

## Mutation analysis of connexin 50 gene among Iranian families with autosomal dominant cataracts

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### ABSTRACT

**Objective(s):** Childhood cataract is a genetically heterogeneous eye disorder that results in visual impairment. The aim of this study was to identify the genetic mutations of connexin 50 gene among Iranian families suffered from autosomal dominant congenital cataracts (ADCC).

**Materials and Methods:** Families, having at least two members with bilateral familial congenital cataract, were selected for the study. Probandes were evaluated by detailed ophthalmologist's examination, and the pedigree analysis was performed. PCR amplifications were performed corresponding to coding region and intron-exon boundaries of *GJA8*, a candidate gene responsible for ADCC. PCR products were subjected to bidirectional sequencing, and the co-segregation of identified mutations was examined and finally, the impact of identified mutations on biological functions of *GJA8* was predicted by in silico examination.

**Results:** Three different genetic alterations, including c.130G>A (p.V44M), c.301G>T (p.R101L) and c.134G>T (p.W45L) in *GJA8* gene were detected among three probands. Two identified mutations, W45L and V44M have been already reported, while the R101L is a novel mutation and its co-segregation was examined. This mutation was exclusively detected in the ADCC and could not be found among the healthy control group. The result of bioinformatic studies of R101L mutation predicted that this amino acid substitution within *GJA8* could be a disease-afflicting mutation due to its potential effect on the protein structure and biological function.

**Conclusion:** Our results suggest that mutations of lens connexin genes such as *GJA8* gene could be one of the major mechanisms of cataract development, at least in a significant proportion of Iranian patients with ADCC.

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### Introduction

Childhood cataract is one of the most frequent causes of treatable childhood blindness. This disease is a genetically heterogeneous eye disorder, which affects the central nucleus of the eye and frequently results in visual impairment or even blindness in children. The prevalence of congenital cataract is estimated between 1 and 15 in 10,000 newborns (1). Congenital cataract occurs in all genders, races and ethnicities. It has been reported that the rate of congenital/infantile cataract in males was 10% higher than females (2-4).

To date, surgery has been the first line of treatment for congenital cataract, but it has many complications such as strabismus, nystagmus, amblyopia, posterior capsular opacity, and glaucoma.

So, despite dramatic improvements in the treatment

of congenital cataracts, still a large proportion (75%) of childhood blindness in developing countries is due to congenital cataract (2-6).

Although the aforementioned disease can be either bilateral or unilateral, and also with or without genetic basis, it has been shown that the unilateral cataract is rarely inherited, whereas approximately 50% of bilateral cases have a genetic background. This trait can be transmitted in different patterns such as autosomal dominant, autosomal recessive and X-linked; however, the autosomal dominant pattern seems to be the most common inheritance pattern (1, 7, 8).

Identification of the genetic variations underlying hereditary cataract and subsequent functional studies will improve our insight into normal and

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abnormal lens development and the mechanisms of cataract pathogenesis. Although identification of genetic variations in congenital cataract has not yet led to establish a better and newer treatment, it may be possible to cure congenital cataracts before birth or in early childhood with advances in genetic science.

So far, several chromosomal regions are suggested to be involved in the molecular etiology of the disease. In addition, several genes such as crystallin genes (*CRYAA*, *CRYAB*, *CRYBB1*, *CRYBB2*, *CRYBA1/A3*, *CRYGA*, *CRYGB*, *CRYGC*, *CRYGD*, and *CRYGS*), *GJA3*, *GJA8*, *MIP* and *AQP0*) have been reported to be associated with the inherited cataract (1, 7-12). Although more than 90% of soluble lens proteins consist of crystallin proteins, connexin proteins play an important role in maintenance of lens structure and transparency by controlling water and ion balance in the lens. The mutations identified in the connexin genes that encode these corresponding proteins have been associated with inheritance of cataracts in human (9-12).

In the present study, we conducted a mutation screening of the entire coding sequences of *GJA8* gene; the gene which encodes connexin proteins, among ten Iranian families with ADCC.

## Materials and Methods

### Ocular examination

This study was approved by the Institutional Review Board Committees (IRB) at Tehran University of Medical Sciences (TUMS, Iran). A written informed consent was obtained from parents or guardians before mutation analysis. In this study, 20 patients from 10 unrelated Iranian families with ADCC were diagnosed and enrolled based on the following criteria: (1) bilateral congenital cataracts that had been approved by detailed ophthalmologist's examination; (2) no other ocular or systemic disease; (3) no other congenital and syndrome related malformation; (4) no history of any teratogenic drug use during pregnancy; (5) compatible family pedigree with an autosomal dominant pattern of the disease. The exclusion criteria were the various diseases, infections, or trauma that mimics inherited cataracts as well as individuals requiring sedation for study procedures.

