Int Ophthalmol (2021) 41:2847–2852
https://doi.org/10.1007/s10792-021-01843-9

ORIGINAL PAPER

Crystalline gene mutations in Turkish children with congenital cataracts

Mine Karahan · Atılım Armağan Demirtaş · Seyfettin Erdem · Sedat Ava · Selahattin Tekeş · Uğur Keklikçi

Received: 11 September 2020 / Accepted: 8 April 2021 / Published online: 16 April 2021

Abstract

Purpose To detect crystallin gene mutations in Turkish children with congenital cataracts.

Methods The present study included 56 children (38 males and 18 females) who were diagnosed with congenital cataract in our ophthalmology clinic. The patients' blood samples were collected and sent to the medical genetics laboratory. The samples were assessed using the sequence analysis method, which covered all exons of *CRYAA*, *CRYAB*, *CRYBB1*, *CRYBB2*, *CRYBB3*, *CRYGC* and *CRYGD*.

Results In total, 56 patients with congenital cataracts were included in the present study. Of these, 68% were male and 32% were female. The age range of the patients was 2 months to 5 years. The mean age of onset was 21.08 ± 15.15 months. All the patients had bilateral congenital cataracts. The female-to-male ratio was 1:2.1. Mutation analysis was performed to detect possible mutations in *CRYAA*, *CRYAB*, *CRYBB1*, *CRYBB2*, *CRYBB3*, *CRYGC* and *CRYGD*. Of the four mutations detected, one was novel (c.383A > T in *CRYGD*) and three were known (c.592C > T in *CRYBB2*, c.164A > G in *CRYGC* and c.592C > T in *CRYBB2*). Two of these three mutations were detected in the same gene (*CRYBB2*). Crystallin gene mutations were detected in 7% of patients with congenital cataracts (four out of 56 patients) in the present study.

Conclusions We think that mutations in crystallin genes are responsible for 7% of congenital cataract cases in our country. The detection of these mutations may help in the molecular diagnosis of congenital cataracts.

Keywords Congenital cataract · Crystalline gene · Genetic

Introduction

Congenital cataract is of the most common treatable causes of paediatric visual impairment. It is caused by metabolic disorders affecting embryonic lens transparency in the early foetal period. Moreover, it is characterised by an increase in the protein content, resulting in the loss of lens transparency or opacity due to the deterioration of the lens microarchitecture [1, 2]. The prevalence of congenital cataracts is 1–6/10.000 live births in developed countries and 5–15/10.000

M. Karahan · S. Erdem · S. Ava · U. Keklikçi
Department of Ophthalmology, Faculty of Medicine, Dicle University, Diyarbakır, Turkey

A. A. Demirtaş (✉)
Department of Ophthalmology, Izmir Tepecik Training and Research Hospital, Health Sciences University, Izmir, Turkey
e-mail: atilimdemirtas77@gmail.com

S. Tekeş
Department of Medical Genetics, Faculty of Medicine, Dicle University, Diyarbakır, Turkey

births in developing countries. It is one of the most common causes of vision loss in infants and children. In most patients, vision loss is caused by amblyopia; however, in some patients, it can be caused by postoperative complications, such as glaucoma and retinal detachment. Approximately half of the congenital cataracts are hereditary and can be a clinical feature of syndromic genetic diseases [3, 4].

