



## Original article

Association of single nucleotide polymorphism variations in *CRYAA* and *CRYAB* genes with congenital cataract in Pakistani populationPriya Jarwar<sup>a,\*</sup>, Yar Muhammad Waryah<sup>b</sup>, Muhammad Rafiq<sup>a</sup>, Ali Muhammad Waryah<sup>c,d</sup><sup>a</sup> Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan<sup>b</sup> Scientific Ophthalmic and Research Laboratory, Sindh Institute of Ophthalmology and Visual Sciences, Hyderabad 71500, Pakistan<sup>c</sup> Department of Pathology, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan<sup>d</sup> Department Molecular Biology and Genetics, Medical Research Center, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan

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## ABSTRACT

**Background:** The purpose of present study was to analyze the association of single nucleotide polymorphism (SNPs) variant in *CRYAA* and *CRYAB* genes with Congenital Cataract.**Method:** Total 196 blood samples of children were collected, out of which 102 samples were congenital cataract (case group) and 94 samples were normal individuals (control group). Genomic DNA was extracted by using optimized inorganic method. Tetra primers for SNPs were designed and TETRA-ARMS assay was performed on both groups. Genotypic, allelic frequency and haplotype analyses were obtained by using SNPstats software.**Results:** The coordination of genotypic and allelic frequencies of *CRYAA* and *CRYAB* genes variants and the association between case and control groups showed increased risk of congenital cataract in children who contained *rs13053109 G > C* variant of *CRYAA* in all models (all  $P > 0.05$ ). This depicts the evident difference between the frequencies of case and control groups. The haplotype analysis of SNPs *rs3761382*, *rs7278468* and *rs13051039* of *CRYAA* gene showed weak linkage disequilibrium between the 3 SNPs ( $r^2 < 0.8$ ). The haplotype CTC indicated the high risk of congenital cataract in infants based of its p value (OR = 1.60 95% CI = 0.11–22.64,  $P > 0.05$ ).**Conclusion:** The variation in *CRYAA* gene can be the risk factor for congenital cataract in infants.© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

The cataract is a clouding on the lens, which causes vision impairment and blindness in humans (Quillen, 1999; Santhiya et al., 2002). According to the World Health Organization (WHO), around 161 million people are visually impaired, while 37 million individuals are blind globally. Blindness in developing countries is increasing with age where its incidences are 5–10 times higher than the developed states. A number of reports also indicated that female are at higher risk than those of males. Cataract is commonly

found in the age-group of more than 60 years old, however, a type congenital cataract is usually found at pediatric stage. Congenital cataract is responsible for 10–30% of blindness in early childhood (Ge et al., 2014). Besides the hereditary factor, other ocular and metabolic disorders are also known factors responsible for cataract in children. In Pakistan, about 570,000 individuals are blind (<3/60) due to cataract, about 3,560,000 eyes with visual acuity of <6/60 due to cataract among the observed population (Jadoon et al., 2007).

Various genetic mutations are associated with congenital cataract. Researchers have identified about 42 loci related to congenital cataract in humans; among them 26 loci have been found causing pathogenic mutations in related genes. It is estimated that about 20 genes present on above 35 loci, are directly associated with congenital cataract. Multiple types of proteins encoded by identified genes, can be the reason of the half of the mutations in crystallin protein family (*CRYAA*, *CRYAB*, *CRYBB1*, *CRYBB2*, *CRYBA1*, *CRYGC* and *CRYGD*). In crystallin protein when pathogenic variation occurs, they are completely responsible for unusual lens metabolic activity during embryonic period of life, which leads to reduction

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in lens development and making of opaque scar tissues in human lens resulting in the formation of congenital cataract (Wang et al., 2020). It is observed that about 90% of lens proteins including major soluble proteins, made up of crystalline  $\alpha$ ,  $\beta$  and  $\gamma$ , are found in newly born babies (Berry et al., 2020). There are families in which hereditary cataracts with inherited mutations occur in the lens where alpha-crystallin is the core soluble protein expressed in lenses responsible for lens transparency. It includes two proteins;  $\alpha$ A- and  $\alpha$ B-crystallin with a molar ratio of 3:1. Congenital cataract also includes the structural protein genes, cell adhesion molecules, transport molecules, and transcription factors (Liu et al., 2006; Zhang et al., 2009; Zhai et al., 2014; Cui et al., 2017).

