The mutation spectrum in familial versus sporadic congenital cataract based on next-generation sequencing

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BMC Ophthalmology  BMC Series

Fan Fan
Eye and ENT Hospital of Fudan University

Yi Luo
Fudan University Eye Ear Nose and Throat Hospital Department of Ophthalmology

eyelu1106@163.com Corresponding Author
ORCiD: https://orcid.org/0000-0001-5971-1200

Jihong Wu
Eye and ENT Hospital of Fudan University

Chao Gao
Eye and ENT Hospital of Fudan University

Xin Liu
Eye and ENT Hospital of Fudan University

Hengjun Mei
Eye and ENT Hospital of Fudan University

Xiyue Zhou
Eye and ENT Hospital of Fudan University

DOI: 10.21203/rs.3.rs-16030/v1

SUBJECT AREAS
Ophthalmology

KEYWORDS
congenital cataract, sporadic, familial, NGS, mutation spectrum
Abstract
Purpose Congenital cataract (CC) is a significant cause of lifelong visual loss. Its genetic diagnosis is challenging due to marked genetic heterogeneity. The purpose of this article is to report the genetic findings in sporadic and familial CC patients.

Methods Patients (n=54) who were clinically diagnosed with CC and their parents were recruited. Blood samples were collected in our hospital. Mutations were detected by high-throughput, next-generation DNA sequencing (NGS) targeting 792 genes frequently involved in common inherited eye diseases.

Results We identified variants in 11/38 cases (28.95%) of sporadic CC and 14/16 cases (87.5%) of familial CC, indicating a significant difference (P=0.000). Of the 14 variants identified in sporadic cases, 9 were previously reported mutations, and 5 were novel mutations, including 2 de novo mutations (CRYBB2 c.487C>T, FYCO1c.215A>T). The most frequent variants in our cohort were in crystallins and cytoskeletal genes (7/30, 23.33%), followed by X-linked syndromic proteins (13.33%) and transcriptional factors (10.00%). Additional information on the possibility of complications with inherited ocular or systemic diseases other than CC was provided in 20/30 (66.67%) variants.

Conclusions These results contribute to expanding the mutation spectrum and frequency. Targeted NGS in CC provided significant diagnostic information and enabled more accurate genetic counseling. This study reports the different distributions of mutation genes in familial and sporadic CC cases.

Background
Congenital cataracts (CCs) are now the most common avoidable cause of childhood blindness worldwide, accounting for 10-35% of such cases with an estimated incidence of 0.63-9.14/10,000 births.(1-4) Management is often difficult due to the risk of amblyopia in the developing visual system and complications of glaucoma, posterior synechia or visual axis opacification, which require additional surgery.(5) CCs occur due to the disruption of the lens microarchitecture or the protein function in the lens.(6) Except for a very few infectious cases, only one-third of CC cases have a
positive family history,(7) with the other two-thirds having an unknown etiology.(8) Therefore, a significant proportion are sporadic cases in which it is not known whether there is an underlying genetic cause for the lens abnormality.

So far, approximately 350 genes have been reported to be associated with CC (Cat-Map; http://cat-map.wustl.edu/); these include mutations in crystallins and gap junction, membrane transport and channel, and cytoskeletal proteins and growth and transcription factors.(9) Locating and identifying the involves genes and mutations is essential to gaining an understanding of the molecular defects and pathophysiologic characteristics underlying inherited CC.

A conventional approach to identifying mutations in CC is usually performed by Sanger sequencing only in familial cases and is time-consuming and costly, with a detection rate of 30%-50% in apparent autosomal dominant cases.(10, 11) Due to marked genetic and phenotypic heterogeneity, determining the precise genetic cause of CC and establishing a robust genotype-phenotype correlation is challenging. Next-generation DNA sequencing (NGS) is increasingly powerful as a diagnostic tool that offers speed, precision, and cost-effectiveness for heterogeneous conditions.(12) This has been demonstrated in studies to determine the cause of other heterogeneous inherited eye diseases, such as congenital macular dystrophy, retinal pigmentosa, etc.(13-16) Recent studies have also shown that NGS allows the efficient identification of genetic causes of CC in the majority of cases, thereby improving its diagnosis and clarifying inheritance patterns(17-19) while guiding genetic counseling and increasing prognostic accuracy.

In this study, we applied targeted NGS in 792 genes involved in common inherited eye diseases to detect causal mutations in a relatively large series of CCs, including a high proportion of sporadic cases, and report the different distributions of mutated genes in sporadic versus familial CC cases (sCC VS fCC), while broadening the mutation spectrum and frequency of genes responsible for CC.

Methods

Ethical Statement

All participants (parents on behalf of their children) provided written informed consent forms for both genetic counseling and molecular genetic testing prior to enrolment. The study was approved by the
Ethics Committee of the Eye and ENT Hospital of Fudan University. All research was conducted in accordance with the Declaration of Helsinki.

