Whole genome sequencing reveals a recurrent missense mutation in the Connexin 46 (*GJA3)* gene causing autosomal dominant lamellar cataract

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Abstract

Purpose: Congenital cataract, opacification of the ocular lens, is clinically and genetically a heterogeneous childhood disease. In this study we aimed to identify the underlying genetic cause of isolated autosomal dominant lamellar cataract in a multi-generation English family.

Methods: Whole genome sequencing (WGS) was undertaken in two affected subjects and one unaffected individual. Segregation analysis was performed and a known cataract causing mutation was identified. Segregation was further validated by sanger sequencing in the entire pedigree.

Results: A heterozygous mutationc.7G>T; p.D3Y was identified in an NH2-terminal region of the gap junction protein GJA3 and found to co-segregate with disease.

Conclusion: We have identified a recurrent mutation in GJA3 in a large British pedigree causing the novel phenotype of autosomal dominant congenital lamellar cataract. Previously, p.D3Y was found in a Hispanic family causing pulverulent cataract. WGS proved an efficient method to find the underlying molecular cause in this large family which could not be mapped due to uninformative markers.

Key Words: Autosomal dominant, congenital, lamellar cataract, *GJA3*, whole genome sequencing.

Introduction

Lens opacity is widely considered to be the primary cause of blindness worldwide. Congenital cataracts are phenotypically and genetically heterogeneous. They are responsible for 1-6/10,000 births in the UK and 5-15/10,000 births in developing countries and are a pronounced factor of vision loss in infants and children[1].

Congenital cataract can occur in isolation, or in association with other non-ocular diseases. Most familial cataracts are associated with an autosomal dominant mode of inheritance[2,3]. Clinical classification depends on the position and type of the lens opacity, such as: blue-dot (cerulean), coralliform, nuclear, cortical, complete, pulverulent and anterior polar, posterior polar, posterior nuclear, polymorphic and lamellar[4.]

So far > 40 genes have been implicated in cataractogenesis including crystallins encoding transparent intracellular lens proteins, water channel proteins (aquaporins), solute carrier protein, cytoskeletal proteins, chromatin modifying protein-4B, transcription factors, transmembrane proteins, lens intrinsic membrane protein, receptor tyrosine kinase gene EPH receptor A2[5], an endoplasmic reticulum membrane-embedded protein, Wolframin[6], and gap junction proteins including GJA8 and GJA3[5].

Gap junction channels and hemi-channels are made by connexins: they play an important role in intercellular communication. Each hemi-channel is formed by six connexin units, called a connexon. Two connexons from neighbouring cells dock to make a gap junction channel through the extracellular loops of connexins, which allows the exchange of ions and small molecules between cells[7.] In humans, at least 21 connexin genes have been associated with several different genetic defects including deafness, skin abnormalities, neurodegenerative diseases, cardiopathies and cataracts[8,9,10,11.]The lens expresses three discrete connexins:Cx43, Cx50, and Cx46, displaying various levels of expression and function in maintaining lens homeostasis (reviewed in reference)[12.]

The lens is a transparent, avascular and biconvex organ in the anterior chamber of the eye, situated behind the cornea. The cornea and lens transmit light onto the retina for

fine focusing. The lens is comprised of two cell types: metabolically active epithelial cells that form a single layer along the anterior surface and fiber cells that form the main bulk of the lens. These fiber cells lose all of their intracellular organelles during differentiation and become metabolically inert. Using the gap junctions to maintain tissue homeostasis and transparency the lens has therefore developed a substantial intercellular communication system.[13.] Cx43 is expressed primarily in the lens epithelial cells, while Cx46 and Cx50 are extensively expressed in lens fiber cells[12.] Mutations in Cx50 and Cx46 lead to congenital cataracts in human and mice[14].

Here we report a recurrent mutation (p.D3Y) in *GJA3* causing an isolated autosomal dominant lamellar cataract in a five generation British family. Previously, this mutation has been found in a Hispanic family causing a different phenotype of pulverulent cataract[15.]

Methods

***Phenotyping*:** The family was identified through the proband attending the Genetic Service at Moorfields Eye Hospital, London, UK. Local ethics committee approval was obtained and all of the participants gave written informed consent. All the family members underwent full ophthalmic examination, including slit lamp examination; all affected individuals were diagnosed as having isolated lamellar cataract.