The human genome consists of about 3.2 billion base pairs, with 99.9% identical genetic make-up among individuals, and has chances of 3.2 million differences in their diploid genome. Mostly this is because of the substitution of one nucleotide into another. More than 1.0% of the population contain these differences with the frequency of every 800 base pairs in human genome with more than 9 million SNPs. The SNPs are not usually responsible for any biological consequence but a fraction of substitution which makes it stable and abundant, has major significance in diversity among human genome. SNPs are also marked as genetic markers, which inherit from generation to generation in chromosomal region and can be associated with any human disease (Blazej et al., 2003; Kwok and Chen, 2003; Medrano and de Oliveira, 2014). Combination of SNPs and microsatellite markers has brought great advancement in identifying mutated genes during linkage analysis studies. The SNPs has been used in clinical trials and forensics by determining loss heterozygosity (Ahmadian et al., 2000). SNP genotyping has now become easy and cost-effective method by just applying PCR amplification, followed by gel electrophoresis is termed as TETRA Amplification Refractory Mutation System (TETRA-ARMS) Assay (Medrano and de Oliveira, 2014). In TETRA-ARMS assay, 4 primers are used during amplification: outer and inner forward (OF, IF) and outer and inner reverse (OR, IR). When SNP locus is generated, the outer fragment is produced by combining OF/OR primers as internal control; and IF/IR primers play their role in obtaining allele-specific yield, which is based on genotypes (Alyethodi et al., 2018).

This study was aimed to observe variations in *CRYAA* gene rs7278468, rs3761382, and rs13053109 and *CRYAB* gene rs370803064 and rs387907338 in order to obtain information regarding the cause of congenital cataract.

## 2. Material and methods

The multicenter collaborative study was conducted from January 2016 to December 2020 at various institutes situated in Sindh province, Pakistan: University of Sindh, Jamshoro, Liaquat University of Medical and Health Sciences LUMHS Jamshoro, and Sindh Institute of Ophthalmology & Visual Sciences (SIOVS), Hyderabad. The study approval was obtained from the Research Ethics Committee and Board of Research and Graduate Studies, University of Sindh, Jamshoro, Pakistan (DRGS/124, dated January 12, 2015).

### 2.1. Sample collection and DNA extraction

This study was conducted at Molecular Biology and Genetics Department (MBGD) lab, LUMHS, Jamshoro. During field work, different patients were observed and identified in OPDs of Pediatric ward of SIOVS Hyderabad, Sindh. Blood samples (10 cc) of 102 patients of non-familial congenital cataract plus 94 samples of normal individuals were collected in EDTA containing tubes and preserved at  $-80^{\circ}\text{C}$  freezer for further analysis. The genomic DNA

was extracted from whole blood through manual inorganic method as reported by Grimberg et al. (1989).

### 2.2. Primer designing

We designed tetra primers for ARMS assay to target the sequences of *CRYAA* gene variants (rs3761382, rs7278468, rs13053109) and *CRYAB* gene variants (rs370803064, rs387907338) by using Primer 1 tool (<http://primer1.soton.ac.uk/primer1.html>) (Table 1).

### 2.3. Detection of SNPs by Tetra-ARMS assay

To perform Tetra-ARMS PCR, we took 2.0 mM  $\text{MgCl}_2$  buffer (2  $\mu\text{l}$ ), 0.125 mM dNTPs (2  $\mu\text{l}$ ), outer forward and outer reverse primers (0.5  $\mu\text{l}$ ), inner forward and inner reverse primers (1  $\mu\text{l}$ ), DNA template (2  $\mu\text{l}$ ) and Taq Polymerase (0.6  $\mu\text{l}$ ) and deionized distilled water (11.9  $\mu\text{l}$ ) in 20  $\mu\text{l}$  reaction mixture. Amplification condition were: 2 min. of initial denaturation at  $95^{\circ}\text{C}$ , followed by 30 cycles of denaturation at  $95^{\circ}\text{C}$  for 45 sec., annealing at  $67^{\circ}\text{C}/69^{\circ}\text{C}$  for 40 sec., extension at  $72^{\circ}\text{C}$  for 50 sec., and the final extension at  $72^{\circ}\text{C}$  for 5 min. The amplified products were resolved on 1.5% agarose gel by gel electrophoresis and observed under gel documentation machine (Bio Rad).