### Molecular genetic studies

Genomic DNA was isolated from five milliliters whole blood using a QIAamp DNA mini kit (Qiagen, Hilden, Germany). The PCR amplification was typically carried out using specific primer pairs of coding regions (<http://simgene.com/Primer3>) and exon-intron boundaries of *GJA8* gene (Table 1), 0.2U Taq DNA polymerase (Roche, Mannheim, Germany), 10 pmole of each primer, 200  $\mu$ M of each dNTPs, 0.67  $\mu$ l of 50 mM  $MgCl_2$ , 60 ng DNA and 2.5  $\mu$ l of PCR buffer in 25  $\mu$ l of PCR reactions. The PCR conditions

included an initial denaturation step for 3 min at 95 °C, 30 sec at 95 °C, 45 sec at 64 °C with a 1 °C decrease every second cycle down to 55 °C, then 55 °C for 14 cycles, 1 min at 72 °C for extension, and finally 10 min at 72 °C (13-15). PCR products were separated on 2% agarose gels and visualized with ethidium bromide, as described previously (15-18).

Subsequently, to determine any mutation the PCR product was subjected to direct sequencing (Gene Fanavaran, Iran). Sequence data searches were performed in non-redundant nucleic and protein databases BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>).

### In silico study

In order to investigate whether the identified mutation could affect the biological function of the altered protein, we analyzed the mutated form of protein using Polyphen2 program (<http://genetics.bwh.harvard.edu/pph2/>).

## Results

According to studied medical records, interviewing the patients, and medical examinations, probands from ten families with ADCC were enrolled in this study. All probands and their families were clinically examined by an ophthalmologist to identify ADCC affected individuals. Genetic analysis of these probands revealed three missense mutations including c.130G>A (p.V44M), c.301G>T (p.R101L) and c.134G>T (p.W45L) in *GJA8* gene.

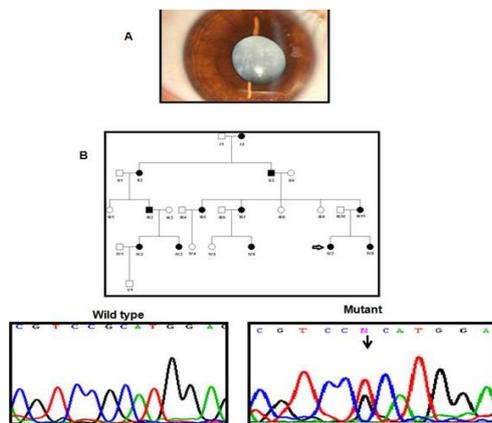
In family 1, the proband was a 3-year-old girl from a family with 12 ADCC affected individuals. She was afflicted with posterior polar cataract and reduced visual acuity (VA). The affected patient underwent slit lamp assessment. Molecular genetic studies revealed a novel mutation c.301G>T (p.R101L) in the *GJA8* gene. The aforementioned mutation also was detected in *GJA8* gene of her mother who suffered from ADCC (Figure 1). This mutation was not observed in any of the unaffected family members or in the 100 healthy control individuals.

In family 2, the proband was a 4-year-old girl from a family with 4 affected individuals with ADCC. Ocular examination using slit-lamp confirmed posterior polar cataract. Through bidirectional sequencing of entire coding regions and intron-exon boundaries of the *GJA8* gene, a missense mutation, c.130 G>A (p.V44M), was identified. In the affected members of family 2, the known mutation, c.130 G>A (p.V44M), was detected in the *GJA8* gene (Figure 2).

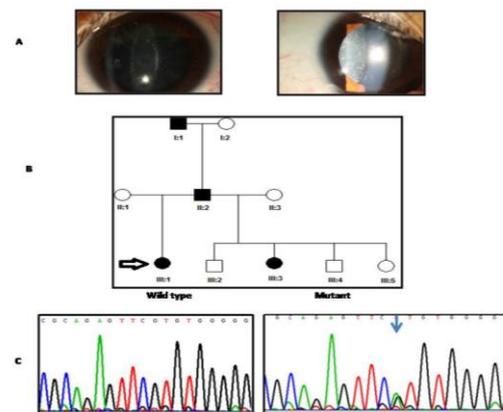
In family 3, the proband was a 4-year old boy from a family with 11 ADCC affected individuals. Mutation analysis of *GJA8* using bidirectional sequencing as described above revealed a missense mutation, c.134 G>T (p.W45L). This mutation was also detected in the *GJA8* gene of other affected family members (Figure 3).

