p.S45G mutation at a conserved phosphorylation site of αA-crystallin in juvenile cortical cataract

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Case Report

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Abstract

Purpose

To identify the potential genetic cause in a patient diagnosed with juvenile cortical cataract.

Observations:

A young patient diagnosed with cortical cataract by ophthalmologic examination was recruited for the current study. DNA isolation was done followed by resequencing of all exons and exon-intron boundaries of 3 genes $CRYAA$, $CRYAB$ and $CRYBB1$, using intron specific primers. A mutation in $CRYAA$ gene in heterozygous condition g.44589342A $>$ G (p.S45G), was identified in the patient. This mutation was predicted to be disease causing by Mutation Taster and other prediction tools. *In-silico* study revealed that this position (S45) was evolutionary conserved and mutation altered phosphorylation pattern of $\alpha$A-crystallin as serine is the site of phosphorylation. Furthermore, this variant was absent in 130 unrelated healthy controls from this population suggesting it to be a disease-causing mutation.

Conclusion and importance:

The $\alpha$A-crystallin mutation (p.S45G) causes juvenile cortical cataract in the patient due to discrepancy in phosphorylation pattern. This mutation is first time reported in juvenile cataract and happened to be the second mutation identified in $CRYAA$ gene responsible for juvenile cataract.

Introduction

Congenital cataract is a lens disorder defined as clouding of lens in the eye that affects vision accounting for one-tenth of the cases of childhood blindness. Cataract can be classified on the basis of age at onset as congenital or infantile cataract (onset within the first year of life), juvenile cataract (onset within the first decade of life), presenile cataract (presents before the age of 45 years) and senile or age-related cataract appears after that. Juvenile cataract is distinguished from congenital cataract in terms of clouding which was clear at birth but opacification progresses while maturation during childhood or adolescence.

There are approximately 25 genes known to be involved in cataract formation and fall into subgroups of genes, like encoding crystallin, beaded filament structural protein gene (BFSP1), lens intrinsic membrane proteins, heat shock factor proteins, transferases and gap junction proteins gene. Three distinct families of crystallin genes viz. $\alpha$-, $\beta$-, and $\gamma$-crystallin were reported that contribute towards the lens transparency with their structure, stability and short-range interaction. The alpha crystallin expressed in two forms: alpha-A($CRYAA$) and alpha-B($CRYAB$) with molar ratio of 3:1, forms a hetero-oligomeric complex in lens fibre, crucial for maintaining the lens transparency, possibly by ensuring the complexes formed by them and other lens proteins remain soluble. $\alpha$A-crystallin also expressed minimally in brain, spleen, lung,
kidney, cornea and skin. Alpha crystallins are the member of small heat shock protein (HSP20) family and can also be induced by heat shock. Alpha crystallin undergoes major post-translational modifications that include truncation, glycosylation, glycation, phosphorylation, carbamylation and acetylation which exerts major effects on the protein stability.

To date 11 CRYAA (αA-crystallin) mutations are reported in cataract of which only one mutation was reported for juvenile cataract. The present study reported another CRYAA (p.S45G) mutation in juvenile cataract that affects the phosphorylation pattern in αA-crystallin.

**Case Report**

A 14 years old male patient with cortical cataract was recruited at Chandra Eye Care, Varanasi. The patient manifested cataract at early age of 8 years as stated by his parents. There was no family history of early onset cataract in the family but age-related cataract was present in a few members. The patient was clinically examined including visual acuity testing, slit lamp examination, intraocular pressure measurement, and fundus examination with dilated pupils and diagnosed with cortical cataract (LOCSIII grading- C3N2) (Fig. 1). Furthermore, the patient was not suffering from cataract associated conditions like diabetes, hypertension, myopia, glaucoma and even not put under medications known to be associated with development of cataract.

