Association of single nucleotide polymorphism variations in CRYAA and CRYAB genes with congenital cataract in Pakistani population

Priya Jarwar, Yar Muhammad Waryah, Muhammad Rafiq, Ali Muhammad Waryah

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 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
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 $-80^\circ$ 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 MgCl2 buffer (2 μl), 0.125 mM dNTPs (2 μl), outer forward and outer reverse primers (0.5 μl), inner forward and inner reverse primers (1 μl), DNA template (2 μl) and Taq Polymerase (0.6 μl) and deionized distilled water (11.9 μl) in 20 μl reaction mixture. Amplification condition were: 2 min. of initial denaturation at 95 $^\circ$ C, followed by 30 cycles of denaturation at 95 $^\circ$ C for 45 sec., annealing at 67 $^\circ$C/69 $^\circ$C for 40 sec., extension at 72 $^\circ$ C for 50 sec., and the final extension at 72 $^\circ$ 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 $<0.05$ was considered as significant. With the help of logistic regression analyses through SNPSstats software (https://www.snpstats.net/start.htm), allelic frequencies, haplotype frequencies and Hardy-Weinberg Equilibrium (HWE) were calculated. The SNPSstats 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-
strated that genotypic and allelic frequencies were 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 C > 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 with HWE (P > 0.05), hence decreased risk of congenital cataract was not found in all models (P < 0.05). In CRYAB gene variant rs13051039 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 rs370803064 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 CGG, TTG and TGG indicated marginally increased risk of congenital cataract among the newborns (CGG: 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). Protein crystallin is made of two main proteins; αA and αB 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-
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 of congenital cataract in newborn babies. 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.
These findings could be helpful in offering a reference line for the congenital cataract studies in future.

**Funding/Sponsorship**

None.

**Authors’ contributions**

All authors contributed in study design, experimentation, data analysis, drafting or revising the article, gave final approval of the version to be submitted for publication and agreed to be accountable for all aspects of work.

**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.

**Acknowledgement**

The author is thankful to Department Molecular Biology and Genetics, Medical Research Center, Liaquat University of Medical
and Health Sciences, Jamshoro Pakistan for providing necessary equipment and lab facilities for this research.

References

Ahmadian, A., Gharizadeh, B., Gustafsson, A.C., Sterky, F., Nyrén, P., Uhlén, M., Lundeberg, J., 2000. Single-nucleotide polymorphism analysis by pyrosequencing. Anal. Biochem. 280 (1), 103–116.

Alyehodhi, R.R., Singh, U., Kumar, S., Alex, R., Deb, R., Sengar, G.S., Raja, T.V., Prakash, B., 2018. T-ARMS PCR genotyping of SNP rs445709131 using thermostable strand displacement polymerase. BMC Res. Notes 11 (1). https://doi.org/10.1186/s13104-018-3236-6.

Berry, V., Ionides, A., Pontikos, N., Georgiou, M., Yu, J., Ocaka, L.A., Moore, A.T., Quinlan, R.A., Michaelides, M., 2020. The genetic landscape of crystallins in congenital cataract. Orphanet. J. Rare Dis. 15 (1). https://doi.org/10.1186/s13023-020-01613-3.

Blazej, R.G., Paegel, B.M., Mathies, R.A., 2003. Polymorphism ratio sequencing: a new approach for single nucleotide polymorphism discovery and genotyping. Genome Res. 13 (2), 287–293.

Cui, X.-J., Lv, F.-Y., Li, F.-H., Zeng, K., 2017. Correlations of single nucleotide polymorphisms of CRYAA and CRYAB genes with the risk and clinicopathological features of children suffering from congenital cataract. Medicine (Baltimore) 96 (25), e7158. https://doi.org/10.1097/MD.0000000000001758.

Devi, R.R., Yao, W., Vijayalakshmi, P., et al., 2008. Crystallin gene mutations in Indian families with inherited pediatric cataract. Mol. Vis. 14, 1157–1170.

Ge, X.-L., Zhang, Y., Wu, Y., Lv, J., Zhang, W., Jin, Z.-B., Qu, J., Gu, F., 2014. Identification of a novel GJA1 (Cx50) point mutation causes human dominant congenital cataracts. Sci. Rep. 4 (1). https://doi.org/10.1038/srep04121.

Graw, J., 2003. The genetic and molecular basis of congenital eye defects. Nat. Rev. Genet. 4 (11), 876–888.

Gromberg, J., Nawoschik, S., Belluscio, L., et al., 1989. A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. Nucleic Acids Res. 17(20), 8390.

