Short Communication

Two New Variants in *FYCO1* Are Responsible for Autosomal Recessive Congenital Cataract in Iranian Population

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Abstract

The purpose of this experimental study was to investigate the genetic etiology of congenital cataract (CC) manifesting an autosomal recessive pattern of inheritance in four Iranian families. Affected individuals and their normal first-degree relatives in each family were included in the present study. The genomic DNA of the blood samples was extracted from all participants, and one affected member belonging to each family was subjected to Whole Exome Sequencing (WES). Using bidirectional Sanger sequencing, the identified variants were validated by co-segregation analysis. Two different mutations were detected in the *FYCO1* gene encoding FYVE and coiled-coil domain-containing protein. A previously reported missense mutation, c.265C>T (p.Arg89Cys), was found in one Iranian family for the first time, and a combination of two variants in a single codon, c.[265C>T;267C>A] (p.Arg89X), was identified in the three other families. On the other hand, accompanying the c.265C>T mutation, the presence of the c.267C>A polymorphism leads to a premature stop codon. In-Silico Analysis of FYCO1 protein demonstrated that RUN domain will be interrupted so that the large part of functional protein will be eliminated due to this novel variant. FYCO1 has been proved to be involved in human lens development and transparency. Its mutations, therefore, result in CC. Herein, we reported the first autosomal recessive CC patients with c.265C>T (p.Arg89Cys) or c.[265C>T;267C>A] variant in Iranian population for the *FYCO1* gene. *FYCO1* mutations could be tracked for preventive objectives or even be targeted as therapeutic candidates via treatment approaches in the future.

Keywords: Congenital Cataract, *FYCO1*, Mutation, Sanger Sequencing, Whole Exome Sequencing

Cataract is defined as any opacity of the transparent lens of the eye, causing blindness and visual impairment (1, 2). Cataracts can occur either in association with a large number of genetic disorders (3, 4) or due to some environmental factors such as infectious agents like cytomegalovirus, toxoplasmosis, rubella, herpes, varicella, and syphilis, drug exposure during pregnancy (corticosteroids, vitamin A), and X-ray radiation. Congenital cataract (CC) is a clinically and phenotypically highly heterogeneous eye disorder (5, 6), which may lead to permanent blindness if remains undetected in childhood. CC refers to the cataract causing by various factors and affecting the development of the lens during the fetal period and it approximately accounts for about 10% of all childhood blindness (7, 8). Hereditary is the leading cause of at least one-third of all these cases. Autosomal dominance is the most frequent pattern of inheritance reported for this disorder, although it could be autosomal recessive or X-linked. More than 15 distinct loci on 14 unique chromosomal locations have been identified to be involved in this disorder (3, 4, 9-11). To date, several genes coding lens proteins, cytoskeletal proteins, transcription factors as well as membrane transport protein genes are known to be involved in this disorder (12). Considerably, 27 genes such as *CRYAA, CRYB1, CONNEXIN 46, PITX, EPHA2,* and *FOXE3, GJA8, FYCO1* have been identified to be linked in non-syndromic autosomal recessive CC (arCC) (1, 11, 13).

The incidence of CC in different populations is around 12-136 per 100000 children (9). Inherited cataracts account for about 8.3-25% of all CC cases (14). While the global prevalence of this disorder is 1 to 15 per 10,000 children (15), the overall risk for this disorder is 92.4 per 100,000 children, 107.9 and 76.2 for males and females, respectively (9). Since consanguineous marriage is of high frequency in Iran, it is not unexpected to observe a high incidence of this condition in the Iranian population.

Considering the meaningful prevalence of CC in

Soozandeh village in Iran, the purpose of this study was to identify the genetic basis of CC in this area using next-generation sequencing (NGS) and Sanger sequencing techniques and assessing risks for the next generation in this population.

This experimental study was initially approved by the Ethical Committee of Sabzevar University of Medical Sciences (IR.MEDSAB.REC.1395.120). Four Iranian families with history of CC were selected from the people of Soozandeh village in Iran. One affected individual of each family was recruited in a high throughput study. Additionally, all affected and unaffected siblings and living parents entered the experiment following the segregation study. The Family members' blood sample was collected after getting written informed consent of all participants. A family medical history was recorded and a pedigree for each family was constructed based on the acquired information. Two families were consanguineous.

