Deciphering the association of intronic single nucleotide polymorphisms of crystallin gene family with congenital cataract

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Purpose: Introns play an important role in gene regulation and expression. Single nucleotide polymorphisms (SNPs) in introns have the potential to cause disease and alter the genotype–phenotype association. Hence, this study aimed to decipher the association of SNPs in the introns of the crystallin gene in congenital cataracts.

Methods: SNPs in the introns of crystallin gene family – CRYAA (rs3788059), CRYAB (rs2070894), CRYBA4 (rs2071861), and CRYBB2 (rs5752083, rs5996863) – were genotyped in 248 participants consisting of 141 congenital cataracts and 107 healthy controls by allele-specific oligonucleotide polymerase chain reaction method. Around 10% of samples for each SNP were sequenced to confirm the genotypes. The allele, genotype, and haplotype frequency were evaluated by the SHEsis online tool.

Results: Using dominant model, the “A” allele of rs3788059 was found to have an increased risk toward congenital cataract development whereas the “G” allele was found to be protective (AA + AG vs. GG; odds ratio [OR] 95% confidence interval [CI] = 3.73 [1.71, 8.15], P = 0.0009). The “A” allele of both rs2070894 (AA + AG vs. GG; OR [95% CI] = 0.49 [0.29, 0.84], P = 0.012) and rs5752083 (AA + AC vs. CC; OR [95% CI] = 0.25 [0.08, 0.76], P = 0.016) were suggested to have a protective role by the dominant model. The A-C-T haplotype (rs2071861, rs5752083, and rs5996863) was found to be a significant risk factor for the development of congenital cataract.

Conclusion: Intronic SNPs in crystallin genes may play a role in the predisposition toward congenital cataract. However, the present findings need to be replicated in a large cohort with more number of samples.

Key words: Crystallin, congenital cataract, intronic, SNP genotyping

Introns, the noncoding segments of DNA (deoxyribonucleic acid) are thought to play a vital role in genome evolution in eukaryotes.[1] Although once considered as junk DNA, introns are gaining importance as they perform a significant role in the regulation of gene expression, mRNA (messenger RNA [ribonucleic acid]) export, splicing, transcription coupling, and enhancing the protein diversity by exon shuffling and alternative splicing.[2–5] With the successful completion of the human genome project and the advent of next-generation sequencing platforms, a large number of intronic single nucleotide polymorphisms (SNPs) have been identified and associated with human diseases through several genome-wide association studies (GWAS).[6–9] Furthermore, introns may be the target for mutations at considerably higher proportion or mutational hotspots because they possess arrays of essential functional elements such as the intron splice enhancers and silencers, trans-splicing elements, and other controlling elements.[10–13] In addition to functional mutations, SNPs in introns may also cause increased susceptibility to disease and modulate the association between genotype and phenotype.[14]

Congenital cataract is characterized by the clouding of the lens, either completely or partially, that significantly affects normal vision either from the beginning or shortly after birth. It is one of the leading causes of treatable childhood blindness and has a prevalence rate of 1 to 6 per 10,000 live birth.[15] It may either be isolated or occur along with other ocular malformations and/or multisystemic disorder.[15] Although both genetic and environmental regulators are well-known causative factors, about 50% of congenital cataracts have been suggested owing to genetic factors.[16] It exhibits autosomal dominant, recessive, X-linked, and mitochondrial mode of inheritance pattern.[17]

More than 90% of the total water-soluble protein in the human eye lens is made up of crystallins that play a vital role in maintaining lens transparency.[18] They are characterized as α-, β-, and γ-crystallin families (encoded by CRYAA; CRYAB, CRYBA1, CRYBA2, CRYBA4, CRYBB1, CRYBB2, CRYBB3; and CRYGA, CRYGB, CRYGC, CRYGD, CRYGS, CRYGN, CRYGEP, CRYGFP, CRYGGP genes, respectively).[19] Mutation in more than 360 genes to be responsible for congenital cataract have been reported in several studies (Cat-Map; https://cat-map.wustl.edu/).[20] Although the majority of mutations that were identified till date in human congenital cataract is in crystallin

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genes, only a few studies have reported the association of intronic SNPs of crystallin genes with congenital cataract. Furthermore, all these studies have been performed in different ethnic groups and different cohorts of the Indian population. In all these studies, the population from the western region of India was always kept isolated. Considering the importance of introns in human genomes and the dearth of genetic association studies in the western Indian population, the present study was designed to elucidate the association of intronic SNPs of crystallin genes (CRYAA, CRYAB, CRYBA4, and CRYBB2) with congenital cataract in a cohort of western India. This study may assist in identifying the disease-associated loci and further help in the implementation of tools for prenatal diagnosis and risk prediction of congenital cataract.