All the exons as well as exon-intron boundaries of CRYAA, CRYAB and CRYBB1 genes were PCR amplified using intron specific primers followed by direct resequencing on ABI-3130 genetic analyzer according to manufacturer’s protocol (ABI®, CA, USA) (Fig. 2A). Primer sequences used in this study were taken from Guo Y et al. 2012.

Resequencing of CRYAA gene in the patient revealed two reported SNP rs872331 and g.44589342A > G (rs765952577). The g.44589342A > G was a non-synonymous single nucleotide substitution in exon 1 of CRYAA gene that causes amino acid change p.S45G (Fig. 2). Frequency of mutation in normal healthy unrelated control individuals was performed using designed ARMS-PCR. For ARMS-PCR, the primers set 5’-AGCAGCCTTCTTCATGAGC-3’(forward primer) and 5’-CAAGACCAGAGTCCATCG-3’(reverse primer) along with either wildtype allele specific primer (WP)- 5’-TTCCTGTCGTCCACCATCA-3’ or mutant allele specific primer (MP)- 5’-TTTCTGTCGTCCACCATCG-3’ were designed to screen g.44589342A > G in control individuals. The forward and reverse primers were used as an internal control to make sure that PCR reaction worked properly whereas allele specific primers (WP and MP) were used to identify the presence of wild type and mutant alleles. The reverse primer produces two PCR products of 441bp and 162bp with forward and allele specific primer, respectively. ARMS PCR was carried out using 50 ng genomic DNA in ABI Veriti 96 well thermal cycler (ABI®, USA) programmed with initial denaturation at 95°C for 5 min, followed by 25 cycles of 95°C for 30 sec, 62°C for 30 sec, 72°C for 30 sec and one final extension at 72°C for 10 min. Amplification products were resolved using 4% agarose gel, stained with Ethidium Bromide and visualized by ultraviolet illumination (Fig. 3). This substitution g.44589342A > G was not observed in 130 (260 chromosomes) unrelated normal controls from the same population (Fig. 3).
Sequencing data were analysed using various online available software. The effect of each SNP was predicted by in-silico analysis using Mutation Taster and other prediction tool like SIFT, Panther, SNAP, Polyphen-1, Polyphen-2, PhD-SNP, MAPP and Predict SNP (Fig. 2C). The Serine was highly conserved and was an important site for phosphorylation (Fig. 2, Fig. 5). The isoelectric point (pI, 5.77), intra-molecular H-bonding (Fig. 4C) and molecular weight (S45-19909.3 Da; G45-19879.2 Da) were unaltered due to mutation. The hydrophobicity was also not significantly affected by the mutation as calculated by ProtScale (Fig. 4A). Effect of mutation on its secondary structure formation was analysed using Chou-Fasman method on ProtScale which revealed reduction in alpha helix formation property (Fig. 4B). The mutant site was a conserved phosphorylation site on protein, identified using Phospho Site Plus online server which predicted a total of 6 phosphorylation sites on αA-crystallin (Fig. 5).