Congenital cataracts may have autosomal dominant, autosomal recessive or X-linked recessive inheritance. Of these, autosomal dominant (44%) is the most frequent type, followed by autosomal recessive (18%), X-linked recessive (6%) and sporadic (32%). Thus, there is significant genetic heterogeneity in congenital cataracts. In recent years, great advances have been made in clarifying the molecular bases of congenital cataracts. Approximately 44 independent loci and 33 genes have been reported in relation to non-syndromic autosomal dominant congenital cataracts. These genes can be subdivided into those encoding intracellular lens proteins (crystallin), cytoskeleton proteins 1 and 2 (*BFSP 1* and *BFSP 2*, respectively), lens inner membrane proteins, heat shock factor proteins, transcription factors (*FOXE3*, *PAX6*, *PITX3* and *MAFA*) and the gap junction protein alpha 8 (*GJA8*) [2, 5]. Crystallins are dominant lens proteins that consist of alpha, beta and gamma crystals. Alpha-crystallin is a large multimeric protein consisting of two types of related subunits: alpha A and alpha B. Both these subunits are similar to other members of the small heat shock protein family and exhibit chaperone-like activity for preventing the clustering of other proteins. Alpha-crystallin is encoded by *CRYAA* on chromosome 21q22.3 and *CRYAB* on chromosome 11q22–q22.3. The beta-crystallin family consists of four acidic (A) forms encoded by *CRYBA2*, *CRYBA1* and *CRYBA4* and three basic (B) forms encoded by *CRYBB3*, *CRYBB2* and *CRYBB1*. Gamma-crystallin is encoded by the gamma gene cluster on chromosome 2q33–35, which includes gamma A–gamma D genes. Fewer mutations have been reported in *CRYGA* and *CRYAGB* than in *CRYGC* and *CRYGD* [6, 7].

In the present study, we aimed to systematically screen cataract-related crystallin genes in order to assess the relative contribution of mutations to the occurrence of congenital cataracts in Turkish children.

Materials and methods

The present study included 56 children (38 males and 18 females) who were diagnosed with congenital cataract in our ophthalmology clinic. The study was approved by the ethics committee (decision date: 5 December 2019, No. 281) and was conducted in accordance with the Declaration of Helsinki. Signed informed consent was obtained from the parents of all the participants.

The inclusion criteria were as follows: patients who were previously operated on for congenital cataracts (which refers to lens opacity detected at birth or at an early stage of childhood) or those who applied to be operated on for congenital cataracts [8]. Patients with drug or radiation exposure during pregnancy; those with cataracts in one eye; a history of ocular injury; congenital ocular anomaly; a mental disorder, and/or other ocular, metabolic or systemic diseases were excluded from the study. Moreover, patients with traumatic cataracts and/or a history of intrauterine infection were excluded from the study.

Blood sampling and DNA extraction

Fasting peripheral venous blood samples (5 mL) were collected from the patients within 24 h of admission. The blood samples were placed in tubes containing ethylenediaminetetraacetic acid (EDTA) and sent to the medical genetics laboratory. Mutations in *CRYAA*, *CRYAB*, *CRYBB1*, *CRYBB2*, *CRYBB3*, *CRYGC* and *CRYGD* and their associations were assessed and recorded using these samples.

Genetic analysis

The sequence analysis method was performed using primers covering all exons of *CRYAA*, *CRYAB*, *CRYBB1*, *CRYBB2*, *CRYBB3*, *CRYGC* and *CRYGD*. The results were analysed using the NextGen analysis programme. Variant classification was performed according to the criteria determined by the American College of Medical Genetics (Table 1) [9].

Statistical analysis

Statistical analysis was performed using SPSS 26.0 (Statistical Package for the Social Sciences, Chicago, Illinois, USA). Categorical variables are presented as

Table 1 Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [9]

Class	Change type	Possibility of pathogenicity (%)	Explanation
1	Pathogenic	> 99	Changes demonstrated by disease-causing efficacy data
2	Possible pathogenic	95–99	Changes with very strong data in favour of the presence of disease effect
3	Changes with unknown pathological effects	5–95	Limited and/or controversial data on the disease-causing effect
4	Possible benign	1–5	Changes in which there are very strong data in favour of having no disease effect
5	Benign	< 1	Changes demonstrated with sufficient data that have no disease effect

frequencies and percentages, while continuous variables are presented as means ± standard deviations.

Results

In total, 56 patients with congenital cataracts were included in the present study. Of these, 68% were male and 32% were female. The age range of the patients was 2 months to 5 years. The mean age of onset was 21.08 ± 15.15 months. All the patients had bilateral congenital cataracts. The female-to-male ratio was 1:2.1.