**Methods**

**Recruitment and ocular examination**

The study participants were recruited essentially from the western region of India. All the study procedures adhered to the tenets of the Declaration of Helsinki and were approved by the Institutional Ethical Committee. Written informed consent was obtained from the parents and/or the guardians.

A thorough ophthalmic examination was performed. Different visual acuity assessments were performed for children of different age-groups, using Cardiff’s acuity test for 1 to 2 years, LEA symbols for 2 to 3 years, Lippman’s HOTV test for 3 to 5 years, and Snellen’s chart for older than 5 years. The distant direct examination was done to look for anterior segment abnormalities such as corneal opacity, shallow anterior chamber, peripheral anterior synchiae (Peter’s anomaly), microcornea, posterior synchiae, the keyhole pupil (iridofundal coloboma), and enlarged ciliary processes with vessels on the lens (PTV). Red reflex screening (Bruckner’s test) with direct ophthalmoscope focusing on each dilated pupil (with homatropine 2%) separately from 30 cm distance was performed to identify lenticular opacity, and both eyes were visualized simultaneously from 3 ft to identify anisometropia, strabismus, and asymmetric cataract and fixation pattern based on different glows. The cataract was classified based on the zone and morphology of lens opacity observed through either slit-lamp biomicroscopy or under an operating microscope.

Participants with no symptoms of cataract and other ocular disorders were considered as controls and those with cataract were identified as cases. Participants with a history of traumatic cataract, viral infection, neurodevelopmental disorder, chromosomal abnormality, systemic diseases, and in-born errors of metabolism were excluded from this study.

**SNP genotyping**

About 2 mL of peripheral venous blood was collected from the cases and healthy controls. Genomic DNA was extracted by the salting out method. A total of 5 SNPs from four different genes – CRYAA (rs3788059), CRYAB (rs2070894), CRYBA4 (rs2071861), and CRYBB2 (rs5752083, rs5996863) – were selected from the 1000 Genomes project (http://www.1000genomes.org/). All the SNPs were genotyped by allele-specific oligonucleotide–polymerase chain reaction (ASO-PCR) method. The ASO primers were designed using the WASP online tool (http://bioinfo.biotec.or.th/wasp) and are listed in Table 1. The PCR reaction for the wild and the mutant allele was carried out in two separate tubes each containing 1X Emerald GT PCR Master Mix (TaKaRa Bio Inc., Japan), 50 ng genomic DNA, 20 pM each of allele-specific primers. The thermal cycling steps consisted of one cycle of initial denaturation at 94°C/1 minute, and 40 cycles of the second denaturation at 94°C/30 seconds, annealing at 53–57°C/30 seconds, extension at 72°C/30 seconds, and a final extension at 72°C/3 minutes. All the amplicons were resolved using 4% agarose gel and visualized by UV (ultraviolet) transilluminator on ethidium bromide staining. The allele and genotype frequencies were scored by direct counting. About 10% of both case and control samples were sequenced to confirm the genotypes.

**In silico analyses**

The effect of intronic SNPs on splicing as well on transcription factor binding was checked using Human Splicing Finder (HSF) and TRANSFAC online tools, respectively.

