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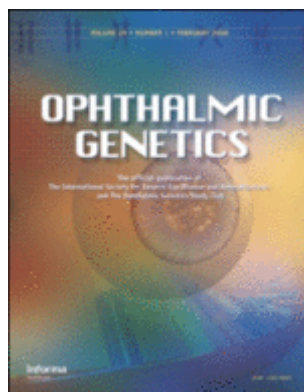
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**A novel missense mutation in LIM2 causing isolated
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A novel missense mutation in *LIM2* causing isolated autosomal dominant congenital cataract

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7 Abstract:

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9 **Introduction:** Congenital cataract is the most common cause of blindness in the world. Congenital
10 cataracts are clinically and genetically heterogeneous and are mostly inherited in an autosomal
11 dominant fashion. We identified the genetic cause of isolated autosomal dominant cataract in a
12 four-generation British family and a Czech family.
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18 **Methods:** Whole exome sequencing (WES) was performed on one affected member in the British
19 family and two affected members in the Czech family.
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23 **Results:** A novel missense variant c.388C>T; p.(R130C) was identified in the Lens integral
24 membrane protein (LIM2) and found to co-segregate with disease in both families.
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28 **Conclusions:** Here we report the first autosomal dominant congenital cataract variant p.(R130C)
29 in LIM2, causing a non-syndromic pulverulent and nuclear phenotype in European families.
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32 33 34 **Introduction**

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37 Cataract accounts for nearly 50% of worldwide blindness ([https://www.who.int/publications-
38 detail/world-report-on-vision](https://www.who.int/publications-detail/world-report-on-vision)). Congenital cataracts are phenotypically and genotypically
39 heterogeneous and can occur in isolation or in association with other systemic disorders.
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42 Significant progress has been made in identifying the molecular genetic basis of cataract, with 38
43 genes identified to date associated with isolated cataract.¹ These genes encode many different
44 proteins including, intracellular lens proteins (crystallins), membrane gap junction proteins
45 (connexins), cytoskeletal proteins (*BFSP1*, *BFSP2* and *VIM2*), transcription factors (*FOXE3*,
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PAX6, *PITX3* and *MAFA*), lanosterol synthase (*LSS*), and lens integral proteins (*MIP* and *LIM2*).

LIM2 also known as *MP19*, is the second most abundant integral membrane protein present in the
ocular lens fiber cells of vertebrates,² consisted of 173-amino-acid with four transmembrane

domains, two extracellular loops, a cytoplasmic loop, and cytoplasmic amino and carboxyl termini.³ It localizes to junction regions of the lens fiber cell membrane as well as throughout the fiber cell membrane, suggesting a role in junction communication^{4 5 6} and shown to interact with calmodulin and galectin.⁷

Mutations in *LIM2* have been associated recessive cataracts and age-related cataracts. To date only three mutations have been identified causing age-related and autosomal recessive forms of cataract. Here we report the first autosomal dominant variant in *LIM2* responsible for an isolated pulverulent and nuclear phenotype in Europeans.

Material and Methods:

Phenotyping

The British family studied was identified through the proband attending the Genetic Service at Moorfields Eye Hospital, London, UK. Local ethics committee approval was obtained and all individuals taking part gave written informed consent. Nine family members including 6 affected, 2 unaffected and one married-in, underwent full ophthalmic examination (Figure 1A). The Czech family studied consisted of a mother and child (Figure 1B). This study was managed in accordance with the Declaration of Helsinki and approved by the Ethical committee of the General University Hospital in Prague. Informed consent was obtained by all participants or their legal guardians prior to the start of the study.

Whole exome sequencing (WES) and Bioinformatics

Genomic DNA was extracted from EDTA sequestered blood samples taken with informed consent and local ethical approval using the Nucleon II DNA Extraction Kit (Scotlab Bioscience, Strathclyde, Scotland, UK). Whole exome sequencing (WES) was undertaken in one affected member (III-2) of the family (Figure 1A), and both (II-2 and III-1) affected members in the Czech family (Figure 1B). The DNA was sequenced at Macrogen Europe. Exon capture and target enrichment was performed using the SureSelectXT Human All Exon V6post, (Agilent, Santa Rosa, CA, USA). Paired-end sequencing was performed on an Illumina Hiseq 2500 high-throughput

sequencer, generating mean exome coverage of 50x. Raw data in fastq format was analysed using the Phenopolis bioinformatics platform⁸. The short-read sequence data were aligned using novoalign (version 3.02.08). Variants and indels were called according to GATK best practices (joint variant calling followed by variant quality score recalibration). The variants were then annotated using the Variant Effect Predictor (VEP). Variants were then filtered to only contain novel variants, not present in public control databases Kaviar and gnomAD (<http://gnomad.broadinstitute.org/>), in known cataract genes (<https://cat-map.wustl.edu/>) and predicted to be moderately or highly damaging. Bi-directional direct Sanger sequencing was performed on ABI 3730 to validate the variant p.(R130C) identified by next-generation sequencing.