Mutation analysis was performed to detect possible mutations in *CRYAA*, *CRYAB*, *CRYBB1*, *CRYBB2*, *CRYBB3*, *CRYGC* and *CRYGD* in the patients. Of the four mutations detected, one was novel (c.383A > T in *CRYGD*) and three were known (c.592C > T in *CRYBB2*, c.164A > G in *CRYGC* and c.592C > T in *CRYBB2*). The c.592C > T mutation in *CRYBB2* was present in two unrelated families. The coexistence of gene mutations (in *CRYAA*, *CRYAB*, *CRYBB1*, *CRYBB2*, *CRYBB3*, *CRYGC* and *CRYGD*) was not detected in the patients with congenital cataracts. It was determined that the hereditary transition of mutations was autosomal dominant.

In *CRYBB2*, the mutation site was found to be exon 6 in two patients; this resulted in the following amino acid change: c.592C > T (p.Arg198Cys). The mutation type was found to be heterozygous, and it showed autosomal dominant inheritance. It was considered a class 3 variant according to variant classification.

In *CRYGC*, the mutation site was found to be exon 2 in one patient; this resulted in the following amino acid change: c.164A > G (p.Gln55Arg). The mutation type was found to be heterozygous, and it showed autosomal dominant inheritance. It was considered a class 3 variant according to variant classification.

In *CRYGD*, the mutation site was found to be exon 3 in one patient; this resulted in the following amino acid change: c.383A > T (p.Glu128Val). Although this change has not been previously reported in the literature, it may be reported to possibly have a damaging effect. The mutation type was found to be heterozygous, and it showed autosomal dominant inheritance. It was considered a class 3 variant according to variant classification.

The clinical data of the patients with congenital cataracts in whom mutations were detected are provided in Table 2.

Discussion

In the present study, the presence of mutations was assessed in crystallin genes in Turkish children with congenital cataracts. One of the four mutations detected was novel (c.383A > T in *CRYGD*), while the other three were known (c.592C > T in *CRYBB2*, c.164A > G in *CRYGC* and c.592C > T in *CRYBB2*). The mutation in exon 3 of *CRYGD* resulted in the following amino acid change: c.383A > T (p.Glu128-Val). This mutation was thought to be closely related to hereditary autosomal dominant cataracts. This

Table 2 Clinical data of children with congenital cataracts with mutations

Patients	Gene	Genomic coordinate	Amino acid change	Exon	Status of mutation	Database status	Inheritance	Variant
1	*CRYBB2*	c.592C > T (rs200845666)	p.Arg198Cys	Exon 6	Heterozygous	Reported	Autosomal dominant	Class 3
2	*CRYGC*	c.164A > G (rs144295934)	p.Gln55Arg	Exon 2	Heterozygous	Reported	Autosomal dominant	Class 3
3	*CRYGD*	c.383A > T	p.Glu128Val	Exon 3	Heterozygous	Novel	Autosomal dominant	Class 3
4	*CRYBB2*	c.592C > T (rs200845666)	p.Arg198Cys	Exon 6	Heterozygous	Reported	Autosomal dominant	Class 3

mutation has not been previously reported in the literature.

Mutations have been reported in more than 40 genetic loci associated with congenital cataracts. While crystallin genes account for half of these mutations, connexin genes account for a quarter of them and other genes account for the rest [2].

Mutations in *CRYAA* and *CRYAB* have been reported to play a critical role in congenital cataracts. Crystallin gene mutations account for most cases of hereditary congenital cataracts [10]. It is believed that crystallin gene mutations can affect the protein structure of the lens and the solubility of the proteins, causing lens opacity [11, 12].

The mutations detected in the present study (c.383A > T in *CRYGD*, c.592C > T in *CRYBB2*, c.164A > G in *CRYGC* and c.592C > T in *CRYBB2*) were thought to affect beta-crystallin and gamma-crystallin proteins in the lens and result in congenital cataracts. Crystallin proteins play an important role in maintaining lens transparency, and as seen in the present study, crystallin gene mutations can cause lens opacity.

Devi et al. [13] reported 16.6% prevalence of crystallin gene mutations by analysing 10 crystallin genes in Indian families, Burdon et al. [14] reported a 5.3% prevalence of crystallin gene mutations by analysing seven crystallin genes in Australian families and Hansen et al. [15] reported a 35.7% prevalence of crystallin gene mutations in Danish families. Moreover, Kumar et al. [16] reported a 20% prevalence of crystallin gene mutations by analysing four crystallin genes in Indian families, while Wang et al. [17] reported a 15% prevalence of crystallin gene mutations by analysing 10 crystallin genes in Chinese families.