**Statistical analysis**

All continuous variables were analyzed by Student’s t test, and the values were presented as mean ± standard deviation (SD). The allele, genotype, haplotype, and Hardy–Weinberg equilibrium (HWE) were analyzed using the SHEsis online tool (http://analysis.bio-x.cn/SHEsisMain.htm). The association of the alleles and the genetic models with the disease was calculated by taking odds ratio (OR) at 95% confidence interval (CI) (http://www.hutchon.net/confidorm.htm). The strength of association of SNPs with the disease between the cases and the controls was tested by Chi-square test (www.socscistatistics.com/pvalues/chidistribution.aspx). Bonferroni’s correction was applied for multiple SNPs testing by dividing the alpha error of 0.05 by the total number of SNPs tested. Hence, a P value <0.01 was considered statistically significant. Genetic models were considered significant if Yates corrected P value is <0.01.

**Results**

**Demography of the participants**

All the study participants were from western India, including Gujarat, Madhya Pradesh, Maharashtra, and Rajasthan. A total of 248 participants were recruited, consisting of 141 congenital cataract cases and 107 age-matched healthy controls. All the cases had isolated congenital cataracts of different phenotypes such as nuclear (18.44%), lamellar (22.70%), posterior subcapsular (31.20%), and total cataract (27.66%). There was no significant difference in age (range 0.1–10 years, P = 0.1) between the cases (5.87 ± 3.37 years) and the controls (6.45 ± 3.77 years). The demography of the recruited participants is shown in Table 2.

**Association of allele, genotype, and haplotype frequencies with disease risk**

A total of five intronic SNPs from four crystallin genes were genotyped in this study. The allele and genotype frequencies of all the polymorphisms in both cases and controls were scored by ASO-PCR followed by agarose gel electrophoresis [Fig. 1].

The allele and genotype frequency of SNP rs3788059 was in HWE, whereas SNPs rs2070894, rs2071861, rs5752083, and rs5996863 showed a deviation from HWE in both cases and controls [Table 3].
Table 1: List of ASO Primers for SNP Genotyping

| Gene and SNP ID | Primer ID | Sequence (5'-3') | Amplicon size (bp) | MAF (1,000 G) |
|-----------------|-----------|------------------|--------------------|---------------|
| CRYAA-rs3788059 (c.190-370G > A) | SNP1-WRP | GTTGGTCCGTTAGGGTCAATA | 174 | A: 0.0004 |
|                 | SNP1-MRP | GTTGGTCCGTTAGGGTCAATA | - | - |
|                 | SNP1-CFP | GTGAGAAGGAGCATGAGAAG | - | - |
| CRYAB-rs2070894 (c.324 + 214G > A) | SNP3-WRP | ATCCCCATCATCCATCTAAGGAG | 185 | A: 0.26 |
|                 | SNP3-MRP | ATCCCCATCATCCATCTAAGGAA | - | - |
|                 | SNP3-CFP | ATATGCCAGGTAGTGCTATGACTT | - | - |
| CRYBA4-rs2071861 (c.159-256A > G) | SNP5-WRP | TGGTCTCTCATCAACCCTGGA | 265 | G: 0.28 |
|                 | SNP5-MRP | TGGTCTCTCATCAACCCTGGA | - | - |
|                 | SNP5-CFP | AGGGTAGAGTGTGCAGGAGGTA | - | - |
| CRYBB2-rs5752083 c.54 + 1112C > A | SNP7-WRP | ATGGCTCTCATCAACCCTGCC | 110 | A: 0.30 |
|                 | SNP7-MRP | ATGGCTCTCATCAACCCTGGA | - | - |
|                 | SNP7-CFP | GAGGGAGGAGGACTGTTGAA | - | - |
| CRYBB2-rs5996863 (g.17486C > T) | SNP8-WFP | CAATTTCCCTTGTCCTGACC | 208 | C: 0.38 |
|                 | SNP8-MRP | CAATTTCCCTTGTCCTGACT | - | - |
|                 | SNP8-CFP | TCAGGGTTTTCTGCTTCTT | - | - |

ASO: Allele-specific oligonucleotide, SNP: Single nucleotide polymorphism, CRYAA: Crystallin alpha-A, CRYAB: Crystallin alpha-B, CRYBA4: Crystallin beta-A4, CRYBB2: Crystallin beta-B2, WRP: Wild-type reverse primer, MRP: Mutant reverse primer, WFP: Wild-type forward primer, MFP: Mutant forward primer, CFP: Common forward primer, CRP: Common reverse primer, MAF: Minor allele frequency.