**Table1.** The primer sequences used in this study

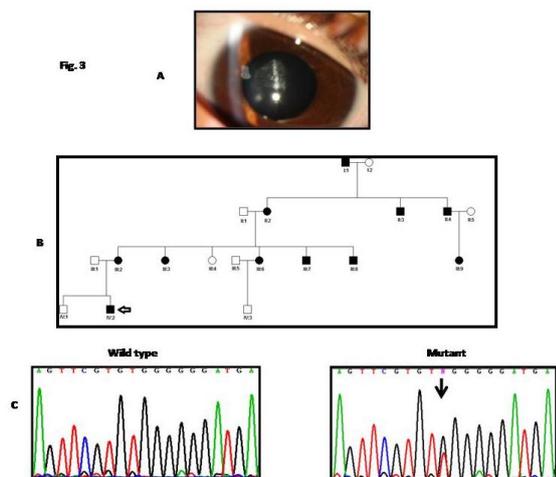
Primer name	Primer sequence; 5' 3' (F: forward, R: reverse)	Product size (bp)
AA1	F: AGCAGCCTTCTTCATGAGC R: CAAGACCAGAGTCCATCG	441
AA2	F: GGCAGGTGACCGAAGCATC R: GAAGCCATGGTGCAGGTG	338
AA3	F: GCAGCTTCTCTGGCATGG R: GGAAGCAAAGGAAGACAGA	376
A8-1	F: CCGCGTTAGCAAAAACAGAT R: CCTCCATGCGGACGTAGT	399
A8-2	F: GCAGATCATCTTCGTCTCCA R: TCGAGGAGAAGATCAGCACA	400
A8-3	F: CCACGGAGAAAACCATCTTC R: GAGCGTAGGAAGGCAGTGTC	378
A8-4	F: TCGAGGAGAAGATCAGCACA R: GGCTGCTGGCTTTGCTTAG	375
BA1-1	F: GGCAGAGGGAGAGCAGAGTG R: CACTAGGCAGGAGAACTGGG	207
BA1-2	F: AGTGAGCAGCAGAGCCAGAA R: GGTCACTCACTGCCTTATGG	293
BA1-3	F: AAGCACAGAGTCAGACTGAAGT R: CCCCTGTCTGAAGGGACCTG	269
BA1-4	F: GTACAGCTCTACTGGGATTG R: ACTGATGATAAAATAGCATGAACG	358
BA1-5	F: CAATGATAGCCATAGCACTAG R: TACCGATACGTATGAAATCTGA	291
BA1-6	F: CATCTCATACCATGTGTTGAG R: GCAAGGTCTCATGCTTGAGG	295
GC-1	F: TGCATAAAATCCCCTTACCG R: CCTCCCTGTAACCCACATTG	556
GC-2	F: TGGTGTGACAAATTCTGGAAG R: CCCACCCATTCACTTCTTA	491
GD-1	F: CAGCAGCCCTCCTGCTAT R: GGGTCTGACTTGAGGATGT	484
GD-2	F: GCTTTTCTTCTCTTTTATTTCTGG R: AAGAAAGACACAAGCAATCAGT	395
AB-1	F: AACCCCTGACATCACCATTC R: AAGGACTCTCCCGTCTAGC	352
AB-2	F: CCATCCCATTCCTTACCTT R: GCCTCAAAGCTGATAGCAC	237
AB-3	F: TCTCTCTGCCTCTTTCCTCA R: CCTTGGAGCCCTCTAAATCA	477



**Figure 1. Ophthalmological evaluation, pedigree analysis and molecular study of family 1.** A: Slit-lamp photographs of eyes from proband revealed congenital proband. B: The pedigree of family 1 shows 12 affected patients (arrow indicates the proband) and co-segregation of c.301G>T (p.R101L) through the family. Filled symbols represent autosomal dominant congenital cataracts (ADCC) patient and open symbols show individuals without clinical ADCC. C: DNA chromatogram showed a heterozygous missense mutation in the codon 101 *GJA8* in which G> T (arrow indicates the position of nucleotide substitution)



**Figure 2. Ocular examination and molecular genetic study of family 2.** A: Slit-lamp images of both eyes from proband shows nuclear cataract. B: The pedigree of family 2 shows 4 affected patients (arrow indicates the proband). Filled symbols represent autosomal dominant congenital cataracts (ADCC) patient and open symbols show individuals without clinical ADCC. C: DNA sequencing revealed a heterozygous mutation in codon 44 for amino acid valin to methionine c.130G>A (p.V44M). Arrow indicates the position of nucleotide substitution



**Figure 3. Clinical examinations and genetic analysis of family 3.** A: Slit-lamp examination shows bilateral congenital cataract in the proband. B: The pedigree of family 3 shows 11 affected individuals. Filled symbols represent autosomal dominant congenital cataracts (ADCC) patients and open symbols show individuals without clinical ADCC. C: heterozygous mutation in coding region of *GJA8* gene showed in DNA chromatogram c.134G>T (p.W45L)

### In silico study

In order to predict the potential biological effect of the novel missense mutation (R101L) within coding region of *GJA8* gene, we utilized the Polyphen 2 program. The mutation analysis predicted this alteration as “probably damaging”.

