THE ASSOCIATION BETWEEN PAX6 GENE AND CONGENITAL CATARACT

Tanmay Srivastava*, Prof. Royana Singh**, Diskha Prakash***
Prof. O.P.S. Maurya **, Pankaj kumar Baranwal *

AIM: To determine association of PAX-6 gene in congenital cataract patients in a cross sectional hospital based study over a period of 1 year.

METHODS: During the study 17 patients and 33 parents were recruited from Ophthalmology, Sir Sunderlal Hospital, Institute of Medical Sciences, Banaras Hindu University, Varanasi after informed consent.All cases underwent Visual acuity using Snellen's chart (if possible),Vision with pin hole (if possible),Retinoscopy under mydriasis,Refraction by autorefractor and best corrected visual acuity,Slit lamp examination,Distant direct ophthalmoscopy,Indirect ophthalmoscopy And Ultrasonography B Scan of both eyes.

RESULTS: The mean age of male and female patients affected with congenital cataract who came in OPD of SS Hospital was 4.3±0.888194 and 4.75±1.258306 respectively.No polymorphism in patient affected with congenital cataract was observed.

CONCLUSIONS: Our study found no link between PAX6 gene polymorphisms and patient affected with congenital cataract in this part of population screened in eastern U.P.

Congenital cataracts are one of the most common treatable causes of visual impairment and blindness during infancy and are responsible for nearly 10% of all vision loss in children worldwide.Its occurrence,depending on the regional socioeconomic development,is of 1 to 6 cases per 10,000 live births in industrialized countries[1-3], and of 5 to 15 per 10,000 in the poorest areas of the world[4].

Congenital cataract is visible at birth or during the first decade of life. About 20,000 to 40,000 new cases of bilateral congenital cataract are diagnosed each year in India[4].

The cataract is usually seen as an isolated abnormality but may occur in association with other ocular developmental or systemic abnormalities.If a cataract goes undetected in an infant, permanent visual loss may ensue.If a lenticular opacity is in the visual axis, it is considered visually significant and may lead to blindness. If the cataract is small, in the anterior portion of the lens, or in the periphery, no visual loss may be present[5].

Congenital cataracts occur in a variety of morphologic configurations, including lamellar, polar, sutural, coronary, cerulean, nuclear, capsular, complete, membranous and are often confined to a portion of the lens, and may be static or progressive.[6] In general, the more posteriorly located and dense an opacity, the greater the impact on visual function.[7]

Congenital cataract is both clinically and genetically heterogeneous; isolated congenital cataract is usually inherited as an autosomal dominant trait although autosomal recessive and X linked inheritance are seen less commonly.[8]

Unilateral cataracts are usually isolated sporadic incidents. They are usually the result of local dysgenesis and may be associated with other ocular dysgenesis such as persistent fetal vasculature (PFV), posterior lenticonus or lentiglobus, persistent hyperplastic primary vitreous, anterior segment dysgenesis, posterior pole tumors, trauma, or intrauterine infection, particularly rubella.

Bilateral cataracts are often inherited and associated with other diseases. They require a full metabolic,

*JR III ,IMS , BHU , Varanasi ,U.P.)
**Department of Anatomy , IMS , BHU , Varanasi , U.P.)
***Ex fellow LVPEI Bhubaneswar)

infectious, systemic, and genetic workup. The common causes are hypoglycemia, trisomy (eg, Down, Edward, and Patau syndromes), myotonic dystrophy, infectious diseases (eg, toxoplasmosis, rubella, cytomegalovirus, and herpes simplex [TORCH]), and prematurity.

It is known that different mutations in the same gene can cause similar cataract patterns, while the highly variable morphologies of cataracts within some families suggest that the same mutation in a single gene can lead to different phenotypes[9].

To date, more than 25 *loci* and genes on different chromosomes have been associated with congenital cataract[10].Mutations in distinct genes, which encode the main cytoplasmic proteins of human lens, have been associated with cataracts of various morphologies[11], including genes encoding crystallins (CRYA, CRYB, and CRYG)[12], lens specific connexins (Cx43, Cx46, and Cx50)[13], major intrinsic protein (MIP) or aquaporine[14], cytoskeletal structural proteins[15], paired-like homeodomain transcription factor 3 (PITX3)[16], avian musculoaponeurotic fibrosarcoma (MAF)[17], and heat shock transcription factor 4 (HSF4)[18].

