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# 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 mutation c.7 G>T; p.D3Y was identified in an NH<sub>2</sub>-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.

## 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 United Kingdom 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 hemichannels are made by connexins: they play an important role in intercellular

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communication. Each hemichannel is formed by six connexin units, called a connexon. Two connexons from neighboring 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 [8–11]. The lens expresses three discrete connexins: Cx43, Cx50, and Cx46, displaying various levels of expression and function in maintaining lens homeostasis (reviewed in ref. 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, whereas 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 ethylenediaminetetraacetic acid-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 coverage of  $35 \times$ . WGS was done by a service provider (MacroGen.Inc., Korea). As described in Berry et al. 2017 [16], raw data in fastq format was analyzed using the Phenopolis platform [17]. The short read sequence data were aligned using novoalign (version 3.02.08). Variants and indels were called according using GATK haplotype caller [18]. The variants were then annotated using the Variant Effect Predictor (VEP) [19]. Variants were then filtered to only contain variants not present in public control databases Kaviar (Glusman et al. 2011) [20] 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 [21] term “lamellar cataract” (HP:0007971) according to OMIM [22]. The mutations were then ordered on CADD score with the highest-ranking mutations at the top.

### Structural bioinformatics

The protein structure of GJA3 was analyzed using SWISS-MODEL <https://swissmodel.expasy.org/repository/uniprot/Q9Y6H8>.

The best PDB match, with a match of 49%, was the structure of 2ZW3 PDB ID, solved with X-ray crystallography (reference <https://www.ncbi.nlm.nih.gov/pubmed/?term=19340074>).

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

### Sanger sequencing

Bi-directional direct Sanger sequencing was performed to validate the variant identified by WGS. Genomic DNA was amplified by PCR using GoTaq 2 × master mix (AB gene; Thermo Scientific, Epsom, UK) and *GJA3*-specific primers designed with Primer3 <http://bioinfo.ut.ee/primer3-0.4.0/primer3/>. PCR conditions were followed as: 94 °C for 10 min of initial denaturation followed by 30 cycles of amplification of 30 s at 94 °C, 30 s at 60 °C, and 45 s 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 (Fig. 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 11 years, 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 <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.7 G>T; p.D3Y, in *GJA3* gene on chromosome 13q11-q12

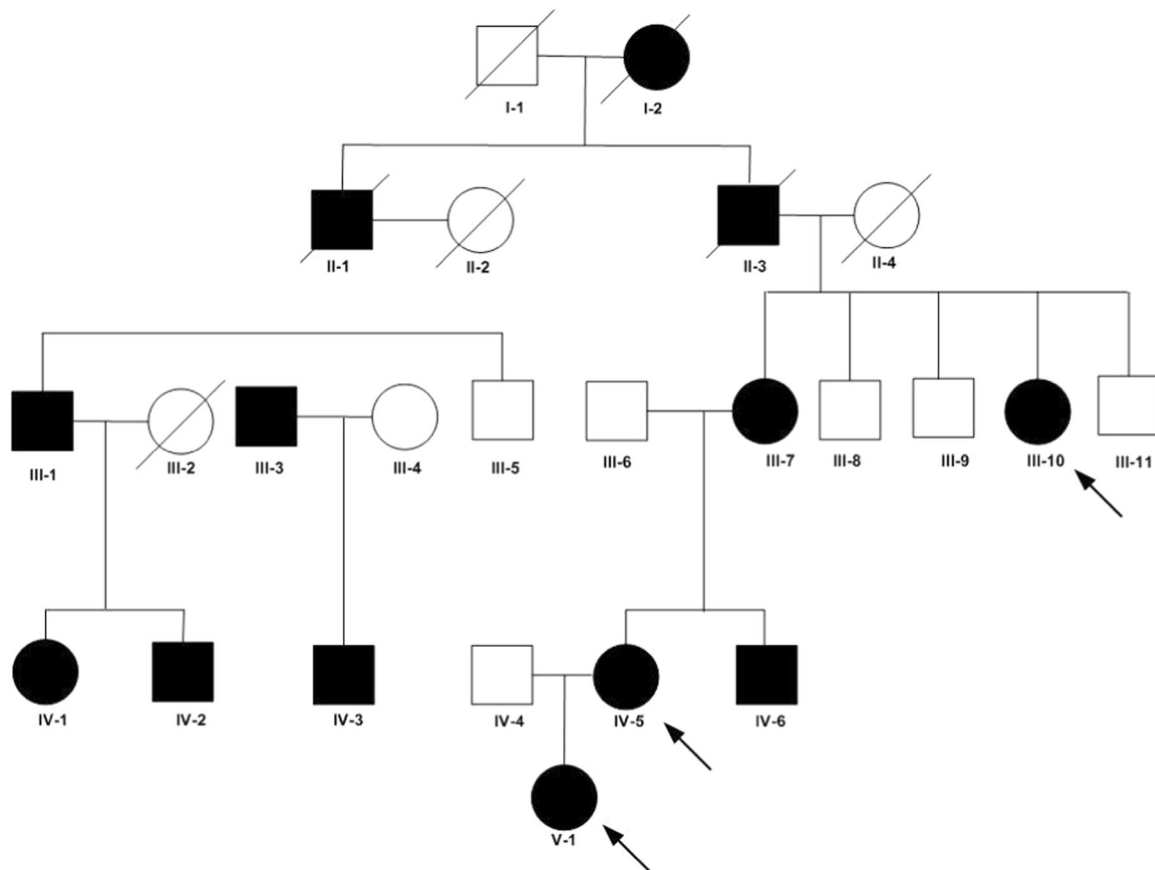
(reference). Direct sequencing confirmed that this missense mutation c.7 G>T in exon 2 of *GJA3* co-segregated with all affected members of the family (Fig. 2).