Jadoon, Z., Shah, S.P., Bourne, R., Dineen, B., Khan, M.A., Gilbert, C.E., Foster, A., Khan, M.D., 2007. Cataract prevalence, cataract surgical coverage and barriers to uptake of cataract surgical services in Pakistan: the Pakistan National Blindness and Visual Impairment Survey. Br. J. Ophthalmol. 91 (10), 1269–1273.

Khidir, F.F., Waryah, Y.M., Ali, F.K., Shaikh, H., Uijjan, I.D., Waryah, A.M., 2019. MTHFR and F5 genetic variations have association with preeclampsia in Pakistani patients: a case control study. BMC Med. Genet. 20 (1). https://doi.org/10.1186/s12881-019-0905-9.

Kong, X.D., Liu, N., Shi, H.R., Dong, J.M., Zhao, Z.H., Liu, J., Li-Ling, J., Yang, Y.X., 2015. A novel 3-base pair deletion of the CRYAB gene identified in a large Chinese pedigree featuring autosomal dominant congenital perinuclear cataract. Genet. Mol. Res. 14 (1), 426–432.

Kwok, P.Y., Chen, X., 2003. Detection of single nucleotide polymorphisms. Curr. Issues Mol. Biol. 5, 43–60.

Liu, M., Ke, T., Wang, Z., Yang, Q., Chang, W., Jiang, F., Tang, Z., Li, H., Ren, X., Wang, X., u., Wang, T., Li, Q., Yang, J., Liu, J., Wang, Q.K., 2006. Identification of a CRYAB mutation associated with autosomal dominant posterior polar cataract in a Chinese family. IOVS 47 (8), 3461. https://doi.org/10.1167/iovs.05-1438.

Ma, X., Jiao, X., Ma, Z., Hejtmancik, J.F., 2016. Polymorphism rs7278468 is associated with age-related cataract through decreasing transcriptional activity of the CRYAB promoter. Sci. Rep. 6 (1). https://doi.org/10.1038/srep23206.

Medrano, R.F.V., de Oliveira, C.A., 2014. Guidelines for the tetra-primer ARMS-PCR technique development. Mol. Biotechnol. 56 (7), 599–608.

Pras, E., Frydman, M., Levy, N.E., et al., 2000. A Nonsense mutation (W89X) in CRYAB causes autosomal recessive cataract in an Inbred Jewish Persian family. IOVS 41 (11), 3511–3515.

Quillen, D.A., 1999. Common causes of vision loss in elderly patients. Am. Fam. Physician 60 (11), 99–108.

Safieh, L.A., Khan, A.O., Alkuraya, F.S., 2009. Identification of a novel CRYAB mutation associated with autosomal recessive juvenile cataract in a Saudi family. Mol. Vis. 15, 980–984.

Santhiya, S.T., Manohar, M.S., Rawlley, D., et al., 2002. Novel mutations in the g-crystallin genes cause autosomal dominant congenital cataracts. J. Med. Genet. 39, 352–358.

Wang, Z., Huang, C., Lv, H., Zhang, M., Li, X., Nagaraj, R., 2020. In silico analysis and high-risk pathogenic phenotype predictions of non-synonymous single nucleotide polymorphisms in human Crystallin beta A4 gene associated with congenital cataract. PLoS One 15 (1), e0227859. https://doi.org/10.1371/journal.pone.0227859.

Xia, X.-Y., Wu, Q.-Y., An, L.-M., Li, W.-W., Li, N.a., Li, T.-F., Zhang, C., Cui, Y.-X., Xue, C.-Y., 2014. A novel P2OR mutation in the alpha-B crystallin gene causes autosomal dominant congenital posterior polar cataracts in a Chinese family. Mol. Ophthalmol. 14 (1). https://doi.org/10.1186/s12886-014-0160-2.

Zhao, X., Li, J., Zhu, Y., Xia, Y., Wang, W., Yu, Y., Yao, K., 2014. A nonsense mutation of D-crystallin associated with congenital nuclear and posterior polar cataract in a Chinese family. Int. J. Med. Sci. 11 (2), 158–163.

Zhang, T., Hua, R., Xiao, W., Burdon, K.P., Bhattacharya, S.S., Craig, J.E., Shang, D., Zhao, X., Mackey, D.A., Moore, A.T., Loo, Y., Zhang, J., Zhang, X., 2009. Mutations of the EPHA2 receptor tyrosine kinase gene cause autosomal dominant congenital cataract. Hum. Mutat. 30 (5), E603–E611.

Zhao, Z., Fan, Q., Zhou, P., Ye, HongFei, Cai, L., Lu, Y., 2017. Association of alpha A-crystallin polymorphisms with susceptibility to nuclear age-related cataract in a Han Chinese population. BMC Ophthalmol. 17 (1). https://doi.org/10.1186/s12886-017-0529-9.