### 2.4. Statistical analysis

To determine statistical analysis for continuous and categorical variables, student's *t*-test and Chi square test were used. A precise *p*-value of  $\leq 0.05$  was considered as significant. With the help of logistic regression analyses through SNPStats software (<https://www.snpstats.net/start.htm>), allelic frequencies, haplotype frequencies and Hardy-Weinberg Equilibrium (HWE) were calculated. The SNPStats software was used to decide the relation of SNPs with affected cases and wholesome manipulate in co-dominant, dominant, recessive, and over-dominant. The odds ratio (OR) and 95% confidence interval were calculated for the determination of affiliation among allelic frequencies of groups (Khidri et al., 2019).

## 3. Results

The demographic distribution of cases of congenital cataract on the basis of gender is shown in Fig. 1 while Fig. 2 shows the data of patients from different age groups, which were selected for analysis of SNPs. TETRA-ARMS assay was examined through a variety of parameters which are mentioned below.

### 3.1. Gel electrophoresis analysis of TETRA-ARMS assay

After PCR amplification, 102 non-familial congenital cataract samples (case group) and 94 ethically matched controls were observed on gel electrophoresis. All the samples were analyzed on 1.5% agarose gel, which were determined through gel documentation machine. Figs. 3 and 4 show the images of amplified products of 3 variants of *CRYAA* gene (rs3761382, rs7278468, rs13053109) and 2 variants of *CRYAB* gene (rs370803064, rs387907338), which were run with ladder of 100–1000 bp in size.

### 3.2. Genotype distribution and allelic frequencies of *CRYAA* and *CRYAB* gene variants

The SNPs of *CRYAA* and *CRYAB* genes have their genotypic and allelic frequencies which were matched with HWE. The test additionally confirmed the association between case and control groups (Table 2). The *CRYAA* gene variant rs3761382 *C > T* demon-

**Table 1**  
Primers sequence, product size and annealing temperature of CRYAA and CRYAB genes.

| Gene  | SNP         | Primers              | Sequence (5'→3')              | Product size                            | Annealing temp                          |       |
|-------|-------------|----------------------|-------------------------------|---|---|-------|
| CRYAA | rs3761382   | FI Primer (C allele) | AACGCTCGAGGGCTTACATCGAGGGTAC  | Product size for C allele: 198          | 69 °C                                   |       |
|       |             | RI Primer (T allele) | CTGGAGCCCCAGCCCATGGCCATAGA    | Product size for T allele: 267          |   |       |
|       |             | FO Primer (5' - 3')  | CAGGGGCCATGGGTCGAGGAGCAAATA   | Product size for two outer primers: 410 |   |       |
|       | rs7278468   | FI Primer (G allele) | AAGGTGCCATAATCCAGCACCTGGGGG   | TCTAACCTCTGTCTAACGGGGGTGCGG             | Product size for G allele: 151          | 67 °C |
|       |             | RI Primer (T allele) | CTCATGGTCCAGAGGAGGGAGAACAA    | CTCATGGTCCAGAGGAGGGAGAACAA              | Product size for T allele: 200          |       |
|       |             | FO Primer (5' - 3')  | GGCACCTTGACGCTTCTTCCACGAGT    | CGACAATGGAGCTTCTCATCTGCCAGTA            | Product size for two outer primers: 295 |       |
|       | rs13053109  | FI Primer (G allele) | TCTTGCCAGGCCCGGTGAGACTCTCAG   | TCTTGCCAGGCCCGGTGAGACTCTCAG             | Product size for G allele: 146          | 67 °C |
|       |             | RI Primer (C allele) | CGTTAGACACAGAGGTTAGACACATCGAG | CGTTAGACACAGAGGTTAGACACATCGAG           | Product size for C allele: 193          |       |
|       |             | FO Primer (5' - 3')  | ATAGGGCTCCCCAGGTCTGGAATT      | ACGTGCCAGGGACCATGCTAGTTCTCTG            | Product size for two outer primers: 281 |       |
| CRYAB | rs370803064 | FI Primer (T allele) | AAGGGCATCTATTTCTTGGGGCGGT     | Product size for T allele: 207          | 67 °C                                   |       |
|       |             | RI Primer (C allele) | CCGTGAAGAGAAGCCTGCTGCAACG     | Product size for C allele: 159          |   |       |
|       |             | FO Primer (5' - 3')  | GCTTGATAATTTGGGCCTGCCTTAGCAT  | Product size for two outer primers: 314 |   |       |
|       | rs387907338 | FI Primer (T allele) | CATGGTTTCATCTCCAGGGAGTTCCACA  | TTTACCTTCGGCCACCTCTTCCGGT               | Product size for T allele: 227          | 67 °C |
|       |             | RI Primer (C allele) | TGCCAGTGTCAAACAGCTGGGTGACAG   | TGCCAGTGTCAAACAGCTGGGTGACAG             | Product size for C allele: 169          |       |
|       |             | FO Primer (5' - 3')  | TGGATCCGCCCCCTTCTTCTTT        | AGTGATGGGGAGGAAGGCAC TAGCAACC           | Product size for two outer primers: 340 |       |