### Discussion

So far, along with the development of molecular genetics, various loci and more than 20 genes such as *CRYAA*, *CRYAB*, *CRYBA1/A3*, and *GJA8* have been reported to be associated with congenital cataracts (7, 9-12). In this study, we conducted mutation detection within *GJA8* gene among ten Iranian families with ADCC. Three missense mutations, including c.130G>A (p.V44M), c.301G>T (p.R101L) and c.134G>T (p.W45L) in the coding region of *GJA8* (Cx50) gene were detected in the patients with ADCC.

Gap junction proteins, are a unique family of transmembrane proteins that form specific channels between cells and are encoded by a gene family with at least 14 members. These proteins connect the cytoplasm of neighboring cells and play an important role in intercellular communication in the lens. The *GJA8* gene encodes 433 amino acid residues of gap junction protein alpha 8 or connexin 50. The encoded protein contains four transmembrane domains that are joined by two extracellular and one cytoplasmic loop and flanked by cytoplasmic N- and C-termini. This protein creates gap junction channels by forming hexamers, or hemi-channels, that can dock between adjacent cells. The normal function of

**Table 2.** Mutations identified in Iranian patients with congenital cataract

Mutation	Nucleotide alteration	Protein alteration	Genotype
R101L	CGC-CTC	Arg-Leu	Heterozygote
V44M	GTG-ATG	Val-Met	Heterozygote
W45L	TGG-TTG	Trp-Leu	Heterozygote

These channels have been shown to be involved in the trafficking of ions and small molecular metabolites (19-24).

Studies have reported that *GJA8* mutations are responsible for a significant proportion of ADCC (1, 7, 25, 26). Up to now, more than 20 mutations in *GJA8* gene have been reported in hereditary congenital cataract (1, 7, 8, 19, 23, 25-27). Even though our results are the first report that demonstrated the genetic alterations of *GJA8* gene in Iranian population, various studies reported the association of *GJA8* mutations with ADCC in different ethnic groups (25-27).

Two out of three identified mutations, c.130G>A (p.V44M) and c.134G>T (p.W45L) (V44M and W45L), through the present work, are located in the extracellular loop 1 near the TM1/E1 border. It has been well documented that this part of *GJA8* gene is supposed to be a hot-spot sequence. In line with our suggestion, several studies reported a high incidence rate of mutation in this region among congenital cataract (18-20, 22-28). Additionally, different studies have shown that mutations in this region impair the normal functions of Cx50 and are introduced as disease-causing mutation in ADCC (29, 30).

Moreover, our *in silico* study of c.130G>A (p.V44M) and c.134G>T (p.W45L) mutations, which are located on the first exon of *GJA8* gene, predicted that these mutations are “probably damaging” to the structure and function of the protein (with a specificity of 0.99 and 1.00, respectively). Then, this is quite understandable that these mutations likely lead to the disruption of the gap junction formation and altered protein trafficking or channel assembly of heteromeric connexins consisting of *GJA8* mutant and wild-type subunits.

The third identified genetic variation c.301G>T (p.R101L) seems to be a novel mutation. Bioinformatic studies using Polyphen program suggested that the R101L is “probably damaging” with a score of 0.997 and specificity of 0.98. This result is justified by the different characteristics of arginine and leucine. Arginine has a basic polarity and positively charged R group, and hydropathy index of -4.5, which is totally different from leucine with a non-polar, neutral R group, and hydropathy index of +3.8. Therefore, this base substitution might completely change the 3D structure, characteristics, and normal function of the resulting protein. Further

studies are required to reveal the possible implication of this genetic alteration with ADCC.

### Conclusion

We identified a novel heterozygous c301G>T (p.R101L) genetic variation and two known mutations in *GJA8* in the three Iranian families with ADCC. Our data also showed that the mutation in *GJA8* gene accounts for ~30% of ADCC in Iranian population. The *in silico* and experimental studies suggested that these genetic alterations might be vital in hemichannel formation. However, further investigations are essential to define the exact molecular mechanisms, which are implicated by these mutations in the abnormal function as well as their pathogenic effects on the development of ADCC.

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