Paired box protein Pax-6 also known as **aniridia type II protein** (AN2) or **oculorhombin** is a protein that in humans is encoded by *PAX6* gene.[19]

Pax6 is a transcription factor present during embryonic development. The encoded protein contains two different binding sites that are known to bind DNA and function as regulators of gene transcription. It is a key regulatory gene of eye and brain development. Within the brain, the protein is involved in development of the specialized cells that process smell. As a transcription factor, Pax6 activates and/or deactivates gene expression patterns to ensure for proper development of the tissue. Mutations of the Pax6 gene are known to cause various disorders of the eyes. Two common disorders associated with a mutation are: aniridia, the absence of the iris, and Peter's anomaly, thinning and clouding of the cornea.[20-25]

The human eye malformation aniridia results from haploinsufficiency of PAX6, a paired box DNA-binding protein.The characteristic paired DNA binding domain of Pax6 utilizes two DNA-binding domains, the paired domain (PD), and the homeodomain (HD). These domains function separately via utilization by Pax6 to carry out molecular signaling that regulates specific functions of Pax6.[26]Because the gene has been sequenced, prenatal diagnosis of aniridia is now possible. In addition, some evidence suggests that *PAX6* may be expressed by damaged eye tissue to induce limited regeneration; artificial upstream regulation of *PAX6* may eventually be used to induce such regeneration. Finally, some cancers, including alveolar rhabdomyosarcoma, may be caused by *PAX* mutations.[27]

These findings suggest potential therapeutic applications for*PAX6* research and may lead to a more complete understanding of its role in eye development.[28]

METHODS- The present study was undertaken to evaluate the association of PAX6 gene polymorphisms in congenital cataract patients in eastern U.P. during the period of July 2015 and June 2016.Ethical approval was obtained for the study. A written consent was taken by the patient mentioning the pros and cons of the treatment, study and duly signed by a witness also. The study is based on data having sporadic cases of congenital cataract as no family history could be elicited. During the study 17 patients and 33 parents were recruited from Ophthalmology, Sir Sunderlal Hospital, Institute of Medical Sciences, Banaras Hindu University, Varanasi after informed consent.

INCLUSION CRITERIA:-

- All cases of congenital cataract as per history and clinical evaluation.
- Patient and guardian consent.

- All patients less than 15 years of age.

EXCLUSION CRITERIA:-

- Unwilling guardians.
- Patients with history of trauma.
- Patients with history of use of steroids.
- Patients with history of previous radiation therapy.
- Patients with history of previous laser therapy.
- Patients with posterior segment pathology.
- Patient receiving treatment for some disease.
- Low birth weight or extremely malnourished child.

Data collected from the patients' records included patients' age, gender, duration of cataract, age at onset of cataract, presence or absence of other associated complaints, use of any medication, antenatal, natal and perinatal history, developmental history and immunization history. All patients undergone biological workup including complete blood count, renal function test, serum electrolytes, liver function test, blood sugar (both fasting and post prandial).

All the patients underwent the following tests on the first day of visit and then at regular follow up at 7th postop day and 4 weeks:

- Visual acuity using Snellen's chart (if possible)
- Vision with pin hole (if possible)
- Retinoscopy under mydriasis
- Refraction by autorefractor and best corrected visual acuity
- Slit lamp examination
- Distant direct ophthalmoscopy
- Indirect ophthalmoscopy
- Ultrasonography B Scan of both eyes.

GENETIC ANALYSIS:

3 to 5 ml of peripheral venous blood was collected from patient along with parents in EDTA coated vials from the subjects and stored at -20 degrees Celsius for less than 3 months before DNA extraction.

DNA isolation was done by "Salting Out" method and dissolved in tris- EDTA (TE) buffer. Primers used for PCR amplification were designed using Primer3 software version 0.4.0 (http://frodo.wi.mit.edu/primer3/) (Rozen S et al., 2000) for PAX6 gene exon5 using sequences from the NCBI Gene (Reference GRCh38.p2 Primary Assembly NC_000002.12) and they were amplified by Thermocycler (Applied Bio system). *In silico* PCR analysis and Blast searches were performed using the UCSC Genome Bioinformatics website (website http://genome.ucsc.edu/).

List of Primers of PAX6

PAX6 Exon		Sequence (5' >3')	Length	Product Length (bp)	Tm
Exon 5	FP	CCTCTTCACTCTGCTCTCTTC TC	23	254	59.87
	RP	AAGAGAGGGCGTTGAGAGTG	20		59.39

PCR was used to amplify DNA with candidate gene primers for sequencing. Primers were purchased from NEB and prepared from dry oligonucleotides to make up a working concentration of 5pmol/µl. Gel electrophoresis was used to enable us to find out if the desired region of the DNA was amplified during PCR reaction. By separating PCR products by size, it allowed us to estimate the size of the amplified product.

DNA Sequencing

Sequencing was used to screen the candidate gene in the relevant affected individuals. PCR products were first purified using the EXOSAP protocol. Purified PCR products were then added to the reaction mixture for sequencing amplification. Sequencing reactions were analysed using 3130*xL* Genetic Analyzer (Applied Biosystems®). Sequencing files obtained from the 3130*xL* Genetic Analyzer (Applied Biosystems®) were analysed using FinchTv viewer.

RESULTS-

A total of 135 children were admitted to Sir Sunderlal Hospital during the period of one year from July 2015 to June 2016 in Department of Ophthalmology.