Whole Genome Sequencing (WGS) and Bioinformatics Analysis: Genomic DNA was extracted from EDTA sequestered blood samples taken with informed consent and local ethical approval using the Nucleon II DNA extraction kit (ScotlabBioscience, Strathclyde, Scotland, UK). Genomic DNA was processed using the Illumina TruSeq DNAPCR-Free Sample Preparation kit (Illumina) and sequenced
using an Illumina Hiseq 2500, generating mean genome coverageof 35x. WGS was done by a service provider (Macrogen.Inc., Korea). As described in Berry et al 2017[35], raw data in fastq format was analysed using the Phenopolis platform[16].The short read sequence data were aligned using novoalign (version 3.02.08). Variants and indels were called according using GATK haplotype caller[17]. The variants were then annotated using the Variant Effect Predictor (VEP)[18]. Variants were then filtered to

only contain variants not present in public control databases Kaviar (Glusman et al. 2011)[19] and gnomAD (http://gnomad.broadinstitute.org/), and predicted to be moderately or highly damaging according to the VEP. Cosegregation of the filtered variants in both affected individuals was then performed. Finally, the list of variants was further screened using Phenopolis, for genes associated with the Human Phenotype Ontology [20] term "lamellar cataract" (HP:0007971) according to OMIM[21.]. The mutations were then ordered on CADD score with the highest-ranking mutations at the top.

Structural bioinformatics: The protein structure of GJA3 was analysed using SWISS-MODELhttps://swissmodel.expasy.org/repository/uniprot/Q9Y6H8

The best PDB match, with a match of 49%, was the structure of 2ZW3 PDB ID, solved with XRay crystallography (referencehttps://www.ncbi.nlm.nih.gov/pubmed/?term=19340074)

All structures were downloaded in PDB format and analysed using Pymol (version 1.8) locally.

***Sanger sequencing*:** Bi-directional direct Sanger sequencing was performed to validate the variant identified by whole genome sequencing. Genomic DNA was amplified by PCR using GoTaq 2X master mix (AB gene; Thermo Scientific, Epsom, UK) and *GJA3*-specifc primers designed with Primer3http://bioinfo.ut.ee/primer3-0.4.0/primer3/. PCR conditions were followed as: 94°C for 10 minutes of initial denaturation followed by 30 cycles of amplification of 30 seconds at 94°C, 30 seconds at 60°C, and 45 seconds at 72°C. After the PCR products were reacted with BigDye Terminator v3.1, they were run on ABI 3730 Genetic Analyzer (both from Applied Biosystems) and analyzed using SeqMan Pro (version 8.0.2 from DNASTAR) sequence analysis. After validating the variant, family segregation was performed in all the individuals.

Results

Sixteen members of a large five generation British family including 10 affected, 4 unaffected and 2 spouses were examined (Figure 1). All the affected family members had bilateral cataract and age of onset varied from birth to age 20 months. One Individual (III-10) was diagnosed at the age of 3 weeks and also had glaucoma. One of the patients (IV-2) had bilateral cataract at birth, surgery at age 11years and suffered bilateral retinal detachment.

WGS was undertaken in two affected (IV-5, V-1) and one unaffected (III-11) member of the family. Variant annotation and filtering was performed using the Phenopolis platform. From a total of 7,096,614 variants in the three individuals, 549,719 were found to co-segregate in the two affected individuals. After filtering for rare variants with a homozygous frequency of 0 and allele frequency less than 0.01 in Gnomad and Kaviar, 33,310 variants remained. A gene list of 97 cataract associated genes was used for gene panel screening, after which, 44 variants remained. The top scoring variant on CADD (score of 27.4) was a known rare heterozygous damaging variant, NM_021954.3:c.7G>T; p.D3Y, in *GJA3*gene on chromosome 13q11-q12 (reference). Direct sequencing confirmed that this missense mutation c.7G>T in exon 2 of *GJA3* co-segregated with all affected members of the family (Figure 2).

The p.D3Y mutation from aspartate (D3Y) to a tyrosine in the in the NH2-terminal cytoplasmic tail of the GJA3 protein is likely to affect the degree of metabolite cell-to-cell coupling and is essential for the voltage sensitivity. The aspartate is a negatively charged amino acid whereas tyrosine is uncharged, which could have some effect on the hemi-channel activity[22,23](Figure 3).

Discussion

Here we report a missense mutation c.7G>T in the gap junction protein (*GJA3)* gene in a five generation English pedigree with autosomal dominant congenital lamellar cataract. All the affected family members had bilateral cataract and age of onset varied from birth to age 20 months.