The p.D3Y mutation from aspartate (D3Y) to a tyrosine in the in the NH<sub>2</sub>-terminal (NT) 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 hemichannel activity [23, 24] (Fig. 3).

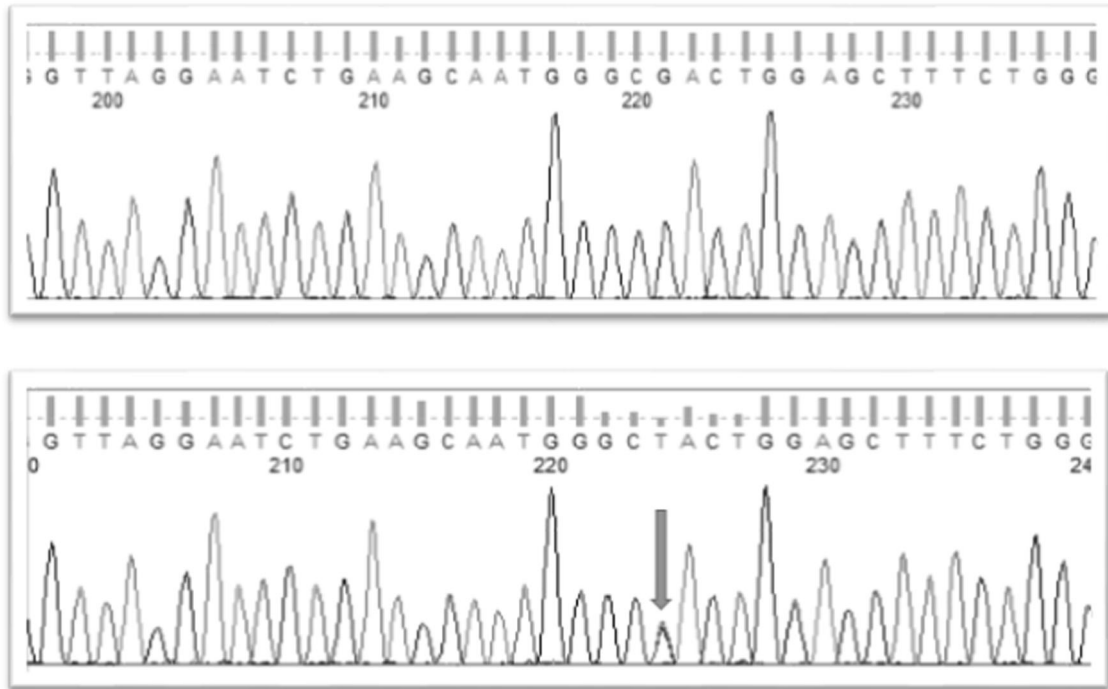
## Discussion

Here we report a missense mutation c.7 G>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. The inner fetal nucleus is made up of a clear lens surrounded by an opacified shell that