Structural bioinformatics

The protein structure of LIM2 was analysed using SWISSMODEL (<https://swissmodel.expasy.org/repository/uniprot/P55344>) (Figure 1). The best PDB match, with a match of 17.83%, was the structure of mouse claudin-3 P134G mutant in complex with C-terminal fragment of Clostridium perfringens enterotoxin, solved with X-ray diffraction by Nakamura et al, in 2019.⁹ All structures were downloaded in PDB format and analysed using Pymol (version 1.8) locally.

Results

All affected family members in the British family had bilateral pulverulent cataract. Individual II-2 had surgery in infancy; III-2 had aphakia and glaucoma. In the Czech family, information on the cataract type was only available for one individual (III-1), who had a nuclear cataract phenotype, and surgery in infancy. The proband (II-2) also underwent cataract surgery in infancy.

After the Phenopolis genetic variant analysis pipeline by allele frequency, in II-2 of the British family, from a total of 123,692 variants, only 403 variants remained. A list of 115 cataract-associated genes was used for gene panel screening. The variants were then sorted by CADD score. The top scoring variant for CADD (score of 35) was a rare heterozygous damaging variant

1 NM_001161748.2 c.388C>T; p.(R130C) in exon 4 of *LIM2*. Direct sequencing confirmed the
2 variant (Figure 2), which co-segregated in all the affected members of the British family (Figure
3 1A), and in two affected individuals from the Czech family. The R130C substitution is located in
4 the second extracellular loop of the LIM2 protein is likely to perturb membrane trafficking and fiber
5 cell-cell communication. It also modifies the structure of the protein compared to wild-type
6 (RMS=0.286) (Figure 3).
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16 Discussion

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18 In this study we report a novel missense variant, c.388C>T; p.(R130C) in *LIM2* in a four-
19 generation British pedigree with non-syndromic AD congenital pulverulent cataract, and a Czech
20 family with a nuclear cataract phenotype. These phenotypes have also been reported in various
21 mouse models with *Lim2* mutations. A *Lim2* missense variant, p.(G15V) has been identified in the
22 To3 mouse and resulted in autosomal semi-dominant congenital cataracts. Heterozygous mice
23 exhibited dense cataracts, whilst homozygous mutant mice suffered microphthalmia, lens rupture,
24 and disorganised primary and secondary fibres¹⁰. *Lim2* homozygous knockout mice have
25 pulverulent nuclear opacities and altered refractive properties of the lens, whereas heterozygotes
26 had normal lenses, suggesting that loss of function of *Lim2* is responsible for the phenotype.¹¹
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41 In humans, to date only three recessive variants have been identified in *LIM2*; first in an Iraqi
42 family p.(F105V)¹² associated with mild pulverulent cataract, with a pre-senile or senile onset. The
43 other two, p.(G154E)¹³ and p.(G78D)¹⁴, in families of South Indian and Pakistani origin
44 respectively (Table 1). Here we report the first autosomal dominant congenital cataract variant
45 p.(R130C) in *LIM2*, causing a non-syndromic pulverulent and nuclear phenotype in European
46 families, which we suggest results in aberrant modification of protein structure.
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1. Shiels A, Bennett TM, Hejtmancik JF. Cat-Map: putting cataract on the map. *Mol Vis* 2010; 16:2007–15.
2. Mulders JW, Voorter CE, Lamers C, de Haard-Hoekman WA, Montecucco C, van d V et al. MP17, a fiber-specific intrinsic membrane protein from mammalian eye lens. *Curr Eye Res* 1988; 7: 207-219.
3. Arneson ML, Louis CF. Structural arrangement of lens fiber cell plasma membrane protein MP20. *Exp Eye Res* 1998; 66:495-509.
4. Louis CF, Hur KC, Galvan AC, TenBroek EM, Jarvis LJ, Eccleston ED et al. Identification of an 18,000-dalton protein in mammalian lens fiber cell membranes. *J Biol Chem* 1989; 264: 19967-19973.
5. Taylor V, Welcher AA, Program AE, Suter U (1995) Epithelial membrane protein-1, Peripheral myelin protein 22, and lens membrane protein 20 define a novel gene family. *J Biol Chem* 270:28824–28833
6. Tenbroek E, Arneson M, Jaris L, Louis C (1992) The distribution of the fiber cell intrinsic membrane proteins MP20 and connexin 46 in the bovine lens. *J Cell Science* 103:245–257
7. Gonen T, Grey AC, Jacobs MD, Donaldson PJ, Kistler J. MP20, the second most abundant lens membrane protein and member of the tetraspanins superfamily, joins the list of ligands of galectin-3. *BMC Cell Biol* 2001; 2:17.
8. 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; 33(15): 2421–2423.
9. Nakamura S, Irie K, Tanaka H, Nishikawa K, Suzuki H, Saitoh Y, Tamura A, Tsukita S and Fujiyoshi Y Morphologic determinant of tight junctions revealed by claudin-3 structures. *Nature Communications* 2019;10: 816. -7https://

