



Congenital Cataract and Its Genetics: The Era of Next-Generation Sequencing

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Abstract

Congenital cataract is a challenging ophthalmological disorder which can cause severe visual loss. It can be diagnosed at birth or during the first year of life. Early diagnosis and treatment are crucial for the visual prognosis. It can be associated with various ocular and systemic abnormalities. Determining whether congenital cataract is isolated or associated with other pathology is an indispensable step for the prediction of potential vision as well as early diagnosis and treatment of conditions that can cause morbidity or mortality. Many genes have been identified in the molecular etiology of congenital cataract. Most mutations have been reported in the crystallin genes. Determination of the genetic cause may not only enable individualized genetic counseling but also help to identify concomitant ocular and/or systemic disorders depending on the characteristics of the genetic test used. Recently, next-generation sequencing in particular has become an evolving technology for determining the molecular etiology of congenital cataract and furthering our knowledge of the disease.

Keywords: Genetics, congenital cataract, crystallin, lens, next-generation sequencing

Introduction

Congenital cataract is lens opacity that presents at birth or early in the postnatal period. It may be unilateral or bilateral. Because it occurs during early vision development, it causes serious vision loss and, more importantly, severe amblyopia. Follow-up and treatment are long-term and important, and an etiology cannot be identified for a substantial proportion of patients.¹ For this reason, one of the main objectives of the Vision 2020: Right to Sight, a global initiative to eliminate preventable blindness worldwide, was to prevent causes of childhood blindness, including congenital cataract.²

Epidemiology and Etiology

In their systematic review and meta-analysis, Wu et al.³ reported that congenital cataract had the highest incidence in Asia (7.43/10,000) and was usually diagnosed after 1 year of

age. They also reported that congenital cataracts were more frequently bilateral and the most common type was total cataract.³ Although most cases of congenital cataract were idiopathic (62.2%), the prevalence of inherited cataract was reported to be 22.3%.³

In their systematic review, Sheeladevi et al.⁴ determined overall prevalence rates of 0.32-22.9 in 10,000 for childhood cataracts and 0.63-9.74 in 10,000 for congenital cataracts.

Factors such as the dynamic genetic infrastructure of societies, socioeconomic and cultural characteristics, access to health services, and the presence of early screening programs may cause major differences in the prevalence of congenital cataracts as well as associated morbidities between populations. This is an important consideration when evaluating statistics.

In congenital cataract, anterior segment structures other than the lens were also shown to differ from noncataractous eyes due to their simultaneous development and mutual interaction

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Received: 13.05.2020 **Accepted:** 27.08.2020

Cite this article as: Taylan Şekeroğlu H, Utine GE. Congenital Cataract and Its Genetics: The Era of Next-Generation Sequencing. Turk J Ophthalmol 2021;51:107-113

during the embryological period.⁵ Congenital cataract may be associated with ocular anomalies such as microcornea, microphthalmia, persistent fetal vascularization, glaucoma, and retinal dystrophies. Twenty-nine percent of congenital cataract cases may be linked to genetic causes, while unilateral cases are more likely to be idiopathic.⁶

In the Vision 2020 global initiative to fight preventable blindness, vitamin A deficiency, measles, neonatal conjunctivitis, and retinopathy of prematurity were also shown to be among the causes of childhood blindness along with congenital cataract.²

Congenital cataract accounts for 7.4-15.5% of all childhood blindness.⁷ Early diagnosis and treatment are very important in terms of visual prognosis. Therefore, one of the most critical steps is recognizing congenital cataract at an early age through postnatal eye screening. The red reflex test is a simple screening test that is important in the detection of many ocular pathologies, especially congenital cataract. Neonatal eye screening has been implemented as routine practice in our country, and the detection of pathologies that disrupt the red reflex test in these examinations and their referral to ophthalmologists enables the recognition of many eye diseases that require early diagnosis and treatment, including congenital cataracts. Despite early surgery and early rehabilitation, visual outcomes of congenital cataract may be limited due to ocular diseases such as glaucoma, nystagmus, or concomitant systemic/neurological anomalies.⁸

Pediatric cataracts can be classified into two main groups, hereditary and nonhereditary.