Clinical Evaluations and Sample Collection

Patients who were clinically diagnosed with CC from June 2018 to May 2019 were recruited. All patients underwent a detailed ophthalmic examination, including slit-lamp examination, B ultrasound, intraocular pressure measurement, and ultrasonic A-scan, as mentioned in our previous study.(20) Visual acuity (VA) was recorded in all patients who were able to cooperate. Patients diagnosed with monocular CC additionally underwent post-eyeball color Doppler ultrasound to help in the differential diagnosis of persistent hyperplastic primary vitreous (PHPV). Children younger than 3 years old were examined under sedation with chloral hydrate. The lens phenotypes of patients and their parents were carefully recorded in all families and included childbirth history, medical history, family history and a detailed history of the gestation period, including high fever, rubella virus [RV] TORCHES ([Toxoplasma gondii; T. gondii], cytomegalovirus [CMV], herpes simplex virus [HSV], syphilis [caused by Treponema pallidum]), tuberculosis infection, exposure to radiation, and drug intake. Additional systemic problems were also recorded in patients and included serum biochemical tests for levels of blood glucose, calcium and phosphorous as well as urine tests. Blood samples were collected in children while under general anesthesia during eye surgery and from their biological parents (Trio sequencing) or other family members in our hospital.

Next-Generation Sequencing

Genomic DNA was extracted from peripheral blood samples using standard methods. The proband was analyzed first; we designed a high-throughput microarray with exon capture and NGS targeting the 792 genes most frequently involved in common inherited eye diseases (the capture probes were custom designed and produced by BGI, Shenzhen, Guangdong, China). Then, library preparation, qualification, and NGS of the targeted sequences were further conducted on an Illumina Hiseq 2000 platform (Illumina, Inc., San Diego, CA, United States) in collaboration with BGI-Shenzhen (Shenzhen, Guangdong, China) as previously described. The following databases were then used to annotate all identified variants with a minor allele frequency (MAF) >0.1% to eliminate benign variants:
dbSNP1371 (http://hgdownload.cse.ucsc.edu/goldenPath/hg19/database/snp137.txt.gz), HapMap Project (ftp://ftp.ncbi.nlm.nih.gov/hapmap), 1000 Genomes Project (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp), YH database (http://yh.genomics.org.cn/), and Exome Variant Server (http://evs.gs.washington.edu/EVS/). Finally, variant prioritization was performed to combine the total depth, quality score, MAF, potential deleterious effect and existence of mutation reports in common databases such as the Human Gene Mutation Database (HGMD), ClinVar or Online Mendelian Inheritance in Man (OMIM).

Results

Participant characteristics

A total of 54 patients with CC and their parents were recruited. These included 38 sporadic and 16 familial cases. The mean ages of the 54 children and their mothers and fathers were 3.0 [2.00-6.00], 30.48±4.95, and 32.62±5.14 years old, respectively. There were more binocular cases than monocular cases and more male than female cases. More detailed information is presented in Table 1. No significant differences were found between sCC and fCC in the mean ages of children and the parents or other constituent ratios (P values are presented in Table 1).

Variants identified

A total of 30 variants were found in 25 of the 54 patients with CC in our cohort, yielding a total detection rate of 46.30%. We identified variants in 11/38 (28.95%) sCC and 14/16 (87.5%) fCC cases, indicating a significant difference (P=0.000, Table 1). The detection rate was lower in monocular cases (4/12, 33.33%) than in binocular cases (21/42, 50.0%), but the differences was not significant (P=0.307). The variants detected are presented in Table 2 and Table 3.

We identified 5 novel mutations in sCC in CRYBB2, NHS (*2), FYCO1, and TSPAN12, 2 of which were de novo mutations in CRYBB2 c.487C>T (p.Gln163*|p. Q163*) and FYCO1 c.215A>T (p.Asp72Val|p.D72V). Eight of the 30 variants detected in our cohort were previously reported pathogenic gene mutations in CC, including loci in CRYGC, CRYGD (*2), CRYAA, CRYBA1, and GJA8 and adjacent loci in CRYGC and PAX6. Another 17 variants involved in additional ocular or systemic diseases that had been reported or included by HGMD or ClinVar were also identified. The remaining 3 genes, CYP27A1,
**TSC1** and **MAPT** (microtubule-associated protein tau), were mainly related to brain neurological conditions, including cerebrotendinous xanthomatosis, tuberous sclerosis complex and frontotemporal dementia, respectively. In addition, we also identified a monoallelic mutation in **BMP4**, which has been associated with isolated hypospadias, a disorder of sexual development. (40)

In terms of gene function, genes encoding crystallins and cytoskeleton were the most frequently identified in our cohort, accounting for 7/30 (23.33%) of the cases, and was followed by X-linked syndromic proteins (13.33%) and transcriptional factors (10.00%).

Differential distribution of mutational genes

A comparison of the distributions of mutational genes between fCC and sCC showed that variants in crystallins accounted for the highest proportion (37.50%) in fCC cases but only 7.14% of sCC cases (Figure 1). The sporadic cases mainly consisted of structural protein genes, including transmembrane, collagen, or microtube-associated proteins.