Lamellar cataract is also referred to as zonular cataract and is one of the most common phenotypes of autosomal dominant congenital cataract (ADCC). The inner fetal

nucleus is made up of a clear lens surrounded by an opacified shell that is in turn surrounded by clear cortex, which may contain opacities referred to as “riders” or “cortical spokes”. Lamellar cataract represents a disturbance in the lens development at a particular time and the cataractous “shell” varies in size according to the stage of fetal development at which the disturbance occurs[4,35]. The elongated fiber cells of the lens constitute the main bulk of the lens’ mass and represent the target cells for cataract formation due to miscommunication; *GJA3* protein mainly functions in gap junction communication between these cells[24] Connexin46 mutations are phenotypically highly heterogeneous[9] (summarised inTable1).

In 1990, Willecke et al were the first group to assign *GJA3* to chromosome 13, and after 9 years, Mackay et al found the first connexion 46 mutations in humans causing autosomal dominant congenital cataract[25,26].Connexin 46 comprises two exons encoding a transmembrane protein of 435 amino acids, containing four transmembrane domains (TM1-TM4), two extracellular loops (E1, and E2), an intracellular loop (CL), and cytoplasmic NH2- and COOH-termini. Connexins share the same membrane topology among all the family members. So far, 50(novel and recurrent) cataract-causing mutations in *GJA3*have been reported in various ethnic groups. Interestingly, half of the mutations are found in China, and the remainder have been found in other ethnic groups; six from India, four from Australia, three from Denmark, ten from UK, two from USA and one from Honduras; and exhibiting different phenotypes (Table1).

In the present study, the recurrent p.D3Y(c.7G->T) change in *GJA3* gene resulted in an aspartate (a negatively charged amino acid) to tyrosine (an uncharged amino acid) at position 3 within the NH2-terminal cytoplasmic tail. The Asp-3 residue of *GJA3* is phylogenetically conserved, hence, this indicates aspartate is likely to be functionally important and that the mutation may therefore have a detrimental physiological effect. Several studies have suggested that the NH2-terminal (NT) along with E1 and TM1 contribute to the pore lining region of the hemi-channel and therefore any compromise in the amino acid residues may interfere with the conformation and flexibility of NT and also with voltage gating[27,28,29,30,31.] Schlingmann and co-workers in 2012 has shown the involvement of Asp3 (D3Y) in the determination of the cell-to-cell coupling and for the voltage dependent Cx46 hemi-channels. This hypothesis is further supported by

Tong et al (2013); they demonstrated the effect of D3Y on reduced hemi-channel activity and alterations in voltage gating and charge selectivity. Lens fiber cells are dependent on intercellular communication for their survival[32,33.]

Ebihara et al 2010 has reported the association of connexin 46 with calcium and sodium influx in fiber cells and their important role on the function and development of the lens[34]. Further, the important role of Cx46 in the delivery of glutathione in the lens nucleus has been demonstrated. Cx46 not only have major role in congenital cataract but also age-related cataract which may give rise to identify new therapeutic strategies[34.]

Here we have found the recurrent p.D3Y (c.7G->T) mutation in the *GJA3* gene in a British family with a different phenotype, lamellar cataract; where previously this variant has only been reported in association with pulverulent cataract. These results show further heterogeneity in inherited cataract, with the same mutation, on a different genetic background, causing a different phenotype, presumably through diverse mechanisms.

Acknowledgments

The present work is supported by grants from the National Institute for Health Research Biomedical Research Centre at Moorfields Eye Hospital National Health Service Foundation Trust and UCL Institute of Ophthalmology (UK), Moorfields Eye Hospital Special Trustees (UK), Moorfields Eye Charity (UK), and the Foundation Fighting Blindness (USA). Michel Michaelides is supported by an FFB Career Development Award. We would like to thank the members of the family for taking part in this study.

Conflict of Interest

The authors report no conflict of Interest.

References

1. Rahi JS, Dezateux C, British Congenital Cataract Interest Group. Measuring and interpreting the incidence of congenital ocular anomalies: lessons from a national study of congenital cataract in the UK. Invest Ophthalmol Vis Sci. 2001

Jun;42(7):1444–8.

2. Reddy MA, Francis PJ, Berry V, Bhattacharya SS, Moore AT. Molecular genetic basis of inherited cataract and associated phenotypes. Surv Ophthalmol. 2004 May;49(3):300–15.

3. Krumpaszky HG, Klauss V. Epidemiology of blindness and eye disease. Ophthalmologica. 1996;210(1):1–84.

4. Ionides A, Francis P, Berry V, Mackay D, Bhattacharya S, Shiels A, et al. Clinical and genetic heterogeneity in autosomal dominant cataract. Br J Ophthalmol. 1999 Jul;83(7):802–8.