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10. Steele EC Jr, Kerscher S, Lyon MF, Glenister PH, Favor J, Wang J, Church RL. Identification of a mutation in the MP19 gene, Lim2, in the cataractous mouse mutant To3. *Mol Vis*;1997 3:5.
11. Shiels A, King JM, Mackay DS, Bassnett S. Refractive defects and cataracts in mice lacking lens intrinsic membrane protein-2. *Invest Ophthalmol Vis Sci* 2007; 48:500-8.
12. Pras E, Levy-Nissenbaum E, Bakhan T, Lahat H, Assia E, Geffen-Carmi N et al. A missense mutation in the LIM2 gene is associated with autosomal recessive presenile cataract in an inbred Iraqi Jewish family. *Am J Hum Genet* 2002;70: 1363-1367. doi: 10.1086/340318 PMID: 11917274
13. Ponnamp SP, Ramesha K, Tejwani S, Matalia J, Kannabiran C. A missense mutation in LIM2 causes autosomal recessive congenital cataract. *Mol Vis*. 2008 14: 1204-1208. PMID: 18596884
14. Irum B, Khan SY, Ali M, Kaul H, Kabir F, Rauf B, Fatima F, Nadeem R, Khan AO, Saif AI, Obaisi SA et al. Mutation in LIM2 Is Responsible for Autosomal Recessive Congenital Cataracts. *PLoS ONE* 2016, 11(11): e0162620. doi: 10.1371/journal.pone.0162620

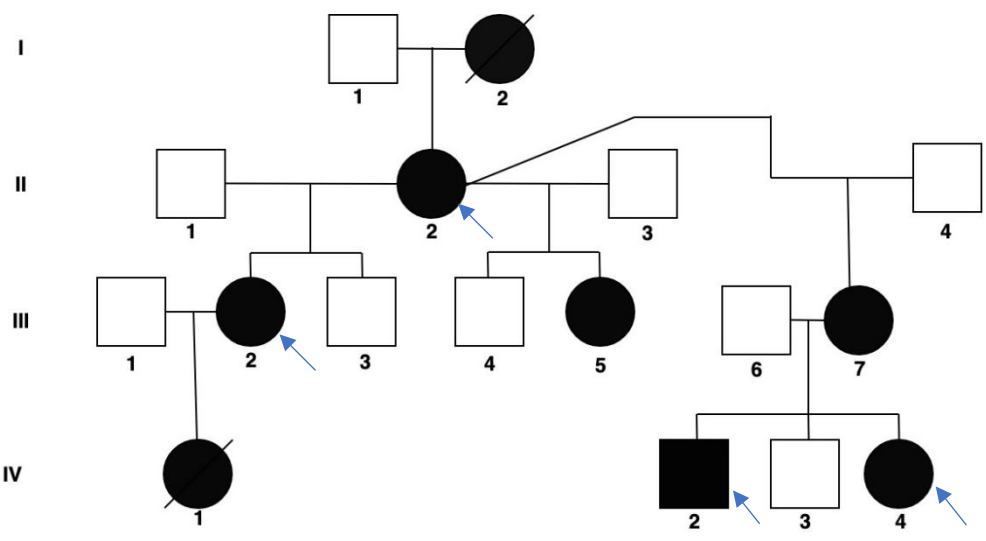
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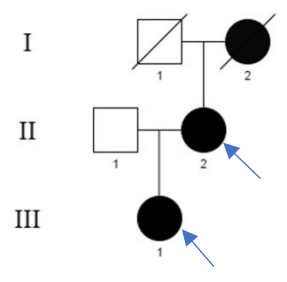
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7 Conflict of interests: The authors declare that they have no conflict of interest.
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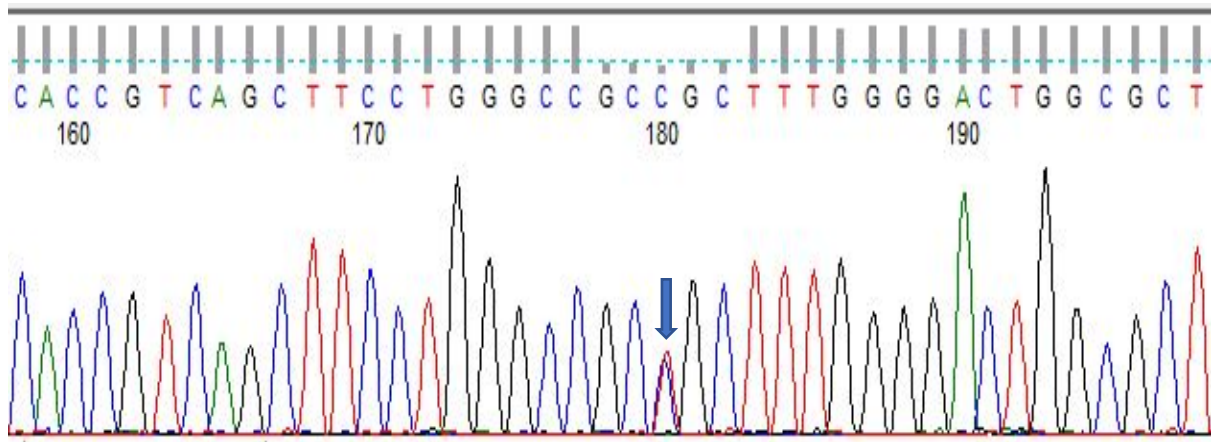