Table 2: Demography of the recruited participants

| Demography | Congenital cataracts (n=141) | Controls (n=107) |
|------------|-----------------------------|-----------------|
| Female, n (%) | 60 (42.55) | 48 (44.86) |
| Male, n (%) | 81 (57.45) | 59 (55.14) |
| Age in years (mean±SD) | 5.87±3.37 | 6.45±3.77 |
| P | 0.1 |
| Cataract type (%) | | - |
| Nuclear | 26 (18.44) | - |
| Lamellar | 32 (22.70) | - |
| PSC | 44 (31.20) | - |
| Total | 39 (27.66) | - |

PSC: Posterior subcapsular cataract, SD: Standard deviation.

SNP1: rs3788059
The frequency of “A” allele (OR [95% CI] = 3.55 [1.68, 7.51], P = 0.0005), and “AG” genotype (OR [95% CI] = 3.53 [1.61, 7.73], P = 0.001) of SNP-rs3788059 were significantly higher in cases than in the controls. The dominant model for SNP-rs3788059 indicated that “A” allele is associated with increased risk (AA + AG vs. GG; OR [95% CI] = 3.73 [1.71, 8.15], P = 0.0009) of disease, whereas “G” allele showed protective effect.

SNP2: rs2070894
The frequency of “AG” genotype of SNP-rs2070894 was significantly different between the cases and the controls (OR [95% CI] = 0.48 [0.28, 0.82], P = 0.007). The dominant model suggested that “A” allele of SNP-rs2070894 is protective (AA + AG vs. GG; OR [95% CI] = 0.49 [0.29, 0.84], P = 0.012).

SNP3: rs5752083
The frequency of “AC” genotype of SNP-rs5752083 was significantly different between the cases and the controls (OR [95% CI] = 0.22 [0.07, 0.68], P = 0.005). The dominant model for rs5752083 indicated a protective effect with “A” allele (AA + AC vs. CC; OR [95% CI] = 0.25 [0.08, 0.76], P = 0.016).

Discussion
Majority of congenital cataracts are manifested as a result of genetic variations in crystallin genes. Crystallin gene clusters...
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are responsible for the synthesis of two major crystallin protein families: α-crystallin and β/γ crystallins. The α-crystallin inhibits lens cell apoptosis and maintains protein stability.\[31\] Mutations in CRYAA is linked to the loss of α-crystallin protein, which ultimately leads to excessive light scattering and lens opacification.\[32,35\] On the other hand, β-crystallins aid in lens development and maintaining lens transparency.\[84\] Mutations in the β-crystallin genes are known to cause abnormality of the protein structure that makes the protein unstable, which precipitates from the solution. This in turn leads to additional protein denaturation and precipitation that subsequently leads to the formation of congenital cataract.\[37\]

Although 90% of the genome comprises introns, to date only very few reports are available on intronic variations or SNPs associated with congenital cataracts. Even if the intronic SNP does not have a functional consequence, it may exist in linkage disequilibrium with other functional SNPs and thereby help recognize the disease loci. Considering the potential association of SNPs with congenital cataract and the dearth of information on genetic association studies using intronic SNPs,\[38,39\] the present study was performed to understand the distribution of intronic SNPs rs3788059 (CRYAA), rs2070894 (CRYAB), rs2071861 (CRYBA4), rs5752083 (CRYBB2), and rs5996863 (CRYBB2) in congenital cataracts and normal healthy controls. Although association studies using these markers have never been reported in congenital cataracts, studies on rs2070894 concerning colorectal and oral cancer\[40,41\] and rs2071861 concerning high myopia\[42,43\] have been reported.