5. Shiels A, Bennett TM, Hejtmancik JF. Cat-Map: putting cataract on the map. Mol Vis. 2010 Oct 8; 16:2007–15.

6. Berry V, Gregory-Evans C, Emmett W, Waseem N, Raby J, Prescott D, et al. Wolfram gene (WFS1) mutation causes autosomal dominant congenital nuclear cataract in humans. Eur J Hum Genet. 2013 Dec;21(12):1356–60.

7. Kumar NM, Gilula NB. The gap junction communication channel. Cell. 1996 Feb 9;84(3):381–8.

8. Beyer EC, Berthoud VM. The Family of Connexin Genes. In Book: Connexins. Humana Press; 2009. p. 3–26.

9. Beyer EC, Ebihara L, Berthoud VM. Connexin mutants and cataracts. Front Pharmacol. 2013 Apr 15;4:43.

10. Gerido DA, White TW. Connexin disorders of the ear, skin, and lens. BiochimBiophysActa. 2004 Mar 23;1662(1-2):159–70.

11. Srinivas M, Verselis VK, White TW. Human diseases associated with connexin mutations. BiochimBiophysActa. 2018 Jan;1860(1):192–201.

12. Berthoud VM, Minogue PJ, Osmolak P, Snabb JI, Beyer EC. Roles and regulation of lens epithelial cell connexins. FEBS Lett. 2014 Apr 17;588(8):1297–303.

13. White TW. Unique and redundant connexin contributions to lens development. Science. 2002 Jan 11;295(5553):319–20.

14. Gong X, Li E, Klier G, Huang Q, Wu Y, Lei H, et al. Disruption of alpha3 connexin gene leads to proteolysis and cataractogenesis in mice. Cell. 1997 Dec 12;91(6):833–43.

15. Addison PKF, Berry V, Holden KR, Espinal D, Rivera B, Su H, et al. A novel mutation in the connexin 46 gene (GJA3) causes autosomal dominant zonular pulverulent cataract in a Hispanic family. Mol Vis. 2006 Jul 20; 12:791–5.

16. Pontikos N, Yu J, Moghul I, Withington L, Blanco-Kelly F, Vulliamy T, et al. Phenopolis: an open platform for harmonization and analysis of genetic and phenotypic data. Bioinformatics. 2017 Aug 1;33(15):2421–3.

17. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010 Sep;20(9):1297–303.

18. McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GRS, Thormann A, et al. The Ensembl Variant Effect Predictor. Genome Biol. 2016 Jun 6;17(1):122.

19. Glusman G, Caballero J, Mauldin DE, Hood L, Roach JC. Kaviar: an accessible system for testing SNV novelty. Bioinformatics. 2011 Nov 15;27(22):3216–7.

20. Köhler S, Vasilevsky NA, Engelstad M, Foster E, McMurry J, Aymé S, et al. The Human Phenotype Ontology in 2017. Nucleic Acids Res. 2017 Jan 4;45(D1): D865–76.

21. Hamosh A, Scott AF, Amberger JS, Bocchini CA, McKusick VA. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic Acids Res. 2005 Jan 1;33(Database issue): D514–7.

22. Schwede T, Kopp J, Guex N, Peitsch MC. SWISS-MODEL: An automated protein homology-modeling server. Nucleic Acids Res. 2003 Jul 1;31(13):3381–5.

23. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN. The Protein Data Bank Nucleic Acids Research, 28, 235-242. View Article PubMed/NCBI. 2000;

24. Paul DL, Ebihara L, Takemoto LJ, Swenson KI, Goodenough DA. Connexin46, a novel lens gap junction protein, induces voltage-gated currents in nonjunctional plasma membrane of Xenopus oocytes. J Cell Biol. 1991 Nov;115(4):1077–89.

25. Willecke K, Eiberger J, Degen J, Eckardt D, Romualdi A, Güldenagel M, et al. Structural and functional diversity of connexin genes in the mouse and human genome. Biol Chem. 2002 May;383(5):725–37.

26. Mackay D, Ionides A, Kibar Z, Rouleau G, Berry V, Moore A, et al. Connexin46 Mutations in Autosomal Dominant Congenital Cataract. Am J Hum Genet. 1999 May 1;64(5):1357–64.

27. Dong L, Liu X, Li H, Vertel BM, Ebihara L. Role of the N-terminus in permeability of chicken connexin45.6 gap junctional channels. J Physiol. 2006 Nov 1;576(Pt 3):787–99.