**Prognosis and Genotype**

We recorded VA before surgery and 3 months postoperatively in patients whose age was older than 3 years old as a main indicator of prognosis. The phenotypes, genotypes and prognoses are listed in Table 4. Of the 9 patients who could cooperate with the VA examination, 4 had mutations in **CYP27A1**, **WFS1**, **OPA3**, and **CYP1B1** as well as **FBN1** and showed significant postoperative improvement in VA, while the other 5 cases had poor prognosis.

**Discussion**

Approximately 70% of CC cases may occur alone, and 15% of such cases may be accompanied by other ocular abnormalities, such as microphthalmia, aniridia, or retinal degeneration. In another 15% of cases, cataracts are one part of a multisystem genetic disorder. (47) To obtain clues related to the noncataractous phenotype or provide a reference for the prognosis of CC surgery, we designed a high-throughput microarray with exon-capture and NGS targeting of the 792 genes most frequently involved in common inherited eye diseases. Compared to related previous studies, our study included the largest numbers of patients and targeted genes. We achieved detection rates in familial and sporadic cases similar to those in a recent study. (37) Although the overall detection rate (46.3%) in
our cohort was apparently lower than that in the other studies listed in Table 5, these rates are not comparable due to differences in the proportions of participants. Most of the studies (17-19) included only binocular cataracts, whereas we enrolled many monocular cases. Regarding the distribution of genes, our result was slightly different from those reported previously. Li et al (37) reported that variants in the crystallin genes were the most frequent mutations found in their study, whether in familial or sporadic cases. We found that variants in crystallins accounted for a similar proportion of fCC cases but only 1 sCC case (Figure 1). Structural protein genes, such as transmembrane, collagen, or microtubule-associated proteins, accounted for most of the sCCs in our study.

In our study, approximately 20/30 (66.67%) variants provided clues regarding the possibility of complication with inherited ocular or systemic diseases other than CC. Among these, 7 identified loci provided additional ophthalmological diagnostic information. For instance, OPA3 mutations are associated with optic atrophy, (22) BEST1 mutations with best vitelliform macular dystrophy (BEST), (25-28) TSPAN12 mutations with familial exudative vitreoretinopathy (FEVR) (33), PAX6 mutations with aniridia and Peter’s anomaly, (48) and CYP1B1 mutations with glaucoma. (45) Six variants were associated with systemic syndrome. WFS1 is the most common causative gene in Wolfram-like syndrome, a rare autosomal dominant disease characterized by congenital progressive hearing loss, diabetes mellitus, and optic atrophy. (49) COL4A5 is one of causing genes in Alport Syndrome, a genetic condition characterized by progressive loss of kidney function, hearing, and eye abnormalities, including misshapen lenses and abnormal retina. (32) JAG1 has been associated with Alagille syndrome, which involves liver damage or a combination of heart defects. Loss-of-function mutations in the BCOR gene have been identified in individuals with oculo-facio-cardio-dental syndrome (OFCD), which comprises microcornea, CC, and facial, cardiac, and dental abnormalities. (38) Mutations in the FBN1 (fibrillin-1) gene may be diagnostic of Marfan syndrome. (46) NHS mutations have been identified in patients with Nance-Horan syndrome (NHS), an X-linked developmental disorder characterized by CC, dental anomalies, facial dysmorphism and, in some cases, mental retardation. (50) Clinically, a new diagnosis was made after surgery and with reference to genetic testing in at least two patients in our cohort. One of the sporadic cases (ID 11 in Table 2)
presented some retinal abnormalities during operations after the removal of cataracts in both eyes, including settled subretinal exudates and dragging of the optic disc. Combined with this clinical manifestation, we have clarified the diagnosis of FEVR with regard for the TSPAN12 mutation, which is a pathogenic gene known to indicate FEVR. We also observed dental, facial and mental anomalies and made a new diagnosis of NHS at 2 years after the first CC operation was performed in one of the sporadic cases with an identified NHS mutation. However, whether other variants are associated with a noncataractous phenotype is difficult to confirm. The relationships between complicated phenotypes and mutations in ocular genes are not explicit. Thus, more cases should be included, and more experiments should be performed to verify these connections.

This study emphasizes the power of and the need for comprehensive parent-child NGS analyses of CC families. Such analyses have the potential to reveal a striking new landscape of inheritance in CC by identifying pathogenic heterozygous and homozygous mutations, de novo mutations, and parental mosaicism, with important implications beyond only the affected child. However, trio sequencing can reveal numerous variants of unknown significance for which thorough functional validation is mandatory, although this remains challenging. Furthermore, based on a parent-child approach, future research is required to determine the clinical implications of non-Mendelian inheritance, the complex interactions between genetic predispositions and environmental factors, and genetic and epigenetic interplay. These studies will provide important insights into the pathogenesis and the complex genotype-to-phenotype association of CC. In the future, these results may also lead to the development of novel gene therapies for some types of congenital cataracts, similar to other inherited eye diseases.