A]-British Pedigree

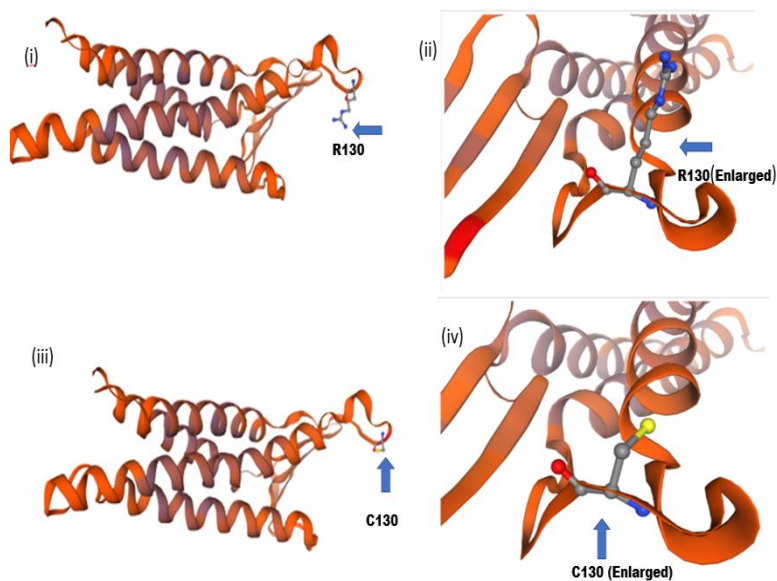


B] Czech Pedigree

Figure 1: (A) British and (B) Czech pedigrees showing a dominant inheritance pattern. Open and filled symbols indicate unaffected and affected individuals.



A] Sequence of LIM2



B] Structure of LIM2 protein

Figure 2: (A) Sequence analysis of LIM2 showing segregation of the p.(R130C) variant in an affected individual. (B) Structure of the LIM2 protein- c.388C>T; p.(R130C) variant affects the second extracellular domain of LIM2 and modifies the structure of wild-type protein (RMS = 0.286).

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Table 1: Spectrum of *LIM2* Mutations causing cataractogenesis.

Exon	HGVSc	HGVSp	Genomic	CADD	Inheritance	Ethnicity/ Origin	Phenotype	Reference
Ex3/5	c.313 T>G	p. F105V	chr19- 51885684- A>C	23.9	AR	Iraqi- Jewish	Presenile Cortical sutural	Pras et al 2002
Ex3/5	c.233G>A	p. G78D	chr19- 51885764 -C>T	31	AR	Pakistan	Nuclear	Irum et al 2016
EX5/5	c.461G>A	p. G154E	chr19- 51883516 -C>T	32	AR	South India	Congenital	Poonam et al 2008
Ex4/5	c.388C>T	p. R130C	chr19- 51883831 -G>A	35	AD	British and Czech	Pulverulent and Nuclear	Present Study

All mutations are on the transcript ENST00000596399.1 of *LIM2* - produces a 173 aa protein (Uniprot: **P55344-1**). CADD=Combined Annotation Dependent Depletion; AR=Autosomal Recessive; AD=Autosomal Dominant