28. Maeda S, Nakagawa S, Suga M, Yamashita E. Structure of the connexin 26 gap junction channel at 3.5 Å resolution. Nature [Internet]. 2009; Available from: https://www.nature.com/nature/journal/v458/n7238/abs/nature07869.html

29. Verselis VK, Ginter CS, Bargiello TA. Opposite voltage gating polarities of two closely related connexins. Nature [Internet]. 1994; Available from: http://link.springer.com/article/10.1038/368348a0

30. Oh S, Rubin JB, Bennett MV, Verselis VK, Bargiello TA. Molecular determinants of electrical rectification of single channel conductance in gap junctions formed by connexins 26 and 32. J Gen Physiol. 1999 Sep;114(3):339–64.

31. Purnick PE, Benjamin DC, Verselis VK, Bargiello TA, Dowd TL. Structure of the amino terminus of a gap junction protein. Arch BiochemBiophys. 2000 Sep

15;381(2):181–90.

32. Schlingmann B, Schadzek P, Busko S, Heisterkamp A, Ngezahayo A. Cataract-associated D3Y mutation of human connexin46 (hCx46) increases the dye coupling of gap junction channels and suppresses the voltage sensitivity of hemichannels. J BioenergBiomembr. 2012 Oct;44(5):607–14.

33. Tong J-J, Minogue PJ, Kobeszko M, Beyer EC, Berthoud VM, Ebihara L. The connexin46 mutant, Cx46T19M, causes loss of gap junction function and alters hemi-channel gating. J Membr Biol. 2015 Feb;248(1):145–55.

34. Slavi N, Rubinos C, Li L, Sellitto C, White TW, Mathias R, et al. Connexin 46 (cx46) gap junctions provide a pathway for the delivery of glutathione to the lens nucleus. J Biol Chem. 2014 Nov 21;289(47):32694–702.

35. Berry V, Pontikos N, Moore A, Ionides A C W, Plagnol V, Cheetham M E and Michaelides M. A novel missense mutation in HSF4 causes autosomal-dominant congenital lamellar cataract in a British family. Eye. 2017; doi:10.1038/eye2017.268

36. Ebihara L, Tong J J, Vertel B, White T W, and Chen T L. Properties of connexin 46 hemichannels in dissociated lens fiber cells. Invest. Ophthalmol. Vis. Sci. 2010. 52, 882–889. doi: 10.1167/iovs.10-6200

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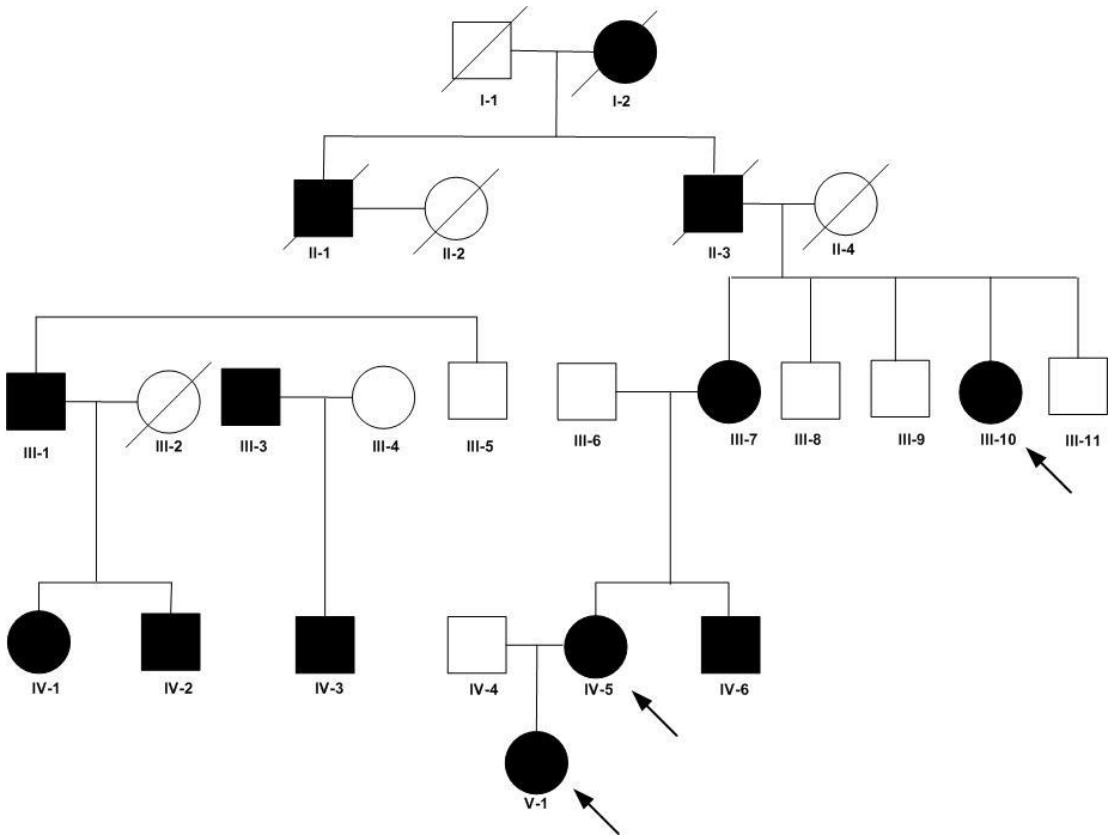