Although we sought to identify links between genotypes and prognoses after uneventful CC surgery to facilitate the provision of accurate prognoses and support clinical decision-making before invasive management, we did not find any clear correlations. Samples in which no mutations were identified in this study could be further submitted to whole-genome sequencing but rarely are because it is challenging to obtain a sufficient amount of blood from infants and young children to meet experimental needs.
Conclusions
In conclusion, our study highlights the benefits of an NGS approach combined with the analysis of a large targeted group of genes in a setting of genetically heterogeneous CC patients. Our findings provide significant diagnostic information and enable more accurate genetic counseling. Our results expand what we know about the mutation spectrum and frequencies of genes responsible for CC as well as the different distributions of genes mutated in familial and sporadic cases in the Chinese population.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of the Eye and ENT Hospital of Fudan University. All research was conducted in accordance with the Declaration of Helsinki. All participants (parents on behalf of their children) provided written informed consent forms for both genetic counseling and molecular genetic testing prior to enrolment.

Consent to publish
All participants (parents on behalf of their children) of the study gave written consent for their personal or clinical details along with any identifying images to be published in this study.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Competing Interest
The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding
This work was supported by grants from the National Natural Science Foundation of China (Grant No 81870645) and National Natural Youth Fund (Grant No 81900839).

Authors’ Contributions
LY and WJ contributed to the conception and design of the study, LY, LX, GC, MH and ZX contributed to sample recruitment and patient clinical evaluation, FF, LX, GC MH and ZX conducted the
experiment, WJ, FF and MH organized the database and performed the statistical analysis, FF wrote the first draft of the manuscript, and WJ wrote sections of the manuscript. All authors contributed to manuscript revisions and read and approved the submitted version.

**Acknowledgments**

This work was carried out in collaboration with BIG (Shenzhen, China). We are grateful to the members of the families for their participation in the study. We would like to thank all the residents and clinicians for their efforts during sample collection.

**References**

1. Daien V, Le Pape A, Heve D, Villain M, Gignac DB, Safety CE. Incidence and Characteristics of Congenital Cataract Surgery in France from 2010 to 2012: The EPISAFE Program. Ophthalmic Res. 2017;58(2):114-6.

2. Wu XH, Long EP, Lin HT, Liu YZ. Global prevalence and epidemiological characteristics of congenital cataract: a systematic review and meta-analysis. Lancet. 2016;388:55-.

3. Sheeladevi S, Lawrenson JG, Fielder AR, Suttle CM. Global prevalence of childhood cataract: a systematic review. Eye (Lond). 2016;30(9):1160-9.

4. Solebo AL, Teoh L, Rahi J. Epidemiology of blindness in children. Arch Dis Child. 2017;102(9):853-7.

5. Vasavada AR, Vasavada V, Shah SK, Praveen MR, Vasavada VA, Trivedi RH, et al. Five-Year Postoperative Outcomes of Bilateral Aphakia and Pseudophakia in Children up to 2 Years of Age: A Randomized Clinical Trial. American Journal of Ophthalmology. 2018;193:33-44.

6. Shiels A, Hejtmancik JF. Mutations and mechanisms in congenital and age-related cataracts. Experimental eye research. 2017;156:95-102.

7. Wirth MG, Russell-Eggitt IM, Craig JE, Elder JE, Mackey DA. Aetiology of congenital and paediatric cataract in an Australian population. Brit J Ophthalmol.
2002;86(7):782-6.

8. Haargaard B, Wohlfahrt J, Fledelius HC, Rosenberg T, Melbye M. A nationwide Danish study of 1027 cases of congenital/infantile cataracts - Etiological and clinical classifications. Ophthalmology. 2004;111(12):2292-8.

9. Shiels A, Bennett TM, Hejtmanck JF. Cat-Map: putting cataract on the map. Mol Vis. 2010;16:2007-15.

10. Sun WM, Xiao XS, Li SQ, Guo XM, Zhang QJ. Mutational screening of six genes in Chinese patients with congenital cataract and microcornea. Molecular Vision. 2011;17(168-69):1508-13.

11. Hansen L, Yao WL, Eiberg H, Kjaer KW, Baggesen K, Hejtmanck JF, et al. Genetic heterogeneity in microcornea-cataract: Five novel mutations in CRYAA, CRYGD, and GJA8. Invest Ophthalmol Vis Sci. 2007;48(9):3937-44.

12. Shendure J, Ji HL. Next-generation DNA sequencing. Nat Biotechnol. 2008;26(10):1135-45.

13. Simpson DA, Clark GR, Alexander S, Silvestri G, Willoughby CE. Molecular diagnosis for heterogeneous genetic diseases with targeted high-throughput DNA sequencing applied to retinitis pigmentosa. J Med Genet. 2011;48(3):145-51.

14. Coppieters F, De Wilde B, Lefever S, De Meester E, De Rocker N, Van Cauwenbergh C, et al. Massively parallel sequencing for early molecular diagnosis in Leber congenital amaurosis. Genetics in Medicine. 2012;14(6):576-85.