In the present study, a higher distribution of the CRYAA-rs3788059 “AG” genotype in congenital cataracts is observed. The dominant model also showed that “A” allele is positively associated with an increased risk. HSF analyses for this SNP showed alteration of auxiliary sequences, whereas TRANSFAC analyses revealed loss of REV-ErbA and gain of HNF-1 (hepatocyte nuclear factor–1) and T3R transcription factor binding site. The CRYAB-rs2070894 “AG” genotype frequency was found to be more in controls than in cases, and the dominant model showed that CRYAB-rs2070894 “A” allele is protective. In two separate studies, Bau et al. (2011)\[40\] and Wu et al. (2018)\[41\] evaluated the association of CRYAB-rs2070894 polymorphism with colorectal and oral cancer, respectively, and did not report any significant association of the allele or genotype with the disease. The distribution of the “AC” genotype of CRYBB2-rs5752083 was found to be significantly less in cases than in controls. The dominant model for CRYBB2-rs5752083 showed that the “A” allele is protective. This SNP showed alteration of auxiliary sequences in HSF analyses and revealed loss of Sp1, Rar-alph, Rev-ErbA, RAR-beta, and ER and gain of YY1 transcription factor binding site in TRANSFAC analyses.

Haplotype analysis of polymorphisms rs2071861, rs5752083, and rs5996863 revealed the association of A-C-T haplotype with the risk of developing congenital cataract. In this study, the association of allele or genotypes of CRYBA4-rs2071861 and CRYBB2-rs5996863 SNPs with congenital cataract was not established. However, in two separate studies, Kawagoe et al. (2017)\[42\] showed a marginal association and Ho et al. (2012)\[43\] showed a significant association of CRYBA4-rs2071861 with high myopia. These observations made a presumption that apart from candidate gene mutations and genetic makeup of an individual, there are additional factors such as environmental factors and gene–gene interactions that might contribute toward the onset and progression of congenital cataract. Nevertheless, in the present study, it is too

**Figure 1:** Four percent agarose gel shows the amplification of wild-type and rare alleles of the polymorphisms (a) rs3788059 (G > A), (b) rs2070894 (G > A), (c) rs2071861 (A > G), (d) rs5752083 (C > A), (e) rs5996863 (C > T) with their appropriate amplicon size.
### Table 3: Allele and genotype distribution of selected SNPs in congenital cataract cases

| Gene (SNP) | Allele/Genotype | Cases (n=141) | Controls (n=107) | OR [95% CI] | \( \chi^2 \) | \( P \) | \( P \) (HWE) |
|------------|-----------------|--------------|-----------------|-------------|----------|------|-------------|
| CRYAA (rs3788059) | G | 244 (0.865) | 205 (0.958) | - | - | - | C=0.69; CT=0.65 |
| | A | 38 (0.135) | 9 (0.042) | 3.55 [1.68, 7.51] | 12.19 | 0.0005 | - |
| | GG | 1.5 (0.745) | 98 (0.916) | - | - | - |
| | AG | 34 (0.241) | 9 (0.084) | 3.53 [1.61, 7.73] | 10.8 | 0.001 | - |
| | AA | 2 (0.014) | 0 (0.000) | 4.67 [0.22, 98.45] | 1.85 | 0.170 | - |
| CRYAB (rs2070894) | G | 202 (0.716) | 137 (0.640) | - | - | - | C=0.0005; CT=7.04-008 |
| | A | 80 (0.284) | 77 (0.360) | 0.7 [0.48, 1.03] | 3.26 | 0.071 | - |
| | GG | 64 (0.454) | 31 (0.290) | - | - | - |
| | AG | 74 (0.525) | 75 (0.701) | 0.48 [0.28, 0.82] | 7.4 | 0.007 | - |
| | AA | 3 (0.021) | 1 (0.009) | 1.45 [0.15, 14.54] | 0.1 | 0.750 | - |
| CRYBA4 (rs2071861) | A | 167 (0.592) | 136 (0.636) | - | - | - | C=0.02; CT=0.02 |
| | G | 115 (0.408) | 78 (0.364) | 1.2 [0.83, 1.73] | 0.96 | 0.327 | - |
| | AA | 56 (0.397) | 49 (0.458) | - | - | - |
| | AG | 55 (0.390) | 38 (0.355) | 1.27 [0.72, 2.23] | 0.68 | 0.411 | - |
| | GG | 30 (0.213) | 20 (0.187) | 1.31 [0.66, 2.60] | 0.61 | 0.440 | - |
| CRYBB2 (rs5752083) | C | 140 (0.496) | 104 (0.486) | - | - | - | C=1.14e-007; CT=1.94e-016 |
| | A | 142 (0.504) | 110 (0.514) | 0.96 [0.67, 1.37] | 0.05 | 0.817 | - |
| | CC | 19 (0.135) | 4 (0.135) | - | - | - |
| | AC | 102 (0.723) | 96 (0.897) | 0.22 [0.07, 0.68] | 8.04 | 0.005 | - |
| | AA | 20 (0.142) | 7 (0.065) | 0.60 [0.15, 2.40] | 0.53 | 0.470 | - |
| CRYBB2 (rs5996863) | C | 120 (0.426) | 100 (0.467) | - | - | - | C=1.24e-013; CT=1.03e-012 |
| | T | 162 (0.574) | 114 (0.533) | 1.18 [0.83, 1.69] | 0.86 | 0.354 | - |
| | CC | 4 (0.028) | 5 (0.047) | - | - | - |
| | CT | 112 (0.794) | 90 (0.841) | 1.56 [0.41, 5.96] | 0.42 | 0.520 | - |
| | TT | 25 (0.177) | 12 (0.112) | 2.60 [0.59, 11.49] | 1.66 | 0.200 | - |