FI Forward inner, RI Reverse inner, FO forward outer, RO Reverse outer.

stated that genotypic and allelic frequencies were in consistent with HWE ( $P > 0.05$ ) and the comparison between both groups (case and control) represents non-evident difference in the frequencies. In CRYAA gene rs7278468 G > T SNP, the demonstration of genotypic and allelic frequencies revealed suggestively decreased risk of congenital cataract in all models (all  $P < 0.05$ ). Hence the association of variant and HWE was not found consistent on the basis of its P-value ( $P < 0.05$ ), but the compared groups (case and control) revealed evident differences between their genotypic and allelic frequencies (Table 2). Furthermore, a profound increased risk of congenital cataract in CRYAA gene variant rs13053109 G > C in all models (all  $P > 0.05$ ) was discovered. This depicts evident difference between the frequencies of case and control groups while the genetic association was not found consistent with HWE ( $P < 0.05$ ). In CRYAB gene variant rs370803064C > T, the genotypic and allelic frequencies confirmed obvious difference in case and control groups and were not found consistent with HWE ( $P < 0.05$ ), hence decreased risk of congenital cataract was discovered. However, rs387907338 C > T variant of CRYAB gene was not found to be associated with HWE, as it resulted in monomorphic SNP in population.

### 3.3. Haplotype analysis of CRYAA gene variants

The haplotype analysis of CRYAA gene variants is presented in Table 3. The haplotype study of SNPs rs3761382, rs7278468 and

rs13051039 of CRYAA gene showed weak linkage disequilibrium between these SNPs ( $r^2 < 0.8$ ). The haplotype results presented that CGG of CRYAA gene might have decreased risk of congenital cataract (OR = 0.34, 95% CI = 0.14–0.84,  $P < 0.05$ ), while CGC, TTG and TGG indicated marginally increased risk of congenital cataract among the newborns (CGC: OR = 0.41, 95% CI = 0.15–1.09,  $P > 0.05$ ; TTG: OR = 0.56, 95% CI = 0.20–1.56,  $P > 0.05$ ; TGG: OR = 0.86, 95% CI = 0.13–5.92,  $P > 0.05$ ). Moreover, another haplotype CTC was observed in infants with highly inclined risk of congenital cataract (OR = 1.60 95% CI = 0.11–22.64,  $P > 0.05$ ). On parallel, there was no any haplotype association found in CRYAB gene variants on account of monomorphic SNP.