15. Shanks ME, Downes SM, Copley RR, Lise S, Broxholme J, Hudspith KAZ, et al. Next-generation sequencing (NGS) as a diagnostic tool for retinal degeneration reveals a much higher detection rate in early-onset disease. European Journal of Human Genetics. 2013;21(3):274-80.

16. Valencia CA, Ankala A, Rhodenizer D, Bhide S, Littlejohn MR, Keong LM, et al.
Comprehensive Mutation Analysis for Congenital Muscular Dystrophy: A Clinical PCR-Based Enrichment and Next-Generation Sequencing Panel. PloS one. 2013;8(1).

17. Gillespie RL, O'Sullivan J, Ashworth J, Bhaskar S, Williams S, Biswas S, et al. Personalized Diagnosis and Management of Congenital Cataract by Next-Generation Sequencing. Ophthalmology. 2014;121(11):2124-U302.

18. Zhai Y, Li JY, Yu WS, Zhu S, Yu YH, Wu MH, et al. Targeted Exome Sequencing of Congenital Cataracts Related Genes: Broadening the Mutation Spectrum and Genotype-Phenotype Correlations in 27 Chinese Han Families. Sci Rep-Uk. 2017;7.

19. Ma AS, Grigg JR, Ho G, Prokudin I, Farnsworth E, Holman K, et al. Sporadic and Familial Congenital Cataracts: Mutational Spectrum and New Diagnoses Using Next-Generation Sequencing. Human Mutation. 2016;37(4):371-84.

20. Liu X, Zheng TY, Zhou XT, Lu Y, Zhou P, Fan F, et al. Comparison between Limbal and Pars Plana Approaches Using Microincision Vitrectomy for Removal of Congenital Cataracts with Primary Intraocular Lens Implantation. Journal of Ophthalmology. 2016.

21. Khan AO, Aldahmesh MA, Alkuraya FS. Phenotypes of Recessive Pediatric Cataract in a Cohort of Children with Identified Homozygous Gene Mutations (An American Ophthalmological Society Thesis). Trans Am Ophthalmol Soc. 2015;113:T7.

22. Chen JQ, Xu K, Zhang XH, Jiang F, Liu LJ, Dong B, et al. Mutation Screening of Mitochondrial DNA as Well as OPA1 and OPA3 in a Chinese Cohort With Suspected Hereditary Optic Atrophy. Invest Ophthalmol Vis Sci. 2014;55(10).

23. Qian YY, Xiao DY, Guo X, Chen HB, Hao LL, Ma XJ, et al. Multiple gene variations contributed to congenital heart disease via GATA family transcriptional regulation. J Transl Med. 2017;15.

24. Warthen DM, Moore EC, Katnath BM, Morrissette JJD, Sanchez P, Piccoli DA, et al.
Jagged1 (JAG1) mutations in alagille syndrome: Increasing the mutation detection rate. Human Mutation. 2006;27(5):436-43.

25. Katagiri S, Hayashi T, Ohkuma Y, Sekiryu T, Takeuchi T, Gekka T, et al. Mutation analysis of BEST1 in Japanese patients with Best's vitelliform macular dystrophy. Brit J Ophthalmol. 2015;99(11):1577-82.

26. Tian L, Sun TY, Xu K, Zhang XH, Peng XY, Li Y. Screening of BEST1 Gene in a Chinese Cohort With Best Vitelliform Macular Dystrophy or Autosomal Recessive Bestrophinopathy. Invest Ophthalmol Vis Sci. 2017;58(9):3366-75.

27. Nakanishi A, Ueno S, Hayashi T, Katagiri S, Kominami T, Ito Y, et al. Clinical and Genetic Findings of Autosomal Recessive Bestrophinopathy in Japanese Cohort. American Journal of Ophthalmology. 2016;168:86-94.

28. Borman AD, Davidson AE, O'Sullivan J, Thompson DA, Robson AG, De Baere E, et al. Childhood-Onset Autosomal Recessive Bestrophinopathy. Arch Ophthalmol-Chic. 2011;129(8):1088-93.

29. Sloan-Heggen CM, Bierer AO, Shearer AE, Kolbe DL, Nishimura CJ, Frees KL, et al. Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. Human Genetics. 2016;135(4):441-50.

30. Prochazkova D, Hruba Z, Konecna P, Skotakova J, Fajkusova L. A p.(Glu809Lys) Mutation in the WFS1 Gene Associated with Wolfram-like Syndrome: A Case Report. J Clin Res Pediatr Endocrinol. 2016;8(4):482-3.

31. Qing J, Yan D, Zhou Y, Liu Q, Wu WJ, Xiao Z, et al. Whole-Exome Sequencing to Decipher the Genetic Heterogeneity of Hearing Loss in a Chinese Family with Deaf by Deaf Mating. PloS one. 2014;9(10).