The living family members underwent ocular examination by a cornea fellowship in Vasei Hospital, Sabzevar University of Medical Sciences. The assessment included: gross appearance, visual acuity measurement, intraocular pressure (IOP) measurement using (Haagsh, Tonometer, Switzerland), eye movements, evaluation of anterior segment consisting of the cornea, pupil, lens, and iris; as well as posterior segment including fundus, vitreous chamber, retina, optic disk, and macula, using a slit lamp (Topcon, Japan).

Whole blood as the source of genomic DNA was processed using a salting out procedure. The DNA concentration was determined by a Nano drop spectrophotometer (BIO INTELLECTICA Nano100, Canada). Two micrograms of DNA were used for whole Exome Sequencing (WES), using Illumina HiSeq 2500, Q30≥80% (Novogene, Beijing). WES using UCSC hg19 as a reference genome, included exome capture using Agilent SureSelect Human All Exon V6 Kit and sequencing depth of 50× through paired-end sequencing with a HiSeq 2500 Genome Analyzer (Illumina), which led to 150 bases sequences from each end of the fragments. SNV and InDels were identified using VarScan version 2.2.5, and MuTec and GATK Somatic Indel Detector, respectively. The prediction of probable effects due to identified variants was performed using Mutation Taster (16). The secondary structure of mutant FYCO1 protein was predicted by GORIV (17).

For extension of the mutated region, conventional polymerase chain reaction (PCR) was performed using Taq DNA Polymerase Master Mix RED (Ampliqon, Odense M DENMARK) and specific primer sequences of

F: 5′-TAAATGCCGGAATGAAGAGC-3′
R: 5′-GCTTTAAGCAGGCAAAAGG-3′ (product size 241 bp).

Primer designation was performed using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/). Amplification was performed using the parameters below: initial denaturation at 95°C for 2 minutes, 35 cycles of 95°C for 30 seconds, 54.9°C for 30 seconds and 72°C for 35 seconds, followed by final 5 minutes extension at 72°C. The cleanup PCR products using ExoSAP-IT® (USB, Cleveland, Ohio, USA) were bi-directionally sequenced using a 3730XL DNA analyzer (ABI, Foster City, CA, USA).

Four families affected by CC (IrCC1, IrCC2, IrCC3, and IrCC4) were included from Soozandeh village of Iran. Drawing the pedigrees demonstrated an autosomal recessive pattern of inheritance (Fig.1). Two families including IrCC1 and IrCC3 showed consanguineous marriage. The medical history of affected members was obtained in detail, and cataracts were validated via the patients' medical records and ocular examination. The individuals affected by CC had already undergone cataract-removal surgery; amongst, some had posterior chamber intraocular lens and some were aphakic. Intra-ocular pressure in both healthy and affected groups was in the normal range of 12-18 mmHg, without any significant difference.