### 4. Discussion

The crystallin protein is a core soluble protein which belongs to small heat shock protein (sHSP) family responsible for lens transparency (Cui et al., 2017). Crystallin protein is made of two main proteins;  $\alpha$ A and  $\alpha$ B crystallin, which get converted into CRYAA and CRYAB. Other crystallin proteins are protected from thermal and stress-induced aggression or inactivation by the help of CRYAA gene chaperone activity (Xia et al., 2014; Ma et al., 2016; Cui et al., 2017; Zhao et al., 2017). Autosomal dominant and recessive mutations are caused by CRYAA gene. It has been observed in humans and mice that recessive phenotype affects N-Terminus and domi-

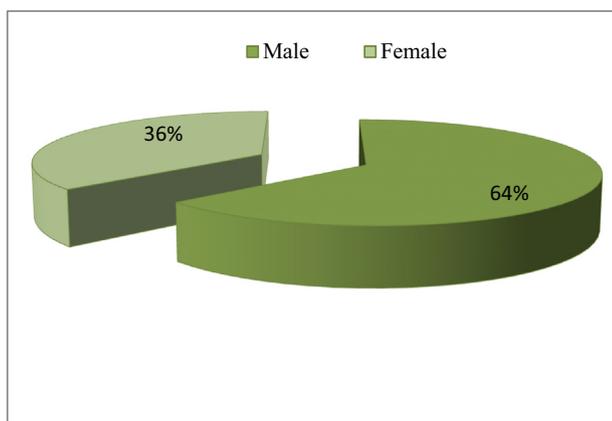


Fig. 1. Gender distribution of cases of congenital cataract.

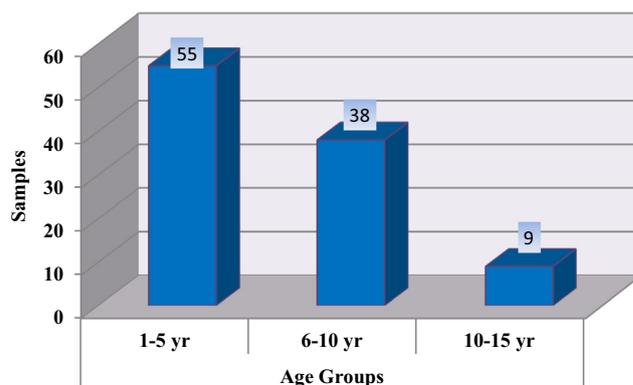
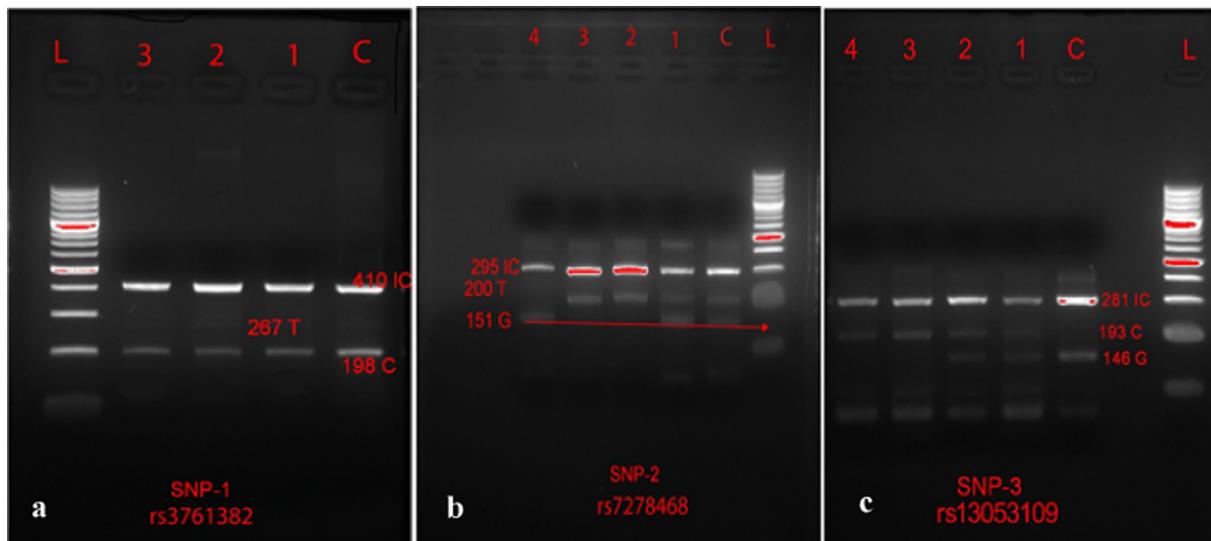
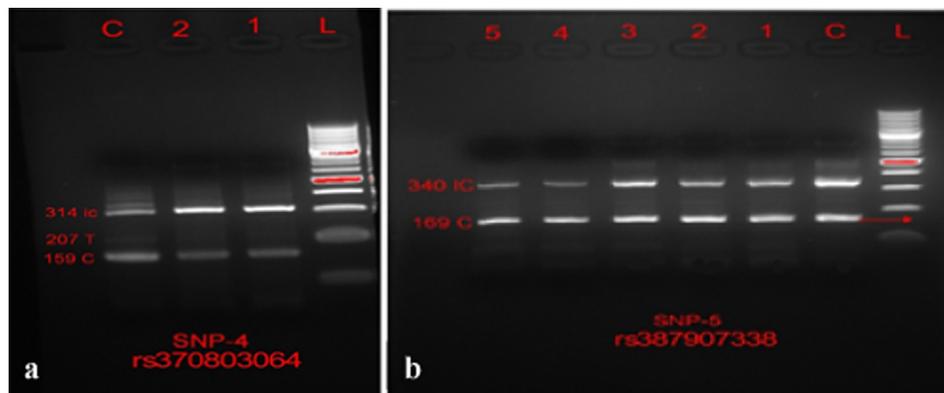