SNP: Single nucleotide polymorphism, C: Cases; CT: Controls, HWE: Hardy-Weinberg equilibrium, OR: Odds ratio, CI: Confidence interval

### Table 4: Dominant and recessive models for the selected SNPs

| Gene (SNP) | Genetic model | Cases (n=141) | Controls (n=107) | OR [95% CI] | \( \chi^2 \) | \( P \) |
|------------|---------------|--------------|-----------------|-------------|----------|------|
| CRYAA (rs3788059) | Dominant | AA + AG | 36 (0.255) | 9 (0.084) | 3.73 [1.71, 8.15] | 12.01 | 0.001 |
| | | GG | 105 (0.745) | 98 (0.916) | - | - | - |
| | | Recessive | AA | 2 (0.014) | 0 (0.000) | 3.85 [0.18, 81.10] | 1.53 | 0.220 |
| | | | AG + GG | 139 (0.986) | 107 (1.000) | - | - | - |
| CRYAB (rs2070894) | Dominant | AA + AG | 77 (0.546) | 76 (0.710) | 0.49 [0.29, 0.84] | 6.94 | 0.008 |
| | | | GG | 64 (0.454) | 31 (0.290) | - | - | - |
| | | Recessive | AA | 3 (0.021) | 1 (0.009) | 2.3 [0.24, 22.47] | 0.55 | 0.460 |
| | | | AG + GG | 138 (0.979) | 106 (0.991) | - | - | - |
| CRYBA4 (rs2071861) | Dominant | GG + AA | 85 (0.603) | 58 (0.542) | 1.28 [0.77, 2.13] | 0.92 | 0.340 |
| | | | AA | 56 (0.397) | 49 (0.458) | - | - | - |
| | | | Recessive | GG | 30 (0.213) | 20 (0.187) | 1.18 [0.63, 2.21] | 0.25 | 0.620 |
| | | | AG + AA | 111 (0.787) | 87 (0.813) | - | - | - |
| CRYBB2 (rs5752083) | Dominant | AA + AC | 122 (0.865) | 103 (0.963) | 0.25 [0.08, 0.76] | 6.85 | 0.009 |
| | | | CC | 19 (0.135) | 4 (0.037) | - | - | - |
| | | | Recessive | AA | 20 (0.142) | 7 (0.065) | 2.36 [0.96, 5.81] | 3.66 | 0.060 |
| | | | AC + CC | 121 (0.858) | 100 (0.935) | - | - | - |
| CRYBB2 (rs5996863) | Dominant | TT + CT | 137 (0.972) | 102 (0.953) | 1.68 [0.44, 6.41] | 0.59 | 0.440 |
| | | | CC | 4 (0.028) | 5 (0.047) | - | - | - |
| | | | Recessive | TT | 25 (0.177) | 12 (0.112) | 1.71 [0.81, 3.57] | 2.03 | 0.150 |
| | | | CT + CC | 116 (0.823) | 95 (0.888) | - | - | - |

SNP: Single nucleotide polymorphism, OR: Odds ratio, CI: Confidence interval
early to predict how the genotype that showed association with congenital cataract can influence the gene to cause congenital cataract. But it is anticipated that these markers might present near other disease-causing functional SNPs that need to be scrutinized further.