1. Hereditary pediatric cataracts may be:⁹

- a) Isolated
- b) Associated with metabolic diseases (e.g., galactosemia, Wilson's disease, diabetes, cerebrotendinous xanthomatosis, Fabry disease, mannosidosis, Refsum disease)
- c) Associated with renal diseases (e.g., Alport syndrome, Lowe syndrome)
- d) Associated with musculoskeletal diseases (e.g., myotonic dystrophy, chondrodysplasia puncta)
- e) Associated with dermatological diseases (e.g., incontinentia pigmenti, Cockayne syndrome, Rothmund-Thomson syndrome)
- f) Associated with craniofacial anomalies (e.g., Hallerman-Streiff syndrome, Rubinstein-Taybi syndrome, Smith-Lemli-Optitz syndrome, cerebro-oculo-facial-skeletal syndrome)
- g) Associated with genetic anomalies (e.g., trisomy 13, 18, 21; 5p deletion, 11p deletion, Norrie disease, Nance-Horan syndrome)

2. Non-hereditary pediatric cataracts:

May occur due to trauma, congenital infections such as TORCH (toxoplasma, rubella, cytomegalovirus, herpes simplex), drugs such as steroids, radiation, or teratogen exposure.

Classification:

Although congenital cataract can be classified according to the timing of development, etiology, location of opacity, or morphological features, morphological classification is most commonly used in clinical practice:⁹

1) Anterior cataract: This group includes anterior polar, anterior pyramidal, and anterior subcapsular cataracts.

2) Central cataract: This group includes nuclear, sutural, lamellar (zonular), cerulean, Christmas tree, pulverulent, aculeiform, polymorphic, crown-shaped, cuneiform, and coralliform cataracts.

3) Posterior cataract: This group includes posterior lenticonus, posterior subcapsular, posterior polar, and oil droplet cataracts, persistent hyperplastic primary vitreous, and Mittendorf dots.

4) Total cataract: This type of cataract involves the entire lens. It is often not possible to identify the morphology at the onset in the absence of additional ocular findings. Congenital Morgagnian and membranous cataract can also be considered in this group.

Perucho-Martinez et al.¹⁰ reported that nuclear cataract was the most common congenital cataract morphology in their study.

The Genetics of Congenital Cataract

Determining the molecular etiology of congenital cataract is essential both to identify and better understand the pathways involved in its pathogenesis and to provide individualized genetic counseling. It has been shown that 47% of unilateral congenital cataracts and 61% of bilateral congenital cataracts are isolated, and the frequency of association with systemic diseases is 6% in unilateral and 25% in bilateral cases.¹¹ In addition, approximately half of congenital cataracts have a genetic etiology.¹² Congenital cataract is characterized by genetic heterogeneity and variable inheritance patterns.¹³ Although the inheritance of congenital cataract is usually autosomal dominant, in rare cases it may be autosomal recessive or X-linked. The etiology of isolated congenital cataract is unknown in 50% of cases, but up to 30% are monogenic and generally have autosomal dominant inheritance.¹⁴

Determining the genetic etiology of congenital cataract in a family member enables a molecular diagnosis to be established and opens the possibility of prenatal diagnosis in pregnancies to be planned in the same or following generations. Non-invasive prenatal testing now makes it possible to collect blood from the mother and diagnose fetal chromosomal aneuploidy through extracellular fetal DNA circulating in the peripheral blood.¹⁵ In addition, knowing the molecular etiology of a hereditary ocular disease in the family may also allow preimplantation genetic diagnosis.¹⁶

Mutations Associated with Congenital Cataract

Some genes that have been associated with congenital cataracts and the mutations demonstrated in these genes are shown in Table 1. Mutations that cause congenital cataracts are categorized into four basic groups:

1) Crystallin mutations

Crystallins comprise over 90% of the lens proteins and have the most fundamental place in the lens structure.¹⁷ Crystallins can be divided into the α , β , and γ groups, although β and γ crystallins can also be considered a single family. α , β , and γ crystallins are water-soluble proteins that

account for the majority of lens proteins. They are found not only in the eye but also in extraocular tissues. Crystallins are essential for maintaining lens stability and transparency due to their antiapoptotic, antidegradation, and antioxidant effects.¹⁸ Crystallin mutations have been associated with congenital cataracts of varying phenotype.¹²

Mutations in crystallin genes account for approximately 50% of autosomal dominant cataracts.¹⁹ Numerous mutations have been detected in various crystallin genes, including *CRYAA*, *CRYAB*, *CRYBB1*, *CRYBB2*, *CRYBB3*, *CRYBA1*, *CRYGC*, *CRYGD*, and *CRYGS*.^{13,20,21}