Figure 1: Abridged pedigree of the British family with lamellar cataract. Squares and circles symbolize males and females respectively. Open and filled symbols indicate unaffected and affected individuals.

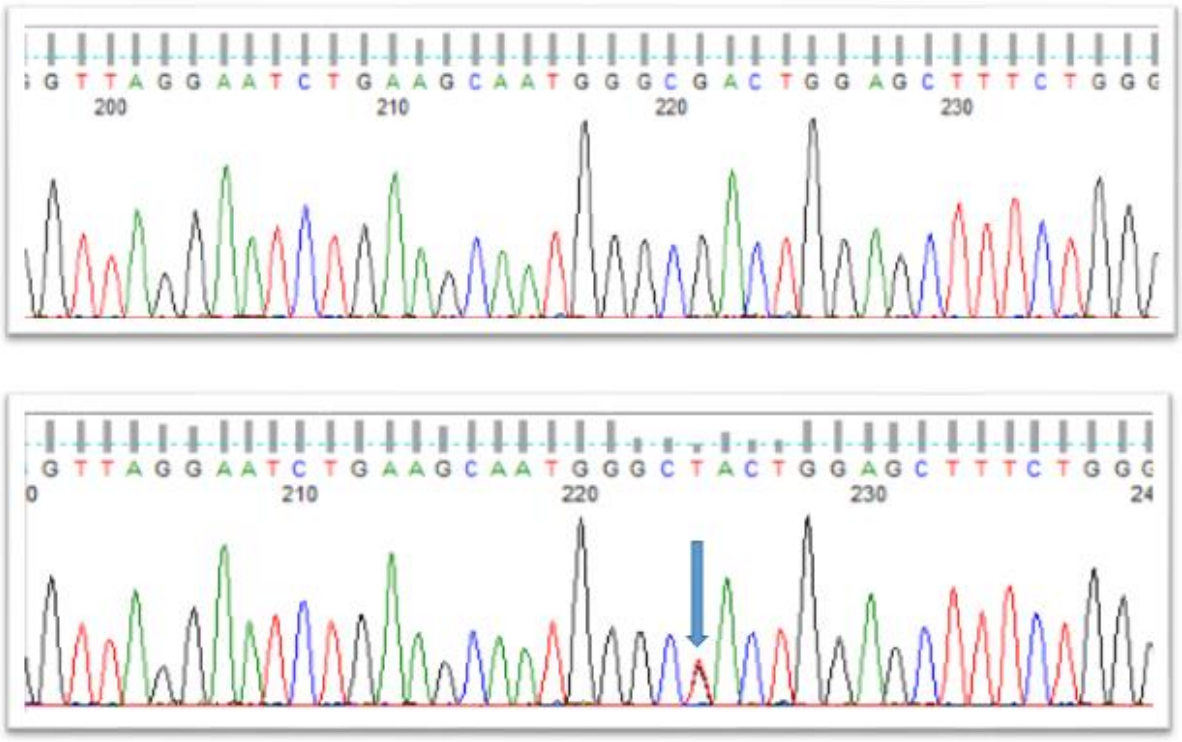

Figure 2: Sequence analysis of *GJA3.* An unaffected individual (upper chromatogram illustrates a normal control and a missense mutation c.7G>T shown in affected member of the family with lamellar cataract.