WES was performed on the genomic DNA of one proband in each family. Regarding the consanguinity in two families under the study and the probability of autosomal recessive inheritance pattern in these families, we prioritized homozygous potentially functional variants residing within the runs of homozygosity (larger than 1 Mb). The genomic variants screening was performed through different population databases of gnomAD and GME, as well as Iranome, an in-house database for genetic variants in the Iranian population. For the filtering approach, synonymous variants, intronic variants (>5 bp from exon boundaries), and variants with >0.01% minor allele frequency were eliminated. Homozygous variants, as well as compound-heterozygous variants, were designated in consanguineous families. Intriguingly, in all four families, we found 2 different homozygous variants in the *FYCO1* gene. c.265C>T (p.Arg89Cys) (NM_024513.4; rs141476300), a previously known missense mutation, was identified in the proband of IrCC1. However, it is the first report of this variant in the Iranian population. Interestingly, c.[265C>T;267C>A] (p.Arg89X) in the same position was detected in the probands of families IrCC2, IrCC3, and IrCC4, resulting in a premature stop codon and subsequent protein truncation (Fig.2). The variants were investigated in affected and unaffected family members by Sanger sequencing (Fig.3). In the aforementioned three families, carriers were heterozygous for c.265C>T and homozygous for c.267C>A. c.267C>A is known to be a frequent allele in the Iranian population-based on Iranome. Table 1 represents the reported pathogenic mutations in the *FYCO1* gene with related amino acid changes in non-syndromic CC.
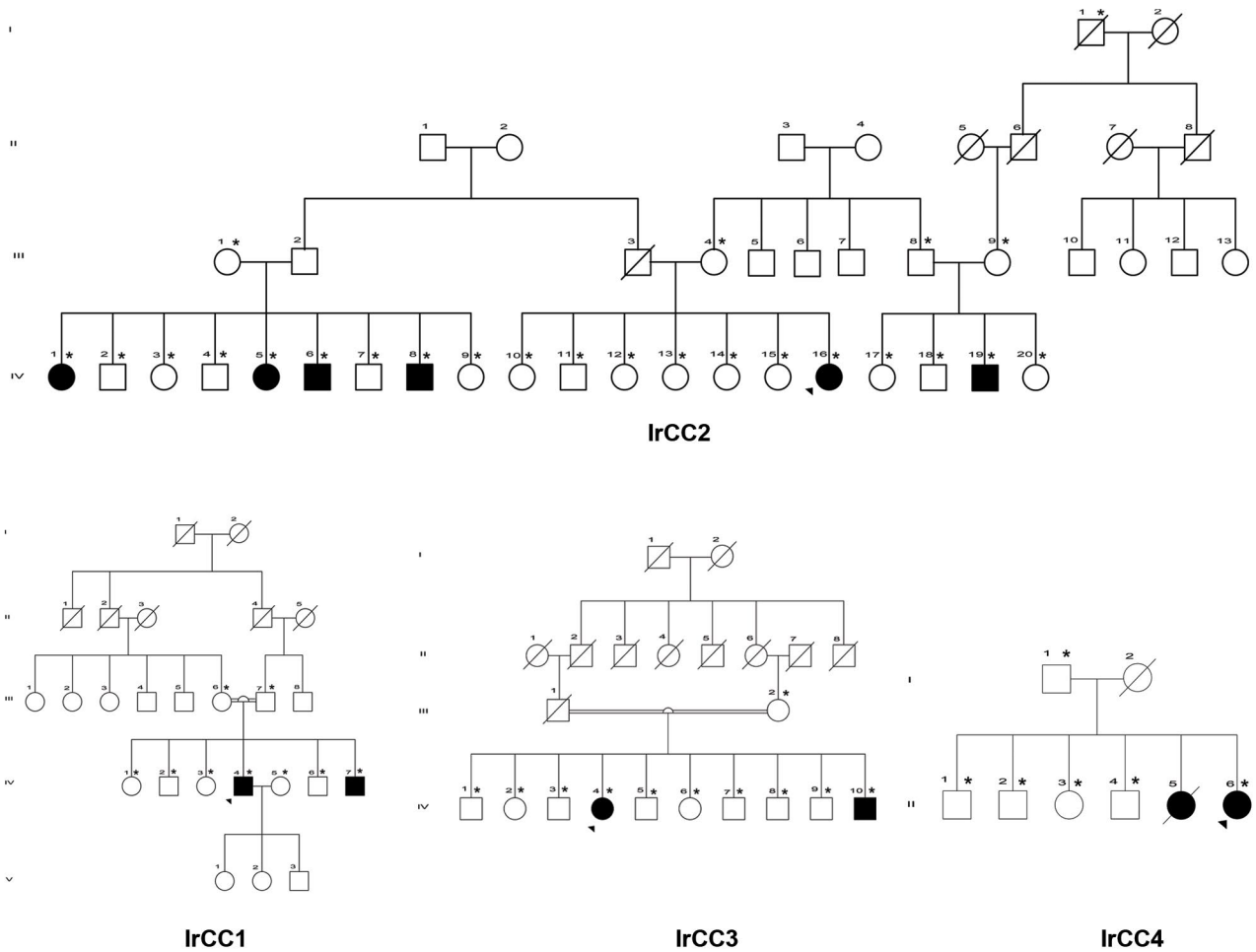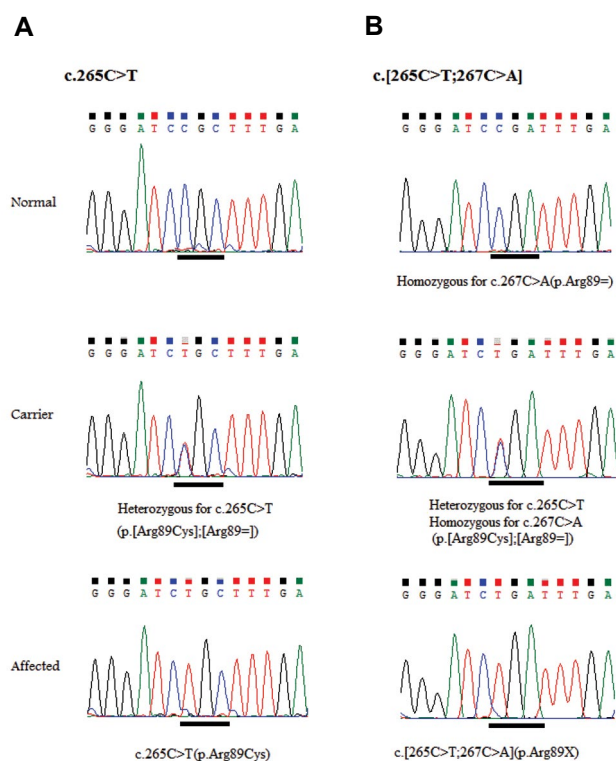
Fig.1: Four pedigrees of CC originated from Iran. One proband per family was selected for WES. The probands' parents of IrCC1 and IrCC3 families were first-degree cousins. Affected individuals have been depicted by filled symbols. Individuals marked with an asterisk were genotyped by Sanger sequencing for mutation screening. IrCC; Iranian family of congenital cataract, WES; Whole exome sequencing, and CC; Congenital cataract.
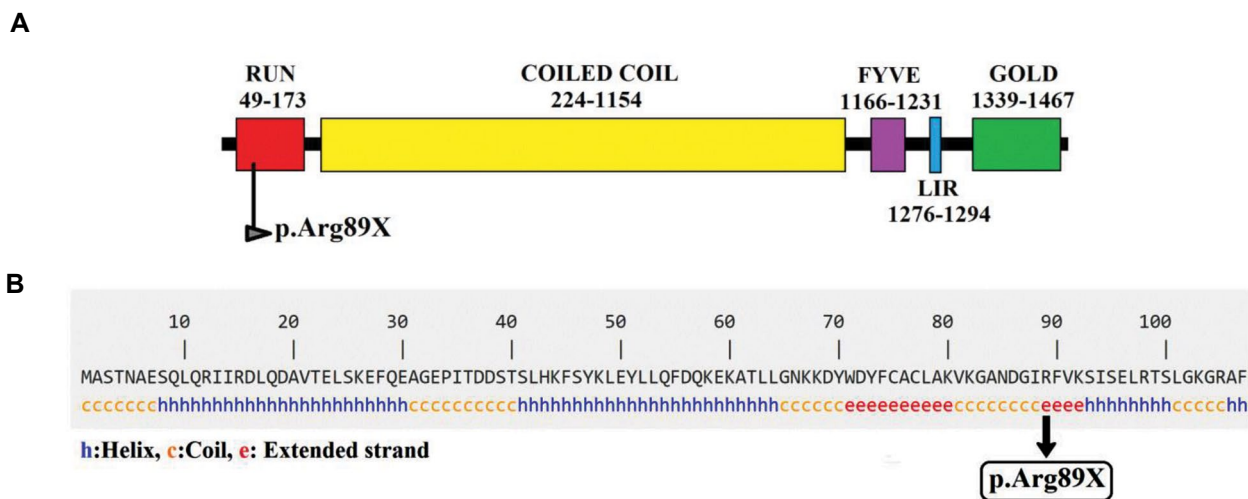
Fig.2: Position of c.267C>A variant in FYCO1 at protein level. **A.** p.Arg89X identified in our study in Run domain of FYCO1 protein in three Iranian families. **B.** Secondary structure prediction of mutant FYCO1 protein. In-silico analysis illustrates that the p.Arg89X mutation results in a truncated product.