Fig. 2. The chart represents the number of non-familial congenital cataract affected individuals of different age group.



**Fig. 3.** Gel images of PCR products of *CRYAA* gene variants showing range of TETRA primers used in ARMS assay. (a) *CRYAA* rs3761382 homozygous CC 2 bands at 410 and 198 bp (lanes 1, 3), and heterozygous CT 3 bands at 410, 267 and 198 bp (lanes 2, C); (b) *CRYAA* rs7278468 heterozygous GT 3 bands at 295, 200 and 151 bp (lanes C & 1), homozygous TT 2 bands at 295 and 200 bp (lanes 2,3), and homozygous GG 2 bands at 295 and 151 bp (lane 4); (c) *CRYAA* rs13053109 heterozygous GC 3 bands at 281, 193 and 146 bp (lanes 1,2), homozygous GG 2 bands at 281 and 146 bp (lane C), and homozygous CC 2 bands at 281 and 193 bp (lanes 3,4).



**Fig. 4.** Gel images of PCR products of *CRYAB* gene variants showing ranges of TETRA primers used in ARMS assay. (a) *CRYAB* rs370803064 homozygous CC 2 bands at 314 and 159 (lanes 1,2), and heterozygous CT 3 bands at 314, 207 and 159 (lane C); (b) *CRYAB* rs387907338 homozygous CC 2 bands at 349 and 169 (lanes C, 1 to 5).

nant phenotype affects C-terminus (Graw, 2003). Furthermore, *CRYAB* is notably expressed in lens, which is accountable for expression of cardiac, neurologic and skeletal muscle tissue besides retinal muscle tissue (Safieh et al., 2009; Xia et al., 2014; Cui et al., 2017). 13 useful genes have been found in humans, out of which 10 are pre-dominant crystallin genes concerned with causing infantile cataract (Devi et al., 2008). Different mutations or variants were found in sufferers and families with congenital cataract where *CRYAA* and *CRYAB* genes were responsible. Likewise, researchers discovered a nonsense mutation in *CRYAA* gene in a Jewish-Persian family, which specified G to A transformation that formed a premature stop codon (W9X) and resulted in autosomal recessive congenital cataract (Pras et al., 2000). Kong et al. (2015) found a disease-inflicting novel mutation in *CRYAA* gene [c.246\_248delCGC (p.117delR)], which was accountable for causing autosomal-type perinuclear congenital cataract into a Chinese family. Safieh et al. (2009) analyzed c.166C > T conversion (which was considered to be pathogenic synonymic variant rs 387907338), responsible for bringing change in amino acid arginine to tryptophan (R56W) that eventually resulted in causing missense mutation in *CRYAB* gene. Ma et al. (2016) and Cui et al. (2017)