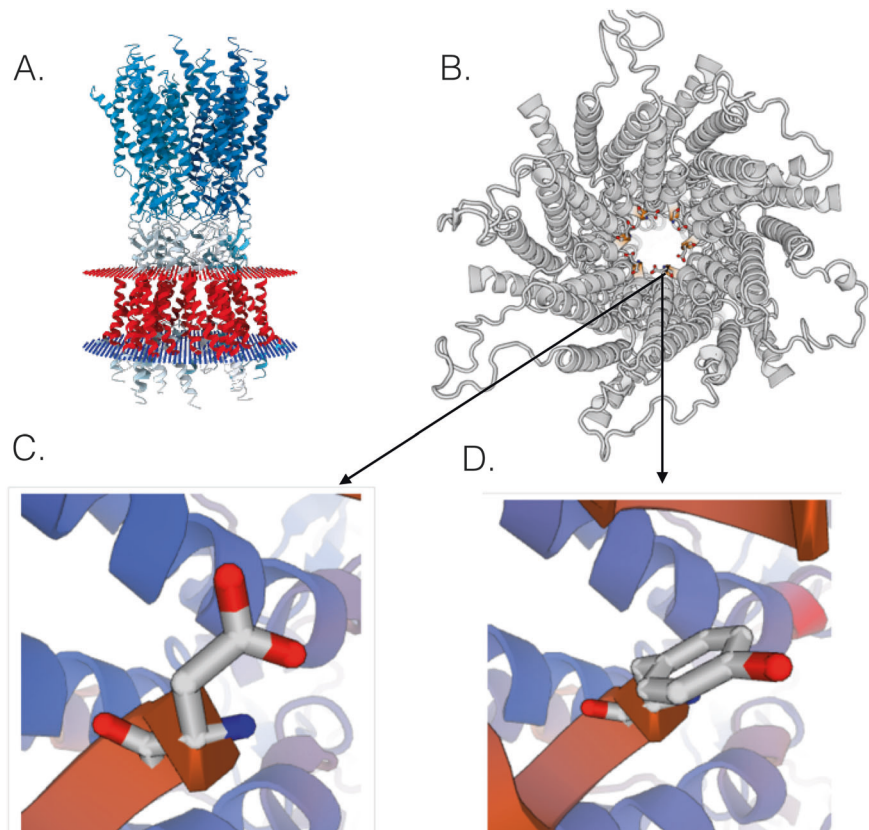


**Fig. 1** Abridged pedigree of the British family with lamellar cataract. Squares and circles symbolize males and females, respectively. Open and filled symbols indicate unaffected and affected individuals



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

**Fig. 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* hemichannel <https://swissmodel.expasy.org/repository/uniprot/Q9Y6H8>. **c** Wild-type amino acid at position 3 (Aspartate) **d** Mutant amino acid at position 3 (Tyrosine). The side chain of the tyrosine interferes with the hemichannel activity