Zhuang et al. [18] studied mutations in *CRYAA*, *CRYAB*, *CRYBA1*, *CRYBA4*, *CRYBB1*, *CRYBB2*, *CRYBB3*, *CRYGC*, *CRYGD* and *CRYGS* in 42 Chinese families with congenital cataracts. They noted 33% prevalence of crystallin gene mutations; four of these mutations were novel (35G > T in *CRYAA*, c.463C > A in *CRYBB2*, IVS1 c.10–1G > A in *CRYGC* and c.346delT in *CRYGD*).

Sun et al. [19] performed mutation analyses on 12 crystallin genes in 25 Chinese families with congenital cataracts and detected nine heterozygous mutations in six genes. Five of these mutations were novel (c.106G > C in *CRYGD*, c.205C > T in *CRYAB*, c.350_352delGCT in *CRYAA*, c.77A > G in *CRYGS* and c.1143_1165del23 in *GJA3*) and four were known (c.292G > A in *CRYAA*, c. 215 + 1G > A and c.272_274delGAG in *CRYBA1* and c.176C > T in *GJA3*).

Moreover, Su et al. [20] reported that the c.161G > C (p.R54P) mutation in *CRYAA* causes autosomal dominant Y-sutural cataracts. Autosomal recessive congenital nuclear cataracts have been reported to be caused by p.R11C and p.R12C mutations in *CRYAB*. The c.59C > G (P20R) mutation in *CRYAB* has been detected in a family of five generations with a history of hereditary posterior polar cataracts [21]. Another study has identified a novel *R11H* mutation in *CRYAB* in a family of four generations with congenital nuclear cataracts [22].

In the present study, crystallin gene mutations were detected in 7% of Turkish children with congenital cataracts (four out of 56 children) by analysing seven crystallin genes (*CRYAA*, *CRYAB*, *CRYBB1*, *CRYBB2*,

CRYBB3, *CRYGC* and *CRYGD*). One of these detected mutations was novel (c.383A > T in *CRYGD*), while the other three were known (c.592C > T in *CRYBB2*, c.164A > G in *CRYGC* and c.592C > T in *CRYBB2*). All these mutations showed autosomal dominant inheritance. To our knowledge, no study has been conducted on the relationship between congenital cataracts and crystallin gene mutations in our country.

Cui et al. [23] investigated the effects of mutations in rs7278468, rs3761382 and rs13053109 loci of *CRYAA* and rs370803064 and rs387907338 loci of *CRYAB* on paediatric congenital cataract risks. They found *CRYAA* rs7278468 and *CRYAB* rs370803064/rs387907338 to be associated with the risk and clinicopathological features of congenital cataracts in children.

In the present study, *CRYBB2* rs200845666, *CRYGC* rs144295934 and *CRYGD* (c.383A > T) mutations were found to be associated with an increased risk of congenital cataracts and with its clinicopathological features in Turkish children. The present study is the first on this topic in our country.

The limitation of the present study was that mutations in the regions specified in *CRYAA*, *CRYAB, CRYBB1*, *CRYBB2*, *CRYBB3*, *CRYGC* and *CRYGD* were scanned. This method does not rule out changes outside these regions or those caused by a different gene. Moreover, this method does not show rare duplications and deletions.

In summary, we detected one novel mutation and three known mutations in crystallin genes in Turkish children with congenital cataracts. Our findings indicate that crystallin gene mutations are responsible for 7% (4/56) of congenital cataract cases in our country. The detection of these mutations may help in the molecular diagnosis of congenital cataracts. More research is needed to better understand the relationship between congenital cataracts and crystallin genes.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by MK, AAD, SE, SA, ST and UK. Genetic analysis was performed by ST. The first draft of the manuscript was written by MK and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding The authors did not receive support from any organization for the submitted work.

Data availability The authors declare that materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for noncommercial purposes, without breaching participant confidentiality. Moreover, the authors ensure that their datasets are presented in the main manuscript.

Declarations

Conflicts of ınterest All the authors declare that they have no conflict of interest and no financial disclosure.

Consent to participate Informed consent was obtained from legal guardians.