2) Lens membrane protein mutations

This group includes connexins, aquaporins, and other cell membrane proteins that enable intercellular communication. Congenital cataracts have also been reported in mutations affecting major intrinsic protein, connexin 46 and 50, and LIM-2 proteins.¹² It is known that 25% of mutations associated with congenital cataract are in connexin genes.²²

3) Mutations of lens cytoskeletal elements

CP49 and filensin, which form beaded filaments, are the cytoskeletal elements. Mutations in the *BFSP-2* (beaded filament structural protein 2) gene encoding CP49 have been associated with cataract.¹²

4) Other mutations

Congenital cataract can also be observed in mutations of the developmental regulatory genes *PITX3* (paired-like homeodomain 3), *PAX6* (paired box 6), and *HSF-4* (heat shock protein factor-4).¹² Narumi et al.²³ described congenital cataract with microcornea and/or iris coloboma in some members of a Japanese family who had a c.908A>C mutation in the *MAF* (*MAF* bZIP transcription factor) gene.

Congenital cataract can also occur due to physical and environmental factors such as infections and teratogens. Many factors, including socio-cultural-economic background, race-specific genetic traits, the frequency of consanguineous marriage, and differences in vaccination and screening programs, result in population-specific patterns of congenital cataract prevalence and molecular etiology.

Hansen et al.¹⁴ identified mutations in 20 of 28 Danish families with hereditary congenital cataract. They determined that 36% of these mutations were in crystallin genes, 22% in connexin genes, and 15% in the transcription factor genes *HSF4* and *MAF*.

Devi et al.²⁴ showed that crystallin gene mutations (*CRYAA*, *CRYAB*, *CRYBA1*, *CRYBB2*, *CRYGC*, *CRYGD*, *CRYGS*) were responsible for 16.6% of cases of hereditary pediatric cataract cases in 60 Indian families.

Chen et al.²⁵ conducted molecular genetic analysis in a homozygosity mapping study of Pakistani families with autosomal recessive congenital cataract and found that mutations were most commonly in the *FYCO1* (*FYVE* and coiled-coil domain autophagy adaptor 1) gene, followed by the *CRYBB3*, *GALK1* (*galactokinase 1*), and *EPHA2* (*EPH* receptor A2) genes.

Li et al.²⁶ investigated the molecular etiology in 74 patients with sporadic congenital cataracts in a Han Chinese population and reported the most common mutations in the *CRYBB3* gene, followed by the *EPHA2*, *NHS* (*NHS actin remodeling regulator*), and *WDR36* (*WD repeat domain 36*) genes.

Investigating the Molecular Etiology of Congenital Cataract

For a patient with bilateral congenital cataract, family history and pedigree tracing are followed by TORCH screening for intrauterine infections, as well as analysis of urine and blood amino acid analysis and reducing substances in the urine. Apart from these, specific genetic tests can be performed if a particular genetic etiology is suspected, and special organic acid analyses can also be performed if a metabolic disease other than galactosemia is suspected. For example, cerebrotendinous xanthomatosis is the result of a *CYP27A1* (cytochrome P450 family 27 subfamily A member 1) gene mutation that causes a cholesterol metabolism disorder. It causes juvenile cataract, and xanthomas and cognitive/neurological disorders later in life. If diagnosed early, initiating oral chenodeoxycholic acid therapy can prevent later symptoms of the disease. Another example is galactosemia, which is also seen in our country. With early diagnosis and a special diet, it may be possible to slow the progression of the cataract to a certain degree.

Various techniques can be used for the evaluation of a patient with congenital cataract:²⁷

1) Conventional cytogenetic methods, especially standard karyotyping, may be preferred in the presence of developmental delay/mental disability, other malformations, growth retardation, and dysmorphic findings, which suggest that the cataract may be a component of a genetic etiology due to structural or numerical abnormalities at the chromosome level (large deletion, duplication, translocation).

2) Molecular cytogenetic methods such as fluorescence in situ hybridization is applicable if the cataract is believed to show a specific phenotypic pattern associated with a genetic etiology involving submicroscopic deletion/duplication.

3) Methods such as multiplex ligation-dependent probe amplification can be used if the cataract is suspected to occur as a result of a genetic alteration associated with a copy number change in a more specific and smaller region.

4) Methods such as array comparative genomic hybridization (array CGH) are preferable if the cataract is believed to be a component of genetic etiologies associated with copy number changes but are not clinically identifiable (e.g., mental disability spectrum).