In the present study, the SNP CRYAA-rs3788059 alone was in HWE in both cases and controls, whereas the other SNPs CRYAB-rs2070894, CRYBA4-rs2071861, and CRYBB2-rs5752083 and rs5996863 were not. Deviations from HWE can occur due to several reasons such as genotyping error, copy number variation, purifying selection, inbreeding, or population substructure. To eliminate potential genotyping error, genotyping was performed thrice by three different observers who were masked for the sample details. Turner et al. (2011) reported a consistent deviation of many SNPs from HWE at any given significant threshold. They suggested that such SNPs should never be eliminated from further evaluations; instead, they should be flagged for advanced analysis once the association analysis has been performed.

Conclusion

In conclusion, the intronic SNPs CRYAA-rs3788059, CRYAB-rs2070894, and CRYBB2-rs5752083 were significantly associated with congenital cataract. However, this study has a limitation of small sample size, and hence the present finding needs to be replicated in large cohorts and in different populations to confirm the association.

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Conflicts of interest

There are no conflicts of interest.

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| Gene and SNP ID | Human Splicing Finder Analyses | TRANSFAC Analyses |
|----------------|-------------------------------|-------------------|
| **CRYAA (rs3788059)** | Signal | Loss of REV-ErbA and gain of HNF-1 and T3R transcription factor binding site |
|                  | Alteration of auxiliary sequences | | |
|                  | New acceptor splice site | | |
|                  | HSF acceptor site (matrix AG) | | |
| **CRYAB (rs2070894)** | No significant impact on splicing signals. | No changes in TF binding sites |
| **CRYBA4 (rs2071861)** | No significant impact on splicing signals. | No changes in TF binding sites |
| **CRYBB2 (rs5752083)** | Alteration of auxiliary sequences | Loss of Sp1, Rar-alph, Rev-ErbA, RAR-beta and ER and gain of YY1 transcription factor binding site |
| **CRYBB2 (rs5996863)** | Upstream variant | Loss of TF Sp1 and CP2 transcription factor binding site |

SNP: Single nucleotide polymorphism, HSF: Human Splicing Finder, HNF: Hepatocyte nuclear factor, TF: Transcription factor, ESS: Exon splicing silencer, ESE: Exon splicing enhancer
**Supplementary Figure 1:** TRANSFAC analysis for CRYAA rs3788059 wild type and mutant shows the loss of binding site for transcription factor REV-ErbA and gain of binding site for transcription factor HNF-1 and T3R in the mutant.
Supplementary Figure 2: TRANSFAC analysis for CRYBB2 rs5752083 wild type and mutant shows the loss of binding site for transcription factor for Sp1, Rar-alph, Rev-ErbA, RAR-beta and ER and gain of binding site for transcription factor YY1.
Supplementary Figure 3: TRANSFAC analysis for CRYBB2 rs5996863 wild type and mutant shows the loss of binding site for transcription factor Sp1 and CP2 in the mutant.

**CRYBB2 r5996863-WT**

```
caattcccttgcccttgagtggctggttggtgagctcctgagctccctgttacatgtc
```

Segments:

|    | Start | Length | Score |
|----|-------|--------|-------|
| 3.1.2.2 | 113 | 122 | 1== |
| 1.1.3.0 | 116 | 125 | BPdel= |
| 2.3.1.0 | 124 | 133 | ===Sp1=== |
| 4.8.1.0 | 136 | 145 | ===CP2=== |
| 9.9.29  | 179 | 188 |

**CRYBB2 r5996863-MT**

```
caattcccttgcccttgagCggctggttggtgagctcctgagctccctgttacatgtc
```

Segments:

|    | Start | Length | Score |
|----|-------|--------|-------|
| 3.1.2.2 | 113 | 122 | 1== |
| 1.1.3.0 | 116 | 125 | BPdel= |
| 9.9.29  | 179 | 188 |