Discussion And Conclusions

Crystallins are water-soluble structural protein responsible for the lens transparency accounting for up to 90% of total soluble protein. CRYAA gene was less explored in terms of its involvement in cataract and only 11 disease causing mutations were reported for this gene of which 9 were reported in congenital cataract and 2 in early age cataract (one in presenile cataract and other in juvenile cataract) (Table 1). The present study reports second CRYAA mutation in juvenile cataract. The identified mutation g.44589342A > G was non-synonymous (p.S45G) and lies in exon 1 of CRYAA gene. In-silico analysis revealed that mutation p.S45G lie at an invariant position and is reported to be a significant site for phosphorylation in native peptide. Although this mutation did not alter various properties like intra-molecular H-bonding pattern, molecular weight, hydrophobicity and isoelectric point but the helix formation capacity of mutant and neighbourhood residue were compromised.
### Table 1
Spectrum of CRYAA mutation in cataract cases.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Age of onset</th>
<th>Mutation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple types, with or without microcornea cataract</td>
<td>Congenital</td>
<td>p.R116C</td>
<td>Littet al., 1998⁹</td>
</tr>
<tr>
<td>Autosomal recessive cataract</td>
<td>Congenital</td>
<td>p.W9X</td>
<td>Pras E et al., 2000⁵</td>
</tr>
<tr>
<td>Nuclear cataract</td>
<td>Congenital</td>
<td>p.R49C</td>
<td>Mackay et al., 2003¹⁰</td>
</tr>
<tr>
<td>Multiple types, with microcornea</td>
<td>Congenital</td>
<td>p.R116H</td>
<td>Shafiee et al., 2006¹¹</td>
</tr>
<tr>
<td>Total cataract</td>
<td>17 years</td>
<td>p.G98R</td>
<td>Santhiya et al., 2006¹²</td>
</tr>
<tr>
<td>Total cataract, with microcornea, autosomal recessive</td>
<td>Congenital</td>
<td>p.R54C</td>
<td>Khan et al., 2007¹³</td>
</tr>
<tr>
<td>Nuclear cataract, with microcornea</td>
<td>Congenital</td>
<td>p.R116H</td>
<td>Hansen et al., 2007¹⁴</td>
</tr>
<tr>
<td>Multiple types, with microcornea</td>
<td>Congenital</td>
<td>p.R12C</td>
<td>Hansen et al., 2007¹⁴</td>
</tr>
<tr>
<td>Multiple types, with microcornea</td>
<td>Congenital</td>
<td>p.R21W</td>
<td>Hansen et al., 2007¹⁴</td>
</tr>
<tr>
<td>Nuclear lamellar cataract</td>
<td>2 years</td>
<td>p.R21Q</td>
<td>Laurie et al., 2013¹⁵</td>
</tr>
<tr>
<td>Suture cataract</td>
<td>Congenital</td>
<td>p.R54P</td>
<td>Su Det al., 2012¹⁶</td>
</tr>
<tr>
<td>Lamellar cataract</td>
<td>4 years</td>
<td>p.R21Q</td>
<td>Javadiyan et al., 2016¹⁷</td>
</tr>
<tr>
<td>Cortical cataract</td>
<td>8 years</td>
<td>p.S45G</td>
<td>Present study</td>
</tr>
</tbody>
</table>

The phosphorylation of α-crystallin is responsible to regulate its intracellular distribution, translocation, chaperone activity, the ability of binding to substrates and protection against stress¹⁸,²¹. α-crystallin protein have six evolutionary conserved phosphorylation sites which reflect its functional importance (Fig. 5). The phosphorylation sites of αA-crystallin were not explored much but αB-crystallin phosphorylations were well defined by a few studies⁷,¹⁸,¹⁹,²¹. Phosphorylation of αB-crystallin showed context and extent dependent beneficial or deleterious effects. Hypo-phosphorylation of αB-crystallin at Ser45 and Ser59 have protective effect, hyperphosphorylation has negative implications and lead to disease while unphosphorylation resulted into the loss of protection⁷. A moderate amount of phosphorylation is crucial for normal protein function and fluctuation in either direction causes disturbance in protein function. Furthermore, phosphorylation of oligomeric proteins also reported to induces charge repulsion which leads to alterations in quaternary structure and consequently, interacting surface and interacting partners ²¹. Thus the protein phosphorylation is an important post translational modification required for its stability and various functions including chaperone activity, intracellular...
distribution, protein-protein interaction, translocation and others. αA-crystallin shared 57% sequence similarity and similar phosphorylation patterns with αB-crystallin (Fig. 5A) and have three serine residues(Ser20, Ser45, Ser122) for phosphorylation. The identified mutation g.44589342A > G (p.S45G) altered the phosphorylation pattern in αA-crystallin and affect protein function and stability. Therefore, we speculated that the identified mutation p.S45G might be responsible for juvenile cortical cataract in the patient possibly by removing the phosphorylation site of αA-crystallin, required for protection against stress leading to reduced phosphorylation and thus abnormal crystallin in the eye-lens.