5) Whole exome sequencing (WES) or whole genome sequencing (WGS) with confirmation by Sanger sequencing is an option if the etiology of the cataract is genetically heterogeneous and specifically associated with indistinguishable clinical presentations.

Table 1. Certain genes associated with congenital cataracts and some mutations/nucleotide changes demonstrated in these genes*

Gene name	Locus	Inheritance	Encoded protein	Nucleotide change
<i>CRYAA</i>	21q22.3	AD/AR	Crystallin, alpha-A	c.61C>T ³⁷ c.34C>T ^{14, 38} c.155C>T ¹⁴ c.337G>A ¹⁴
<i>CRYBA1/A3</i>	17q11.2	AD	Crystallin, beta-A1/A3	c.279-281delGGA ³⁹ c.272-274delGAG ⁴⁰ c.590-591delAG ⁴¹ IVS3+1 G>A ⁴² c.215+1G>A ⁴³ IVS3+2 T>G ⁴⁴
<i>CRYBA2</i>	2q35	AD	Crystallin, beta-A2	c.148G>A ³⁸
<i>CRYBB1</i>	22q12.1	AD/AR	Crystallin, beta-B1	c.286G>T ³⁸
<i>CRYBB2</i>	22q11.23	AD	Crystallin, beta-B2	c.563G>A ¹⁷ c.498C>A ¹⁴ c.[433C>T;440A>G;449C>T] ¹⁴
<i>CRYBB3</i>	22q11.23	AD/AR	Crystallin, beta-B3	c.581T>A ³⁸ c.493G>C ⁴⁵ c.224G>A ¹⁶
<i>CRYGC</i>	2q33.3	AD	Crystallin, gamma-C	c.124delT ³⁷ c.157_161 dupGC GGC ³⁸ c.417C>G ³⁸ c.143G>A ⁴⁶
<i>CRYGD</i>	2q33.3	AD	Crystallin, gamma-D	c.418C>T ³⁸ c.70C>A ⁴⁷ c.418C>A ¹⁴
<i>GJA3</i>	13q12.11	AD	Gap junction protein alpha 3	c.32T>C ¹⁴ c.176C>T ¹⁴ c.227G>A ¹⁴
<i>GJA8</i>	1q21.2	AD	Gap junction protein alpha 8	c.200A>G ³⁸ c.226C>T ³⁸ c.218C>T ¹⁴ c.565C>T ¹⁴ c.836C>A ¹⁴
<i>HSF4</i>	16q22.1	AD	Heat shock transcription factor 4	c.341T>C ¹⁴ c.355C>T ¹⁴
<i>MIP</i>	12q13.3	AD	Major intrinsic protein of lens fiber	c.605G>A ³⁸
<i>EYA1</i>	8q13.3	AD	EYA transcriptional coactivator and phosphatase 1	c.121G>A ³⁸
<i>MAF</i>	16q23.2	AD	MAF bZIP transcription factor	c.895C>A ¹⁴ c.958A>G ¹⁴

AD: Autosomal dominant, AR: Autosomal recessive

*Information about genes/variants involved in the molecular etiology of congenital cataract is constantly being updated. The table presents a portion of the available information to the reader. However, current platforms such as those mentioned in the text should be monitored for emerging data.

6) Sanger sequencing of a particular gene may be preferred if there is a strong and specific suspicion that the cataract is of genetic etiology and the suspect gene is known.

With all of these options, the key step is a thorough description of the phenotype, detailed evaluation of associated systemic anomalies/diseases, and identification of a preliminary clinical diagnosis.

Next-Generation Sequencing to Determine the Molecular Etiology of Congenital Cataract

Over the years, there has been a shift from genetic tests to genomic tests for many diseases with complex inheritance and genotypes, especially rare pediatric diseases. Genome-wide tests include aCGH, gene panels, and next-generation sequencing technologies. Traditional genetic tests include high-resolution single-gene tests (e.g., Sanger sequencing) that can identify diseases with a very specific phenotype which are caused by

mutations in one or a few genes, and genome-based low-resolution cytogenetic analyses.²⁸ Modern genetic tests, on the other hand, involve next-generation sequencing technologies that enable rapid and simultaneous sequencing of a large number of genes.²⁸

Encoding regions of gene are called exons and noncoding regions are called introns. All of the exons in the human genome are referred to as the exome. Although the exome represents approximately 2% of the human genome, it contains 85% of variants known to cause disease.²⁹ Next-generation sequencing technologies are called whole exome and whole genome sequencing, although these techniques can also be targeted to a specific region of the exome or genome instead of the whole.