32. Miyagawa M, Naito T, Nishio S, Kamatani N, Usami S. Targeted Exon Sequencing Successfully Discovers Rare Causative Genes and Clarifies the Molecular
33. Kondo H, Kusaka S, Yoshinaga A, Uchio E, Tawara A, Hayashi K, et al. Mutations in the TSPAN12 gene in Japanese patients with familial exudative vitreoretinopathy. Am J Ophthalmol. 2011;151(6):1095-100 e1.

34. Hoogeveen-Westerveld M, Wentink M, van den Heuvel D, Mozaffari M, Ekong R, Povey S, et al. Functional Assessment of Variants in the TSC1 and TSC2 Genes Identified in Individuals with Tuberous Sclerosis Complex. Human Mutation. 2011;32(4):424-35.

35. Henz S, Ackl N, Knels C, Rominger A, Flatz W, Teipel S, et al. A Pair of Siblings with a rare R5H-Mutation in Exon 1 of the MAPT-Gene. Fortschr Neurol Psyc. 2015;83(7):397-400.

36. Mackay DS, Bennett TM, Culican SM, Shiels A. Exome sequencing identifies novel and recurrent mutations in GJA8 and CRYGD associated with inherited cataract. Human Genomics. 2014;8.

37. Li JY, Leng YJ, Han SR, Yan LL, Lu CX, Luo Y, et al. Clinical and genetic characteristics of Chinese patients with familial or sporadic pediatric cataract. Orphanet J Rare Dis. 2018;13.

38. Danda S, van Rahden VA, John D, Paul P, Raju R, Koshy S, et al. Evidence of Germline Mosaicism for a Novel BCOR Mutation in Two Indian Sisters with Oculo-Facio-Cardio-Dental Syndrome. Mol Syndromol. 2014;5(5):251-6.

39. Sun ZX, Zhou Q, Li HJ, Yang LZ, Wu SJ, Sui RF. Mutations in crystallin genes result in congenital cataract associated with other ocular abnormalities. Molecular Vision. 2017;23:977-86.

40. Wang H, Zhang LL, Wang N, Zhu H, Han B, Sun F, et al. Next-generation sequencing reveals genetic landscape in 46, XY disorders of sexual development patients with variable phenotypes. Human Genetics. 2018;137(3):265-77.
41. Zhong ZL, Wu ZH, Han LY, Chen JJ. Novel mutations in CRYGC are associated with congenital cataracts in Chinese families. Sci Rep-Uk. 2017;7.

42. Sarkar D, Ray K, Sengupta M. Structure-Function Correlation Analysis of Connexin50 Missense Mutations Causing Congenital Cataract: Electrostatic Potential Alteration Could Determine Intracellular Trafficking Fate of Mutants. Biomed Res Int. 2014.

43. Azuma N, Yamada M. Missense mutation at the C terminus of the PAX6 gene in ocular anterior segment anomalies. Invest Ophthalmol Vis Sci. 1998;39(5):828-30.

44. Azuma N, Hotta Y, Tanaka H, Yamada M. Missense mutations in the PAX6 gene in aniridia. Invest Ophthalmol Vis Sci. 1998;39(13):2524-8.

45. Gong B, Qu C, Li XL, Shi Y, Lin Y, Zhou Y, et al. Mutation spectrum of CYP1B1 in Chinese patients with primary open-angle glaucoma. Brit J Ophthalmol. 2015;99(3):425-30.

46. Comeglio P, Johnson P, Arno G, Brice G, Evans A, Aragon-Martin J, et al. The importance of mutation detection in Marfan syndrome and Marfan-related disorders: report of 193 FBN1 mutations. Hum Mutat. 2007;28(9):928.

47. Hejtmancik JF. Congenital cataracts and their molecular genetics. Seminars in cell & developmental biology. 2008;19(2):134-49.

48. Davis LK, Meyer KJ, Rudd DS, Librant AL, Epping EA, Sheffield VC, et al. Pax6 3' deletion results in aniridia, autism and mental retardation. Human Genetics. 2008;123(4):371-8.

49. De Franco E, Flanagan SE, Yagi T, Abreu D, Mahadevan J, Johnson MB, et al. Dominant ER Stress-Inducing WFS1 Mutations Underlie a Genetic Syndrome of Neonatal/Infancy-Onset Diabetes, Congenital Sensorineural Deafness, and Congenital Cataracts. Diabetes. 2017;66(7):2044-53.

50. Coccia M, Brooks SP, Webb TR, Christodoulou K, Wozniak IO, Murday V, et al. X-linked...
cataract and Nance-Horan syndrome are allelic disorders. Human Molecular Genetics. 2009;18(14):2643-55.