presented in their studies that T allele on rs7278468 and TA allele of *CRYAB* gene variants rs370803064 and rs 387907338 increased the risk of age related cataract and congenital cataract, which is inconsistent with our results. Our study data showed that GC genotype of rs13053109 SNP of *CRYAA* gene may increase the risk factor of congenital cataract among the newborn. Moreover, CTC haplotype of rs3761382 C > T, rs7278468 G > T, rs13053109 G > C in *CRYAA* gene (OR = 1.60 95 %CI = 0.11–22.64, P > 0.05) was found in newborn babies, highly capable of causing congenital cataract (Ma et al., 2016; Cui et al., 2017).

## 5. Conclusion

This study identified three SNPs of *CRYAA* gene that appeared to be associated with congenital cataract. Overall, this study concluded that variant rs13053109 G > C of *CRYAA* gene could be considered to increase the risk of congenital cataract in new born babies. The haplotype analysis of *CRYAA* gene variants showed that the haplotype CTC indicated the high risk (OR = 1.60 95 %CI = 0.11–22.64, P > 0.05) while haplotypes CGC, TTG and TGG exhibited slightly increased risk of congenital cataract among the infants.

**Table 2**  
Genotypic and allelic frequencies of CRYAA and CRYAB genes SNPs in congenital cataract.

| SNP/rs                                      | Genetic Model/HWE | Allele/Genotype   | Case n (%)        | Control n (%) | OR (95% CI)       | p Value           |
|---|-------------------|-------------------|-------------------|---------------|-------------------|-------------------|
| <b>CRYAA</b><br><b>rs3761382 C &gt; T</b>   | N/A               | C                 | 168 (52.1%)       | 153 (47.5%)   | –                 | –                 |
|   |                   | T                 | 34 (48.5%)        | 35 (50%)      | –                 | –                 |
|   | HWE               | C/C               | 67 (53.1%)        | 59 (46.8%)    | 1                 | 0.6               |
|   |                   | C/T               | 34 (48.5%)        | 35 (50%)      | 0.86 (0.48–1.54)  |                   |
|   |                   |                   | <b>0.067</b>      | <b>0.035</b>  |                   |                   |
| <b>CRYAA</b><br><b>rs7278468 G &gt; T</b>   | N/A               | G                 | 88 (48.3%)        | 94 (51.6%)    | –                 | –                 |
|   |                   | T                 | 114 (55.3%)       | 92 (44.6%)    | –                 | –                 |
|   | Codominant        | T/T               | 21 (20.8%)        | 5 (5.4%)      | 1                 | <b>0.0037</b>     |
|   |                   | G/T               | 72 (71.3%)        | 82 (88.2%)    | 0.21 (0.07–0.58)  |                   |
|   |                   | G/G               | 8 (7.9%)          | 6 (6.5%)      | 0.32 (0.08–1.34)  |                   |
|   | Dominant          | T/T               | 21 (20.8%)        | 5 (5.4%)      | 1                 | <b>0.0011</b>     |
|   |                   | G/T-G/G           | 80 (79.2%)        | 88 (94.6%)    | 0.22 (0.08–0.60)  |                   |
|   | Recessive         | T/T-G/T           | 93 (92.1%)        | 87 (93.5%)    | 1                 | 0.69              |
|   |                   | G/G               | 8 (7.9%)          | 6 (6.5%)      | 1.25 (0.42–3.74)  |                   |
|   | Over-dominant     | T/T-G/G           | 29 (28.7%)        | 11 (11.8%)    | 1                 | <b>0.0032</b>     |
|   |                   | T/G               | 72 (71.3%)        | 82 (88.2%)    | 0.33 (0.16–0.71)  |                   |
| Log-additive                                | –                 | –                 | –                 | –             | 0.49 (0.25–0.95)  | <b>0.029</b>      |
| HWE   |                   | <b>&lt;0.0001</b> | <b>&lt;0.0001</b> | –             | –                 |                   |
| <b>CRYAA</b><br><b>rs13053109 G &gt; C</b>  | N/A               | G                 | 169 (51.5%)       | 159 (48.4%)   | –                 | –                 |
|   |                   | C                 | 33 (51.5%)        | 31 (48.4%)    | –                 | –                 |
|   | Codominant        | G/G               | 75 (74.3%)        | 66 (69.5%)    | 1                 | 0.094             |
|   |                   | G/C               | 19 (18.8%)        | 27 (28.4%)    | 0.62 (0.32–1.21)  |                   |
|   |                   | C/C               | 7 (6.9%)          | 2 (2.1%)      | 3.08 (0.62–15.35) |                   |
|   | Dominant          | G/G               | 75 (74.3%)        | 66 (69.5%)    | 1                 | 0.46              |
|   |                   | G/C-C/C           | 26 (25.7%)        | 29 (30.5%)    | 0.79 (0.42–1.47)  |                   |
|   | Recessive         | G/G-G/C           | 94 (93.1%)        | 93 (97.9%)    | 1                 | 0.096             |
|   |                   | C/C               | 7 (6.9%)          | 2 (2.1%)      | 3.46 (0.70–17.11) |                   |
|   | Over-dominant     | G/G-C/C           | 82 (81.2%)        | 68 (71.6%)    | 1                 | 0.11              |
|   |                   | G/C               | 19 (18.8%)        | 27 (28.4%)    | 0.58 (0.30–1.14)  |                   |
| Log-additive                                | –                 | –                 | –                 | –             | 1.00 (0.61–1.65)  | 1                 |
| HWE   |                   | <b>0.0043</b>     | <b>1</b>          | –             | –                 |                   |
| <b>CRYAB</b><br><b>rs370803064 C &gt; T</b> | N/A               | C                 | 101 (34%)         | 190 (64%)     | –                 | –                 |
|   |                   | T                 | 101 (100%)        | 0 (0%)        | –                 | –                 |
|   | HWE               | C/C               | 95 (100%)         | 0 (0%)        | –                 | <b>&lt;0.0001</b> |
|   |                   | C/T               | 0 (0%)            | 101 (100%)    | –                 |                   |
|   |                   |                   | <b>&lt;0.0001</b> | <b>1</b>      | –                 | –                 |
| <b>CRYAB</b><br><b>rs387907338 C &gt; T</b> | N/A               | C                 | 202 (50.7%)       | 190 (47.7%)   | –                 | –                 |
|   |                   | C/C               | 101 (50.7%)       | 95 (47.7%)    | –                 | –                 |
|   | HWE               |                   | –                 | –             | –                 | –                 |

