----------|-----------------|-------------------|
| 5 | First 6 weeks | Light Perception | Yes | Yes | Extinguished |

Table 2

Mutation information

| Genome-level consequence | Gene | Gene-level consequence | | | |
|---|----------------------------------|------------------------------|----------------------------|-------------------------|--|
| NC_000016.9:g.3573261C>T | <i>CLUAP1</i> | NM_024793:c.319C>T;p.L107F | NM_015041:c.817C>T;p.L273F | | |
| <i>In silico</i> nonsynonymous mutation deleteriousness prediction algorithms and corresponding predictions | | | | | |
| SIFT | Polyphen2 HDIV | LRT | MutationTaster | MutationAssessor | |
| Damaging | Damaging | Damaging | Damaging | Medium impact | |
| FATHMM | MetaSVM | VEST3 | PROVEAN | CADD | |
| Tolerated | Damaging | Damaging | Damaging | Damaging | |
| Frequency of variant in large-scale population studies (mutant allele count/total alleles) | | | | | |
| 1000 Genomes Project | Human Genetic Variation Database | Exome Aggregation Consortium | CHARGE | | |
| 0 (0/5,008) | 0 (0/2,416) | 0,0008% (1/121,086) | 0 (0/21,880) | | |