In the present study, we investigated the genetic cause of CC which is very common over multiple generations in Soozandeh, a small village in Iran. To assess the exact etiology of CC in these populations, we performed gene analysis through WES. We identified c.265C>T (p.Arg89Cys) in the *FYCO1* gene in the non-consanguineous IrCC4 family. According to the in-house Iranome database, this missense mutation was detected for the first time, in the Iranian population. In the other three families, two of which were consanguineous, a combination of two variants in codon 89, as c.[265C>T;267C>A] (p.Arg89X), was detected in the *FYCO1* gene. Genotyping the affected and carrier members of these families revealed that carriers were heterozygous for c.265C>T and homozygous for c.267C>A (rs4682801). The allele frequency of c.267C>A in the Iranian population is 0.8931, being found in certain ethnicities including Kurd, Persian Gulf Islander, Turkmen, Lur, Arab, Persian, Azeri and Baloch. According to The Genome Aggregation Database (gnomAD), 1000 Genomes Project, and NHLBI Exome Sequencing Project (ESP) Exome Variant Server, the allele frequency of c.265C>T is 0.00041, 0.00040, and 0.00031, respectively.

Cataracts are caused by alterations in either lens structure, intracellular proteins order, or development of lens fibers. *FYCO1*, located on 3p21.3, encodes a protein containing disparate domains and regions including a α-helical RUN domain, a FYVE-type zinc finger domain, a Golgi dynamics (GOLD) domain, four coiled-coil regions, and a LC3-interacting region (LIR) (18). FYVE and coiled-coil domain containing 1 (FYCO1) is a member of the phosphatidylinositol-3-phosphate (PI3P)-binding protein family which is expressed in eye tissue. FYCO1 functions in the transportation of microtubule plus-end-directed of autophagic vesicles through interactions with the small GTPase RAB7, PI3P, and the autophagosome marker LC3 (19). CC and dysmorphic lens development disorders may be caused by mutation of the *FYCO1* gene via disruption of autophagosomal transportation to lysosomes and resultant defective degradation of mitochondria and other organelles in lens fibroblasts (18, 20, 21).

Mutations in *FYCO1* were first reported by Pras et al. (22) as a cause of arCC-2 (CATC2, OMIM: 610019) or cataract 18 in consanguineous Arab families. Subsequently, various mutations have been identified in different loci of *FYCO1* in association with cataract 18 in different ethnicities. A large number of mutations in *FYCO1* occurs with higher frequency for frame-shift and nonsense mutations such as c.1045C>T (p.Gln349X), c.1546C>T (p.Gln516X), c.2206C>T (p.Gln736X), c.2761C>T (p.Arg921X), c.2830C>T (p.Arg944X), c.3755delC (p.Ala1252AspfsX71), c.3858_3862dupGGAAT (p.Leu1288TrpfsX37) (18, 23), and c.808C>T (p.Gln270X) (24). In the Iranian population, the first report of a homozygous mutation in *FYCO1 was* related to c.l056_1071delGGCCACACGGGACTCA (p.E352DfsX9) (25). In our study, the c.[265C>T;267C>A] (p.Arg89X) and c.265C>T (p.Arg89Cys) mutations are the first reports in the Iranian population. According to the bioinformatics analysis of our findings, c.[265C>T;267C>A] (p.Arg89X) mutation resulting in premature stop codon interrupts the RUN domain in the beginning portion of FYCO1 protein so that the large parts of the functional protein are eliminated (Fig.2). Due to this huge elimination, bioinformatics analysis on the three-dimensional structure of the truncated protein was not feasible. Nonetheless, premature stop codons mostly terminate in mRNA degradation via nonsense-mediated mRNA decay (NMD). The c.265C>T (p.Arg89Cys) mutation deleteriously affects the protein structure and function so that is considered damaging based on bioinformatics prediction tools including SIFT, Polyphen, PROVEAN, CADD (PHRED score for c.265C>T: 28.1) and Mutation Taster. In addition, this mutation has been recently established in ClinVar as a likely pathogenic variant.