**Table 1** Published mutations in GJA3 that cause cataract

Variant id	cDNA	Protein change	inheritance	Origin	Phenotype	Study	CADD	GERP NR
13-20717423-C-T	c.-39C>G	p.G2D	Complex	China	Age-related nuclear	Zhou et al. 2011		
13-20717421-C-G	c.5 G>A	p.D3H	AD	China	Nuclear pulverulent, Posterior polar	Yao et al. 2011	26.6	5.44
13-20717421-C-A	c.7 G>C	p.D3Y	AD	Australia		Ma, et al. 2015	26	5.44
13-20717421-C-A	c.7 G>T	p.D3Y	AD	Honduras	Zonular pulverulent	Addison, et al. 2006	27.4	5.44
<b>13-20717421-C-A</b>	<b>c.7 G&gt;T</b>	<b>p.D3Y</b>	<b>AD</b>	<b>UK</b>	<b>Lamellar</b>	<b>Present Study</b>	<b>27.4</b>	<b>5.44</b>
13-20717396-A-G	c.32 T>C	p.L11S	AD	Denmark	“Ant-egg”	Hansen, et al. 2006	22.5	5.59
13-20717372-G-A	c.56 C>T	p.T19M	AD	India	Posterior polar	Santhiya et al. 2010	29	5.59
13-20717346-C-T	c.82 G>A	p.V28M	AD	India	Total, anterior capsular, cortical	Devi, et al. 2005	31	5.59
13-20717334-A-G	c.96 C>A	p.F32L	AD	China	Nuclear pulverulent	Jiang, et al. 2003	27.6	5.59
13-20717330-C-A	c.98 G>T	p.R33L	AD	India	Granular embryonal	Guleria, et al. 2007	33	5.59
13-20717303-T-G	c.125 A>C	p.E42A	AD	China	Pulverulent	Yang et al 2014	24.1	5.36
13-20717298-C-T	c.130 G>A	p.V44M	AD	China	Central nuclear with punctate cortical	Zhou et al. 2010	26.1	5.36
13-20717298-C-T	c.130 G>A	p.V44M	AD	USA	?	Bennett, et al. 2011	26.1	5.36
13-20717298-C-T	c.130 G>A	p.V44M	AD	China		Ma, et al. 2016	26.1	5.36
13-20717294-C-G	c.134 G>C	p.W45S	AD	China	Nuclear	Ma, et al. 2005	24.9	5.36
13-20717289-C-T	c.139 G>A	p.D47N	AD	China	Nuclear	Yang et al. 2011	25.8	5.36
13-20717289-C-T	c.139 G>A	p.D47N	AD	China	Nuclear	Guo et al 2013	25.8	5.36
13-20717285-T-C	c.143 A>G	p.E48G	AD	China	Nuclear	Li, et al. 2015	24.1	5.36
13-20717265-T-C	c.163 A>G	p.N55D	AD	China	Central Nuclear	Hu et al 2014	22.9	5.36
13-20717280-A-G	c.148 T>C	p.S50P	AD	UK	Y-sutural and Lamellar	Gillespie, et al. 2014	22.9	5.36
13-20717252-G-A	c.176 C>T	p.P59L	AD	USA	Nuclear punctate	Bennett, et al. 2004	29.7	5.36
13-20717252-G-A	c.176 C>T	p.P59L	AD	Denmark	?	Hansen et al. 2009	29.7	5.36
13-20717252-G-A	c.176 C>T	p.P59L	AD	China	?	Sun et al. 2011	29.7	5.36
13-20717252-G-A	c.176 C>T	p.P59L	AD	UK		Gillespie, et al. 2014	29.7	5.36
13-20717252-G-A	c.176 C>T	p.P59L	AD	Australia		Ma, et al. 2015	29.7	5.36
13-20717252-G-A	c.176 C>T	p.P59L	AD	China	Pulverulent	Wang, et al. 2016	29.7	5.36
13-20717244-C-T	c.184 G>A	p.E62K	AD	UK		Gillespie, et al. 2014	33	5.36
13-20717240-T-C	c.188 A>G	p.N63S	AD	UK	Variable pulverulent	Mackay, et al. 1999	24	5.36
13-20717202-G-C	c.226 C>G	p.R76G	AD	India	Total	Devi, et al. 2005	29	5.36
13-20717201-C-T	c.227 G>A	p.R76H	AD	Australia	Nuclear lamellar pulverulent	Burdon, et al. 2004	34	5.36
13-20717201-C-T	c.227 G>A	p.R76H	AD	Denmark	Lamellar, sutural	Hansen et al. 2009	34	5.36
13-20717168-G-A	c.260 C>T	p.T87M	AD	India	“Pearl-box”	Guleria, et al. 2007	28.7	5.36
13-20717168-G-A	c.260 C>T	p.T87M	AD	Australia		Ma, et al. 2015	28.7	5.36
13-20717160-G-A	c.268 C>T	p.L90F	AD	China		Yang et al 2014	26.5	5.36