**Declarations**

**Patient Consent**

Blood sample, detail clinical history and clinical photographs were collected from the patient after receiving written informed consent from his father.

**Acknowledgments and Disclosures**

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**Conflicts of interest**

The authors have no conflicts of interest.

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**References**


Figures
Figure 1

Slit-lamp image of the patient left eye before surgery showing cortical cataract.
Figure 2

A) Electropherograms showing g.44589342A>G substitution in CRYAA gene causing amino acid shift from Serine to Glycine at an invariant position. B) Multiple sequence alignment of αA-crystallin protein across species revealing its evolutionary conservation. C) In-silico prediction of the variant by prediction tools (SIFT, SNAP, Polyphen-1, Polyphen-2, PhD-SNP, MAPP and Predict SNP).

Figure 3

Screening of g.44589342A>G mutation using designed ARMS-PCR. Amplification products of internal control primers set with wild allele specific primer were marked as WP whereas internal control primers set with mutant allele specific primer were designated as MP. Patient and normal control individuals were indicated as P and C) in the lane, respectively. Both wild and mutant allele primer set were amplified in
patient (lane 2 and lane 3) which confirmed the presence of g.44589342A>G mutation in heterozygous condition. In normal control individual mutant allele specific primer set was not amplified but wild allele specific primers were amplified (lane 4 to lane 5) which confirmed the absence of mutant allele in normal population.

![Hydrophobicity profile of wild-type CRYAA protein using ProtScale program available at Expasy server. Circle represents the hydrophobicity around serine in wild-type protein. B) Circle represents the hydrophobicity around the glycine in mutant protein showing mild shift in hydrophobicity compare to wild-type. C) Secondary structure formation (i.e. alpha helix) property of wild-type CRYAA proteins predicted by ProtScale program. Alpha helix forming property of each amino acid in wild type-protein and circle represents helix forming property of serine in wild-type protein. D) Alpha helix forming property of each amino acid in CRYAA mutant protein and circle represents helix forming property of mutant glycine. The mutation (p.S45G) reduces the helix formation capacity at mutant site as well its neighbouring reduces. E) Picture showing tertiary structure of wild type CRYAA protein with S45 predicted by ModBase and visualized on standalone DeepView - Swiss-PdbViewer. F) Tertiary structure of mutant CRYAA protein with mutant G45. There was no disturbance in intra-H-bonding pattern was observed due top. S45G mutation.]

**Figure 4**

**A)** Hydrophobicity profile of wild-type CRYAA protein using ProtScale program available at Expasy server. Circle represents the hydrophobicity around serine in wild-type protein. **B)** Circle represents the hydrophobicity around the glycine in mutant protein showing mild shift in hydrophobicity compare to wild-type. **C)** Secondary structure formation (i.e. alpha helix) property of wild-type CRYAA proteins predicted by ProtScale program. Alpha helix forming property of each amino acid in wild type-protein and circle represents helix forming property of serine in wild-type protein. **D)** Alpha helix forming property of each amino acid in CRYAA mutant protein and circle represents helix forming property of mutant glycine. The mutation (p.S45G) reduces the helix formation capacity at mutant site as well its neighbouring reduces. **E)** Picture showing tertiary structure of wild type CRYAA protein with S45 predicted by ModBase and visualized on standalone DeepView - Swiss-PdbViewer. **F)** Tertiary structure of mutant CRYAA protein with mutant G45. There was no disturbance in intra-H-bonding pattern was observed due top. S45G mutation.
Figure 5

Phosphorylation pattern of α-crystallin. A) Comparison of phosphorylation pattern of αA-crystallin and αB-crystallin. Upper and lower panel shows phosphorylation pattern of αA-crystallin and αB-crystallin, respectively. Both α-crystallin have similar phosphorylation pattern. B) Phosphorylation pattern of αA-crystallin in different species showing this phosphorylation at position S45 was conserved during evolution.