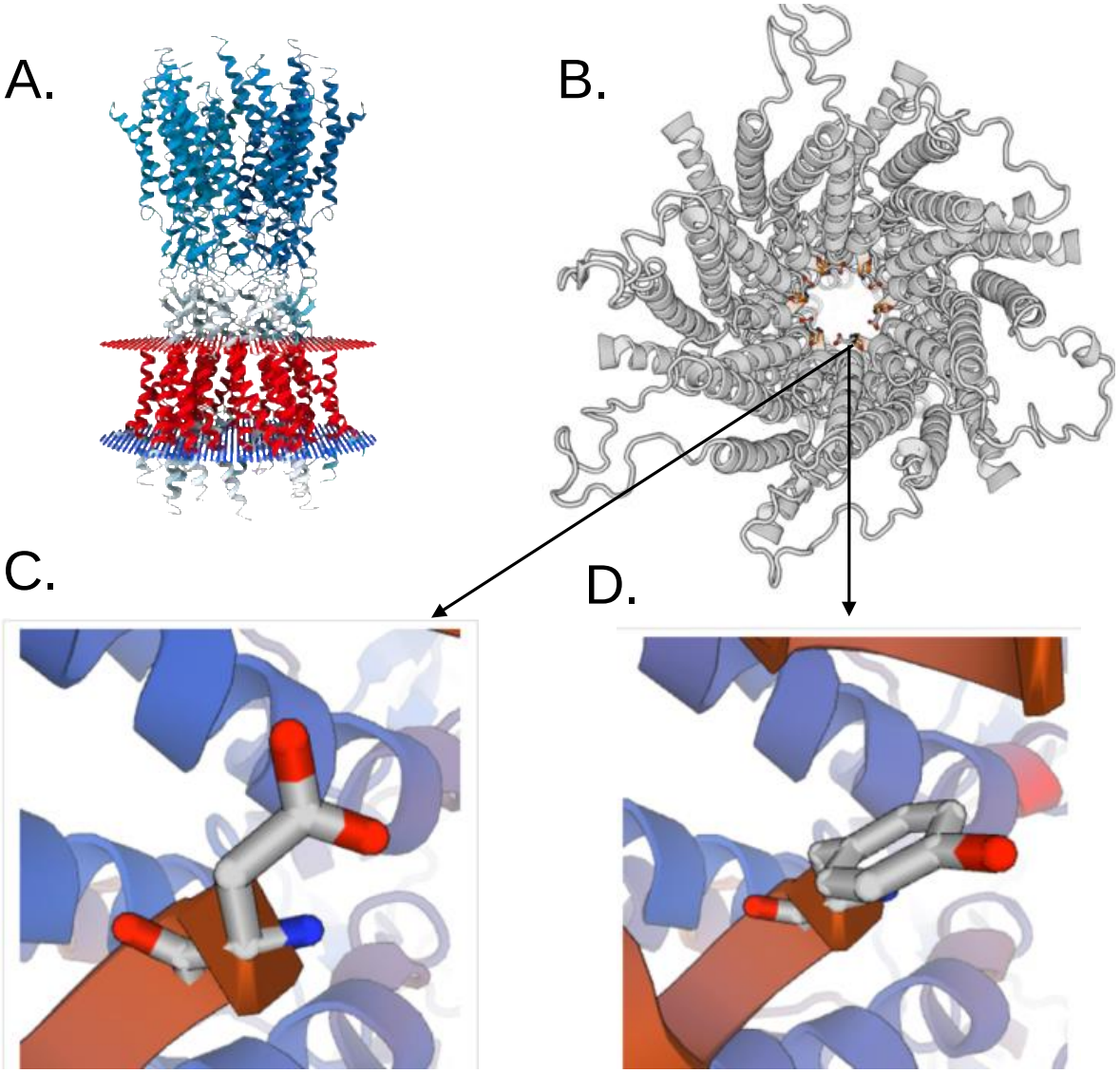

Figure 3: Structure of the GJA3 protein. A. Transmembrane view of GJA3 https://www.rcsb.org/pdb/explore/explore.do?structureId=2zw3. **B.** View of the GJA3 hemichannelhttps://swissmodel.expasy.org/repository/uniprot//Q9Y6H8 **C.** Wild type amino at position 3 (Aspartate) **D.** Mutant amino acid at position 3 (Tyrosine). The side chain of the tyrosine interferes with the hemi-channel activity.

Table 1: Published mutations in GJA3 that cause cataract. Mutations are ordered by amino acid position; Genomic Evolutionary Rate Profiling (GERP) NR corresponds to the neutral rate conservation score of the site. Combined Annotation Dependent Depletion (CADD) is score for the deleteriousness of a variant. A CADD score over 20 is considered damaging.