Tables

Table 1. Basic characteristics of the participants in our study

|                          | sCC          | FCC          |
|--------------------------|--------------|--------------|
| Number of patients       | 38           | 16           |
| Male: female             | 21:17        | 10:6         |
| Mean age of patients     | 3.00 (2.00-6.00) | 3.00 (1.63-6.75) |
| mothers                  | 31.55 ±4.95  | 29.00 ±4.88  |
| fathers                  | 33.59±5.36   | 30.71±4.38   |
| Binocular: monocular     | 27:11        | 15:1         |
| Detected cases           | 11/38 (28.95)| 14/16 (87.50)|
| Detected variants        | 14           | 16           |

Values are shown as n (%) and medians (IQRs) or medians± standard deviation for normally distributed data.

Bold text is used for p values under 0.01, indicating statistical significance.

Table 2. Sporadic cases with likely causative variants

| Family | Phenotype | Inheritance: Before/After testing | Gene    | Refseq ID       | Nucleotide change | Predicted amino acid change | Heterozygosity |
|--------|-----------|----------------------------------|---------|----------------|-------------------|-----------------------------|----------------|
| 1      | Bi, Sub, Juvenile | Sporadic/AR | CYP27A1 | NM_000784.3 | c.1263+1G>A | -                           | Hom            |
| 2      | Bi, All   | Sporadic/new AD                  | CRYBB2  | NM_000496.2 | c.487C>T       | p.Gln163*|p.Q163* | Het            |
| 3      | Bi, All   | Sporadic/ new AR                 | OPA3    | NM_001017989.2 | c.123C>G    | p.Ile41Met|p.I41M   | Het            |
|        |           |                                  | JAG1    | NM_000214.2  | c.1511A>G     | p.Asn504Ser|p.N504S  |                |
| 4      | Mo, Sub+Post, Sub+Post | Sporadic/AR or AD | BEST1|BEST1 | NM_001139443.1|NM_004183.3 | c.20G>A | p.Ser7Asn|p.S7N  | Het            |
|   | Description | Disease Status | Gene | Reference | Mutation | ClinVar | Allele | Outcome |
|---|-------------|----------------|------|-----------|----------|---------|--------|---------|
| 5 | Mo, All, PHPV | Sporadic/? AR or AD | BEST1 | NM_001139443.1/NM_004183.3 | c.584C>T | p.Ala195V | all/p.A195V | Het |
| 6 | Bi,Nuc | Sporadic/X-linked | NHS | NM_001291867.1 | c.2774_2775del | p.Gln926Lefu*3 | Hemi/He |
| 7 | Bi,OD-Dot,OS-Ant+Dot | Sporadic/X-linked | NHS | NM_001291867.1 | c.2933T>C | p.Ile978Thr| p.I978T | Het/Hem |
| 8 | Mo,Sub, Junvenile | Sporadic/AR or AD | WFS1 | NM_006005.3 | c.2603G>A | p.Arg868His | Het |
| 9 | Mo,Nuc | Sporadic/X-linked | COL4A5 | NM_033380.2 | c.4003C>T | p.Pro1335Ser| p.P1335S | Hemi/He |
| 10 | Bi,Sub | Sporadic/AR | FYCO1 | NM_024513.3 | c.215A>T | p.Asp72Val | p.D72V | Hom |
| 11 | Bi,Cort+Sub,F EVR, Cleft Lip and Palate | Sporadic/AD | TSPAN12 | NM_012388.3 | c.194C>T | p.Pro65Leu | p.P65L | Het |
|   |   |   | TSC1 | NM_000368.4 | c.1460C>G | p.Ser487Cys| p.S487C | Het |
|   |   |   | MAPT | NM_001123066.3 | c.14G>A | p.Arg5His | p.R5H | Het |