Whole exome sequencing is especially important in identifying mutations in Mendelian diseases with genetic heterogeneity. The main speed-limiting step in these technologies is the evaluation, interpretation, and validation of the data, which is difficult to review due to its scale. When the first genome/exome information is obtained from the patient, it is compared with the reference genome/exome to detect deviations/variants; in other words, variants are called. The next step is variant filtering by evaluating the variants' frequency in the population and their likely relationship and effect on phenotype and inheritance. After this process, some variants are prioritized.³⁰ The clinical presentation and variant are evaluated together and deep phenotyping is performed if necessary; i.e., additional clinical/laboratory/imaging examinations are requested.³⁰ This is followed by Sanger sequencing to validate the likely causative variant and segregation analysis based on demonstrating its presence in other affected family members.

Variants are classified as pathogenic, likely pathogenic, of unknown significance, likely benign, and benign according to the data in different platforms (e.g., Varsome, Genome Data Viewer, Ensemble, The 1000 Genomes Browsers, Variation Viewer, gnomAD).^{31,32}

Next-generation sequencing is advantageous over other technologies in that it does not require the time-consuming and error-prone steps of older systems, DNA fragments are reproduced with special systems, and millions of sequences can simultaneously be read base by base (massive parallel sequencing) by various methods.³³ As a result, technology has gained speed, increased the reading length, and significantly reduced the frequency of errors over time.

With the technological capacity to screen the entire genome, incidental findings and/or variants of unknown significance can also be detected. These analyses produce extraordinary amounts of data, but major ethical and social issues may arise in reporting the results, especially in clinical conditions related to children.³⁴ In this case, providing genetic counseling can also become more complicated.

Although next-generation sequencing technology, WES or WGS, enables evaluation by comparing with the reference genome/exome, the large data burden poses a substantial challenge in the interpretation phase, especially with WGS.

Deviations from the reference genome/exome do not always mean disease; they may need to be classified as normal variants or variants of unknown significance. In addition, it should be kept in mind that the continuing development and widespread use of this technology will increase global knowledge and experience, and as more data is obtained using this technology, earlier data will be updated and new information may emerge that results in laboratory results changing in significance and classification over time. A variant classified as unknown may later be included in the pathogenic or benign group, or a variant classified as benign may be moved to the pathogenic group. Therefore, considering that new generation technologies are a living system that are constantly evolving, it is extremely important before the test to inform the family in detail and clarify how the results could change the life of the individual and his/her family now and in the future.

A more practical implementation of next-generation sequencing technology in clinical use, which involved contacting the genetics department and informing the patient and family shortly after the patient was seen in the ophthalmology clinic, sample collection and rapid transfer to the laboratory for next-generation sequencing, was reported to increase the rate of children with congenital cataracts who received a diagnosis within 6 months from 26% to 71%.³⁵ The reduction in turnaround time was achieved by accelerating the steps that delay the workflow between clinic and laboratory and facilitating collaboration between clinicians and geneticists.³⁵

In a research project-based study, it was reported that 70% of patients with congenital cataract could be diagnosed with next-generation sequencing technology.³⁶ This high rate may not always be possible in clinical practice, but appropriate selection of patients for genetic testing and the test to perform will increase the diagnosis rate. In addition, in cases where WES is insufficient, the diagnosis rate may be increased by the use of WGS methods, which are just now becoming widespread and still have some limitations in terms of data burden and interpretation.

Although some genes/mutations have been reported in conjunction with certain types of congenital cataracts, there is not yet a direct relationship with which to establish a valid and common genotype-phenotype correlation. One of the main reasons for this is that people diagnosed with congenital cataracts often present for investigation of the molecular etiology after surgery, and the cataract morphology cannot be determined because they are pseudophakic.

In conclusion, congenital cataract is rare but causes severe morbidity, and its diagnosis and treatment are a race against time. Molecular diagnosis will provide a better understanding of the pathogenesis of the disease and enable more detailed and individualized genetic counseling, including prenatal diagnosis. Next-generation sequencing technologies are a useful and reliable method for detecting and evaluating the underlying molecular etiology of this heterogeneous genetic disease, and seem likely to continue to provide more data in the future.

Ethics

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: H.T.Ş., G.E.U., Design: H.T.Ş., G.E.U., Data Collection or Processing: H.T.Ş., Analysis or Interpretation: H.T.Ş., G.E.U., Literature Search: H.T.Ş., Writing: H.T.Ş., G.E.U.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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