Variant id	cDNA	Protein change	inheritance	Origin	Phenotype
	c.-39C>G		Complex	China	Age-related nuclear
13-20717423-C-T	c.5G>A	p.G2D	AD	China	Nuclear pulverulent, Posterior polar
13-20717421-C-G	c.7G>C	p.D3H	AD	Australia	
13-20717421-C-A	c.7G>T	p.D3Y	AD	Honduras	Zonular pulverulent
13-20717421-C-A	**c.7G>T**	**p.D3Y**	**AD**	**UK**	**Lamellar**
13-20717396-A-G	c.32T>C	p.L11S	AD	Denmark	"Ant-egg"
13-20717372-G-A	c.56C>T	p.T19M	AD	India	Posterior polar
13-20717346-C-T	c.82G>A	p.V28M	AD	India	Total, anterior capsular, cortical
13-20717334-A-G	c.96C>A	p.F32L	AD	China	Nuclear pulverulent
13-20717330-C-A	c.98G>T	p.R33L	AD	India	Granular embryonal
13-20717303-T-G	c.125A>C	p.E42A	AD	China	Pulverulent
13-20717298-C-T	c.130G>A	p.V44M	AD	China	Central nuclear with punctate cortical
13-20717298-C-T	c.130G>A	p.V44M	AD	USA	?
13-20717298-C-T	c.130G>A	p.V44M	AD	China	
13-20717294-C-G	c.134G>C	p.W45S	AD	China	Nuclear
13-20717289-C-T	c.139G>A	p.D47N	AD	China	Nuclear
13-20717289-C-T	c.139G>A	p.D47N	AD	China	Nuclear
13-20717285-T-C	c.143A>G	p.E48G	AD	China	Nuclear
13-20717265-T-C	c.163A>G	p.N55D	AD	China	Central Nuclear
13-20717280-A-G	c.148T>C	p.S50P		UK	Y-sutural and Lamellar
13-20717252-G-A	c.176C>T	p.P59L	AD	USA	Nuclear punctate
13-20717252-G-A	c.176C>T	p.P59L	AD	Denmark	?
13-20717252-G-A	c.176C>T	p.P59L	AD	China	?
13-20717252-G-A	c.176C>T	p.P59L		UK	
13-20717252-G-A	c.176C>T	p.P59L	AD	Australia	
13-20717252-G-A	c.176C>T	p.P59L	AD	China	Pulverulent
13-20717244-C-T	c.184G>A	p.E62K		UK	
13-20717240-T-C	c.188A>G	p.N63S	AD	UK	Variable pulverulent
13-20717202-G-C	c.226C>G	p.R76G	AD	India	Total
Variant id	**cDNA**	**Protein change**	**inheritance**	**Origin**	**Phenotype**
13-20717201-C-T	c.227G>A	p.R76H	AD	Australia	Nuclear lamellar pulverulent
13-20717201-C-T	c.227G>A	p.R76H	AD	Denmark	Lamellar, sutural
13-20717168-G-A	c.260C>T	p.T87M	AD	India	"Pearl-box"
13-20717168-G-A	c.260C>T	p.T87M	AD	Australia	
13-20717160-G-A	c.268C>T	p.L90F	AD	China	

13-20717013-C-T	c.415G>A	p.V139M	Complex	China	Age-related cortical
13-20717001-C-T	c.427G>A	p.G143R	AD	China	Granular central disc (Coppock-like)
13-20717000-C-T	c.428G>A	p.G143E	AD	China	Nuclear
13-20716985-G-A	c.443C>T	p.T148I	AD	China	Pulverulent Nuclear
13-20716868-G-A	c.560C>T	p.P187L	AD	UK	Zonular pulverulent
13-20716869-G-A	c.559C>T	p.P187S	AD	China	Nuclear pulverulent
13-20716865-T-G	c.563A>C	p.N188T	AD	China	Nuclear pulverulent
13-20716865-T-A	c.563A>T	p.N188I	AD	China	Coraliform
13-20716850-A-G	c.578T>C	p.F193S		UK	Cataract and Macrocephaly
13-20716850-A-G	c.578T>C	p.F193S	AD	UK	Dense Nuclear
13-20716839-G-A	c.589C>T	p.P197S	AD	India	Lamellar
13-20716832-T-G	c.596A>C	p.E199A		UK	
13-20716812-A-T	c.616T>A	p.F206I	AD	China	Embryonal nuclear
13-20716288-GCT-TTG	c.1137insC	p.S380QfsX87	AD	UK	Punctate
13-20716287-T-G	c.1143_1165del23	p.S381RfsX48	AD	China	Punctate nuclear
13-20716238-G-C	c.1189dupG	p.A397GfsX71	AD	China	Coralliform
13-20716229-GT-TG	c.1196dupC	p.T400HfsX68	AD	China	