Bi -binocular; Mo-monocular; Sub-subcapsular; Cort-cortical; Post-posterior polar; Nuc-nuclear; Ant-anterior polar; All-all white; Dot-dot-like; Peri-perinuclear; Micro-microphthalmia; Hom-homozygosis; Het- heterozygosis; P-pathogenic; LP-likely pathogenic; VUS-variant of unknown significance; D-damaging; B-benign; T-tolerated; NK-not known
| Family ID | Phenotype | Inheritance | Gene   | Refseq ID | Nucleotide change | Predicted amino acid change | Heterozygosity |
|----------|-----------|-------------|--------|-----------|-------------------|----------------------------|---------------|
| 1        | Bi, All   | AD/AD       | CRYGC  | NM_02098 9.3 | c.497C>T           | p.Ser166Phe|p.S166f           | Het           |
| 2        | Bi, Lam   | AD/AD       | CRYGD  | NM_00689 1.3 | c.70C>A            | p.Pro24Thr|p.P24T           | Het           |
| 3        | Bi, OS-All; OD-Dot | AD/X-linked | BCOR   | NM_00112 3385.1 | c.3490C>T          | p.Arg1164*|p.R1164*         | Het           |
| 4        | Bi, Nu    | AD/AD       | CRYAA  | NM_00039 4.3 | c.61C>T            | p.Arg21Trp|p.R21W           | Het           |
| 5        | Bi, Cora  | AD/AD       | CRYGD  | NM_00689 1.3 | c.70C>A            | p.Pro24Thr|p.P24T           | Het           |
| 6        | Bi, Nu    | AD/AD       | BMP4   | NM_00120 2.4 | c.751C>T           | p.His251Tyr|p.H251Y          | Het           |
|          |           |             | CRYBA1 | NM_00520 8.4 | c.626C>G           | p.Ser209Trp|p.S209W          | Het           |
| 7        | Bi, All   | AD/AD       | CRYGC  | NM_02098 9.3 | c.192del           | p.Asp65Thrfs38|p.D65Tfs38     | Het           |
| 8        | Bi, Peri+Cor | AD/?AD or AR | OPA3   | NM_00101 7989.2 | c.123C>G          | p.Ile41Met|p.I41M          | Het           |
|   | Bi | Binocular; All-all white; Lam-lamellar; Sub-subcapsular; Cort-cortical; Post-posterior polar; Nuc-nuclear; ASD-anterior segment dysplasia; Dot-dot-like; Peri-perinuclear; Micro-microphthalmia; Cora-coralliform; Nys-nystagmus; Hom-homozygosis; Het- heterozygosis; P-pathogenic; LP-likely pathogenic; VUS- variant of unknown significance; D-damaging; B-benign; T-tolerated; NK-not known; OFCD-Oculofaciocardiodental syndrome
|---|---|---|---|---|---|---|---|---|---|
| 9 | Bi+Micro | AD/AD | GJA8 | NM_005267.4 | c.136G>A | p.Gly46Arg|p.G46R | Het |
| 10 | Bi+Dot | AD/AD | PAX6 | NM_001310158.1 | c.52G>A | p.Gly18Arg | Het |
| 11 | Bi All | AD/AD | PAX6 | NM_001310158.1 | c.113G>C | p.Arg38Pro|p.R38P | Het |
| 12 | Bi,Nys,Post +OD-ASD | AD/AD | PAX6 | NM_001310158.1 | c.966del | p.Phe323Serfs56|p.F323Sfs56 | Het |
| 13 | Bi,Post+Sub | AD | CYP1B1 | NM_000104.3 | c.319C>G | p.Leu107Val|p.L107V | Hom/het |
|   |   |   | FBN1 | NM_000138.4 | c.7559C>T | p.Thr2520Met|p.T2520M | Het |
| 14 | Bi | AD/AD | WFS1 | NM_006005.3 | c.449C>T | p.Ala150Val|p.A150V | Het |

**Table 4.** The phenotype, genotype and prognosis of patients older than 3 years old.
| Family ID | Patient age (y) | Sex | Phenotype | Genotype |
|-----------|----------------|-----|-----------|----------|
| s1        | 10             | F   | Bi,Sub    | CYP27A1 (c.1263+1G>A) |
| s4        | 4              | F   | Mo,Sub+Post | BEST1|BEST1 (c.20G>A) |
| s8        | 9              | F   | Mo,Sub    | WFS1 (c.2603G>A) |
| s10       | 6              | M   | Bi,Sub    | FYCO1 (c.215A>T) |
| s11       | 7              | M   | Bi,Cort+Sub,Nys, FEVR, CLP | TSPAN12 (c.194C>T) |
|           |                |     |           | TSC1 (c.1460C>G) |
|           |                |     |           | MAPT (c.14G>A) |
| f8        | 7              | M   | Bi, Peri+Cor, | OPA3 (c.123C>G) |
| f10       | 5              | F   | Bi, Dot   | PAX6 (c.52G>A) |
| f12       | 14             | M   | Bi,Nys,Post +OD-ASD | PAX6 (c.966del) |
| f13       | 8              | F   | Bi,Post+Sub | CYP1B1 (c.319C>G) |
|           |                |     |           | FBN1 (c.7559C>T) |

s-sporadic; f-familial; F-female; M-male; Bi-binocular; Mo-monocular; Sub-subcapsular; Post-posterior polar; Cort-cortical; Peri-perinuclear; Dot-dot-like; Nys-nystagmus; FEVR- familial exudative vitreoretinopathy; CLP-cleft lip and palate; ASD-anterior segment dysplasia; VA-visual acuity.

**Table. 5** Studies related to the mutation spectrum of CC obtained using NGS in the past 5 years.

|                  | Out cohort | Li et al, 2018(37) | Gillespie et al, 2014(17) | Ma et al., 2011 |
|------------------|------------|----------------------|--------------------------|-----------------|
| **Target genes** | 792 inherited eye diseases | 80 cataract-associated genes | 115 genes associated with CC | 32 cataract-associated genes |
| **Detection rate** | Familial, 87.5% sporadic, 28.95% | Familial, 75% sporadic, 47.8% | 70% | 70% |
| **Participants** | 38 sporadic and 16 familial cases, 42 bilateral and 12 unilateral | 23 sporadic and 16 familial cases, all bilateral | 15 sporadic and 21 familial cases, all bilateral | 24 sporadic; 22 familial cases, all bilateral nonsyndromic |

**Figures**
Figure 1

Different distributions of mutational genes in familial versus sporadic congenital cataracts