**Table 1** (continued)

Variant id	cDNA	Protein change	inheritance	Origin	Phenotype	Study	CADD	GERP NR
13-20717013-C-T	c.415 G > A	p.V139M	Complex	China	Age-related cortical	Zhou et al. 2011	22.8	5.09
13-20717001-C-T	c.427 G > A	p.G143R	AD	China	Granular central disc (Coppock-like)	Zhang et al. 2012	25.7	5.09
13-20717000-C-T	c.428 G > A	p.G143E	AD	China	Nuclear	Yuan et al 2015	24.9	5.09
13-20716985-G-A	c.443 C > T	p.T148I	AD	China	Pulverulent Nuclear	Yao, et al. 2017	25.7	5.09
13-20716868-G-A	c.560 C > T	p.P187L	AD	UK	Zonular pulverulent	Rees, et al. 2000	27.1	5.07
13-20716869-G-A	c.559 C > T	p.P187S	AD	China	Nuclear pulverulent	Ding et al. 2011	26.5	5.07
13-20716865-T-G	c.563 A > C	p.N188T	AD	China	Nuclear pulverulent	Li, et al. 2004	25.8	5.07
13-20716865-T-A	c.563 A > T	p.N188I	AD	China	Coraliform	Zhang et al. 2012	27.2	5.07
13-20716850-A-G	c.578 T > C	p.F193S		UK	Cataract and macrocephaly	Gillespie, et al. 2014	28.8	5.07
13-20716850-A-G	c.578 T > C	p.F193S	AD	UK	Dense nuclear	Muslehi, et al. 2016	28.8	5.07
13-20716839-G-A	c.589 C > T	p.P197S	AD	India	Lamellar	Ponnam et al 2013	26.9	5.07
13-20716832-T-G	c.596 A > C	p.E199A		UK		Gillespie, et al. 2014	26.6	5.07
13-20716812-A-T	c.616 T > A	p.F206I	AD	China	Embryonal nuclear	Wang et al. 2012	27.8	5.07
13-20716288-GCT-TTG	c.1137msC	p.S380QfsXX87	AD	UK	Punctate	Mackay, et al. 1999		4.02
13-20716287-T-G	c.1143_1165del23	p.S381RfsXX48	AD	China	Punctate nuclear	Sun et al. 2011		4.26
13-20716238-G-C	c.1189dupG	p.A397GfsXX71	AD	China	Coraliform	Zhou et al 2013		4.82
13-20716229-GT-TG	c.1196dupC	p.T400HfsX68	AD	China		Cui, et al. 2017		4.84

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

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, 16]. The elongated fiber cells of the lens constitute the main bulk of the lens’ mass and represent the target cells for cataract formation owing to miscommunication; *GJA3* protein mainly functions in gap junction communication between these cells [25]. Connexin 46 mutations are phenotypically highly heterogeneous [9] (summarized in Table 1).

In 1990, Willecke et al. [26, 27] were the first group to assign *GJA3* to chromosome 13, and after 9 years, Mackay et al. found the first connexin 46 mutations in humans causing autosomal-dominant congenital cataract. 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 NH<sub>2</sub>- 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; 6 from India, 4 from Australia, 3 from Denmark, 10 from UK, 2 from USA, and 1 from Honduras; and exhibiting different phenotypes (Table 1).

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 NT 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 NT along with E1 and TM1 contribute to the pore lining region of the hemichannel and therefore any compromise in the amino-acid residues may interfere with the conformation and flexibility of NT and also with voltage gating [28–32]. Schlingmann and co-workers in 2012 has shown the involvement of Asp-3 (D3Y) in the determination of the cell-to-cell coupling and for the voltage dependent Cx46 hemichannels. This hypothesis is further supported by Tong et al. (2013); they demonstrated the effect of D3Y on reduced hemichannel activity and alterations in voltage gating and charge selectivity. Lens fiber cells are dependent on intercellular communication for their survival [33, 34].

Ebihara et al. 2010 [35] 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. 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 [36].

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.

## Summary

### What was known before

- Opacification of the ocular lens is clinically and genetically a heterogeneous childhood disease.
- Previously, p.D3Y mutation in *GJA3* gene was found in a Hispanic family causing pulverulent cataract.

### What this study adds

- In this study we have identified a recurrent mutation in *GJA3* in a large British pedigree causing the novel phenotype of autosomal-dominant congenital lamellar cataract.
- Our study show further heterogeneity in inherited cataract, with the same mutation, on a different genetic background, causing a different phenotype, presumably through diverse mechanisms.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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