By the time, 157 different variants in *FYCO1* have been established in Iranome found in different ptable arts of *FYCO1* including exons, introns, splice sites, and untranslated region (UTR); of which 42 variants change protein sequence. However, only c.3150+1G>T

Fig.3: Sequencing chromatograms demonstrating two homozygous mutations in the *FYCO1* gene related to cataract 18. **A.** Forward sequence chromatograms related to c.265C>T (p.Arg89Cys) mutation in the normal, carrier, and affected individuals in family IrCC4. **B.** Forward sequence chromatograms of c.[265C>T;267C>A] (p.Arg89X) mutation in the normal, carrier, and affected individuals in families IrCC1, IrCC2, and IrCC3. Probands are homozygous and unaffected individuals are heterozygous. Black bars mark the variant position.

has been reported to be likely pathogenic based on ClinVar. Other identified variants of Iranome have been known as benign or of uncertain significance since these variations have been found in a healthy population. Being detected in the Iranian population, following out the mutations of *FYCO1* might have prenatal or postnatal diagnostic values since a cataract as a visual problem can affect the quality of neonatal life. Early diagnosis could be helpful to families to manage their child's requirements at the right time.

In conclusion, we report two mutations of the *FYCO1* gene including c.265C>T (p.Arg89Cys) and c.[265C>T;267C>A] (p.Arg89X) in arCC in 4 originally Iranian families. Our study provides a new understanding of arCC pathogenesis in the Iranian population, and therefore it might be helpful to consider *FYCO1* mutations in genetic diagnostic methods in pediatric cataracts. Moreover, *FYCO1* mutations could be tracked for preventive objectives or even be targeted as therapeutic candidates for gene therapy approaches in the future.

Table 1: List of reported *FYCO1* gene mutations associated with non-syndromic CC

Exon/Intron	Nucleotide changes	Amino acid changes	Type	Reference(s)
Ex6	c.449T>C	p.I150T	Missense	(26)
Ex8	c.808C>T	p.Q270X	Compound heterozygous	(27)
Ex8	c.1045C>T	p.Q349X	Nonsense	(18)
Ex8	c.1546C>T	p.Q516X	Nonsense	(18)
Ex8	c. 2206C>T	p.Q736X	Nonsense	(18, 23)
Ex8	c.2345delA	p.Q782RfsX32	frame shift	(23)
Ex8	c.2345delA/ c.2714_2715delCA	p.Q782RfsX32/ p.T905SfsX2	Compound heterozygous	(28)
Ex8	c.2506delG	p.A836PfsX80		(29)
Ex8	c.2761C>T	p.R921X	frame shift	(18)
Ex8	c.2830C>T	p.R944X	Nonsense	(18)
Ex8	c.l056_1071delGGCCACACGGGACTCA	p.E352DfsX9	Nonsense	(25)
IVS9	c. 3150＋1G>T	splice variant	frame shift	(18)
IVS9	c.3151-2A>C	p.A1051DfsX27	splice variant	(23)
Ex10	c.3196delC	p.H1066IfsX10	frame shift	(13)
Ex13	c.3670C>T	p.R1224X	frame shift	(30)
Ex13	c.3755delC	p.A1252DfsX71	Nonsense	(18)
Ex14	c.3858_3862dupGGAAT	p.L1288WfsX37	frame shift	(18)
IVS14	c.3945-1G>C	splice variant	frame shift	(30)
Ex16	c.4127T>C	p.L1376P	splice variant	(13, 18)
Ex17	c.4270C>T	p.R1424X	Missense	(13)

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Authors' Contributions

E.Sh., Z.B.; Designed and supervised the study. E.Sh.; Funded the study, recruited the patients, and did the ocular examination. F.P., H.N.; Performed the molecular analysis, the bioinformatics analysis, and drafted the manuscript, which was revised by Z.B. All authors read and approved the final manuscript.

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