Consent to publish Additional informed consent was obtained from all legal guardians for whom identifying information is included in this article.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the Medical University of Dicle University, Diyarbakır, Turkey (decision date: 5 December 2019, No. 281).

References

1. Yi J, Yun J, Li ZK et al (2011) Epidemiology and molecular genetics of congenital cataracts. Int J Ophthalmol 4:422–432
2. Hejtmancik JF (2008) Congenital cataracts and their molecular genetics. Semin Cell Dev Biol 19:134–149
3. Sheeladevi S, Lawrenson JG, Fielder AR et al (2016) Global prevalence of childhood cataract: a systematic review. Eye 30:1160–1169
4. Messina-Baas O, Cuevas-Covarrubias SA (2017) Inherited congenital cataract: a guide to suspect the genetic etiology in the cataract genesis. Mol Syndromol 8:58–78
5. Scott MH, Hejtmancik JF, Wozencraft LA et al (1994) Autosomal dominant congenital cataract: interocular phenotypic variability. Ophthalmology 101:866–871
6. Horwitz J, Bova MF, Ding LL et al (1999) Lens alpha-crystallin: function and structure. Eye (Lond) 13(Pt 3b):403–408
7. Sandilands A, Hutcheson AM, Long HA et al (2002) Altered aggregation properties of mutant gamma-crystallins cause inherited cataract. EMBO J 21(22):6005–6014
8. Uemura Y (1971) Diagnosis of congenital cataract. Ganka 13:613–620
9. Richards S, Aziz N, Bale S et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–424
10. Shiels A, Hejtmancik JF (2013) Genetics of human cataract. Clin Genet 84:120–127

11. Bharat SV, Shekhtman A, Pande J (2014) The cataract-associated V41M mutant of human γS-crystallin shows specific structural changes that directly enhance local surface hydrophobicity. Biochem Biophys Res Commun 443:110–114
12. Banerjee PR, Puttamadappa SS, Pande A et al (2011) Increased hydrophobicity and decreased backbone flexibility explain the lower solubility of a cataract-linked mutant of γD-crystallin. J Mol Biol 412:647–659
13. Devi RR, Yao W, Vijayalakshmi P et al (2008) Crystallin gene mutations in Indian families with inherited pediatric cataract. Mol Vis 14:1157–1170
14. Burdon KP, Wirth MG, Mackey DA et al (2004) Investigation of crystallin genes in familial cataract, and report of two disease associated mutations. Br J Ophthalmol 88:79–83
15. Hansen L, Mikkelsen A, Nurnberg P et al (2009) Comprehensive mutational screening in a cohort of Danish families with hereditary congenital cataract. Invest Ophthalmol Vis Sci 50:3291–3303
16. Kumar M, Agarwal T, Khokhar S et al (2011) Mutation screening and genotype phenotype correlation of alpha-crystallin, gamma-crystallin and GJA8 gene in congenital cataract. Mol Vis 17:693–707
17. Wang KJ, Wang BB, Zhang F et al (2011) Novel beta-crystallin gene mutations in Chinese families with nuclear cataracts. Arch Ophthalmol 129:337–343
18. Zhuang J, Cao Z, Zhu Y et al (2019) Mutation screening of crystallin genes in Chinese families with congenital cataracts. Mol Vis 25:427–437
19. Sun W, Xiao X, Li S et al (2011) Mutation analysis of 12 genes in Chinese families with congenital cataracts. Mol Vis 17:2197–2206
20. Su D, Guo Y, Li Q et al (2012) Concurrent chemoradiotherapy plus adjuvant chemotherapy versus concurrent chemoradiotherapy alone in patients with locoregionally advanced nasopharyngeal carcinoma: a phase 3 multicentre randomised controlled trial. Lancet Oncol 13:163–171
21. Jiaox X, Khan SY, Irum B et al (2015) Missense mutations in CRYAB are liable for recessive congenital cataracts. PLoS ONE 10:e0137973
22. Chen Q, Ma J, Yan M et al (2009) A novel mutation in CRYAB associated with autosomal dominant congenital nuclear cataract in a Chinese family. Mol Vis 15:1359–1365
23. Cui XJ, Lv FY, Li FH, 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