**Table 3**  
Haplotype analyses for three SNPs in CRYAA gene.

| Haplotype association with response (n = 196, crude analysis) |      |      |      |        |         |             |                   |         |
|---|------|------|------|--------|---------|-------------|-------------------|---------|
|   | SNP1 | SNP2 | SNP3 | Case   | Control | Total Freq. | OR (95% CI)       | p value |
| 1   | C    | T    | G    | 0.4448 | 0.3236  | 0.3985      | 1                 | –       |
| 2   | C    | G    | G    | 0.2402 | 0.3448  | 0.2797      | 0.34 (0.14–0.84)  | 0.02    |
| 3   | C    | G    | C    | 0.1219 | 0.1279  | 0.1238      | 0.41 (0.15–1.09)  | 0.075   |
| 4   | T    | T    | G    | 0.0908 | 0.1372  | 0.1042      | 0.56 (0.20–1.56)  | 0.27    |
| 5   | T    | G    | G    | 0.0608 | 0.0311  | 0.0543      | 0.86 (0.13–5.92)  | 0.88    |
| 6   | C    | T    | C    | 0.0247 | 0.0176  | 0.0212      | 1.60 (0.11–22.64) | 0.73    |

CI = Confidence interval, OR = Odds ratio, SNP = Single nucleotide polymorphism.

These findings could be helpful in offering a reference line for the congenital cataract studies in future.

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None.

**Authors' contributions**

All authors contributed in study design, experimentation, data analysis, drafting or revising the article, gave final approval of

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**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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