During July 2015 to June 2016 different cases of congenital cataract were recruited from different districts in and around eastern Uttar Pradesh. These patients hailed not only from Varanasi but also other nearby districts; Chandauli, Jaunpur, SantRavidas Nagar, Ghazipur, Mirzapur and Sonbhadra of Uttar Pradesh.

Of this 135, 17 were affected with congenital cataracts. These comprise of 11 males (77%) and 6 (23%) females, a sex ratio of 1.83:1.The mean age of male and female patients affected with congenital cataract who came in OPD of SS Hospital was 4.3±0.888194 and 4.75±1.258306 respectively. During the study according to religion the patients were categorized into two categories Hindu (14) and Muslims (3). We observed that maximum patient suffering from congenital cataract were Hindu in this part of country.

The study also includes the type of cataract during one year period. The type of cataract included zonular, nuclear and total. During the period zonular (14 or 82.3%), nuclear (2 or 11.7%) and total (1 or 5.8%) were the major type of cataract encountered in our study. As the percentage of zonular cataract was maximum in our study; hence we also checked the distribution pattern of zonular cataract according to sex. We observed that the percentage of male candidate were maximum in zonular cataract.

Our study also recorded preoperative visual acuity and retinoscopy values of all the cases. We found that most of our cases had vision of range between 6/60 to 6/18 (14, 82.3%) followed by 2 cases having vision of < 6/60 (11.7%) and 1case having vision of > 6/18 (5.8%). In retinoscopy done at one arm distance we found that 15 (88.2%) of them had values between range of + 2.50 DS to + 5.00 DS in both meridians while one each had value < +2.50 DS and > +5.00 DS values in both meridians respectively.

The study also recorded preoperative fundus details of patients among which in 10 (58.8%) of them we could easily see all the fundus details very clearly through cataract while in 3 (17.6%) of them we had some difficulty in visualizing fundus details. We could barely see fundus in remaining 4 (23.5%) of them. Subsequently, these 4 cases underwent Ultrasound B Scan of their eyes which were found to be absolutely normal.

Our study also looked for preoperative axial length of cases for planning of surgery and prediction of postoperative visual outcomes. We found majority of our cases had axial length between 20 – 22 mm (88.2%) and one each had axial length less than 20 and greater than 22 mm.

All our 17 cases underwent cataract extraction followed by intraocular lens implantation. Subsequently, post-operative visual acuity was also calculated after one month of surgery. Majority (14, 82.3%) of our cases developed visual acuity ranging between 6/9 – 6/18 and two of them obtained visual acuity of 6/9.One case attained visual acuity of < 6/18 after surgery.

PCR-Genotyping:

We examined exon5 of PAX6 gene for novel polymorphisms that could be used to detect an association of

	Tm
	59.87
	59.39

PAX6 gene with congenital cataract patients and their parents. The amplified products for exon5 were subjected to 2% agarose gel electrophoresis (Fig.1).

Figure 1: 2% Agarose Gel Electrphoresis (AGE) amplified product of congenital cataract family sample (360, 361, 362, 363, 364, 365, 366, 393, 394, 395) of PAX6 Exon5.

We were unable to detect any polymorphism in patient affected with congenital cataract.

DISCUSSION- A cross sectional hospital based study was done involving patient from different parts of eastern Uttar Pradesh that were referred for treatment in SS Hospital, BHU. In our study, it was observed that during the last 1 year the male to female ratio was 1.83:1. However, the ratio is very high this may be due to an underestimation of the true situation since; in India many people do not come to hospital if a girl child has this type of disease. This needs a door to door study of a location. In our study we did observe that the occurrence of zonular cataract was most prevalent and that of nuclear, total, sutural were least. We conclude that zonular or lamellar cataract is the most common type of congenital cataract as also demonstrated in other studies.

The distribution according to religion showed maximum number of affected patients belong to Hindu religion as in Indian scenario majority belongs to Hindu community.Study involving more cases from the general population may confirm the same.

Majority of our cases had preoperative visual acuity between 6/18 – 6/60. This is due to the fact that zonular cataract demonstrates different grades of opacities in different areas of lens with possibly clear area in between zones. One of them who had visual acuity of < 6/60 may have been due to total cataract in which whole lens was opaque thus did not allow any light to pass through.

Majority of the cases who underwent retinoscopy with eye ointment atropine at one arm distance revealed a hypermetropic fundus. This correlates well with age of presentation of disease. As majority of these patients are children their eye ball is in continuous phase of development hence have a hypermetropic fundus till age of 8 years. One of them who demonstrated a myopic fundus may have been due to lenticular myopia induced by cataract. Axial length also followed the same rule of hypermetropia as majority of fundus demonstrated axial length ranging from 20-22 mm.

Fundus details could be deciphered in majority of cases as zonular cataract usually allow for fundus examination through clear portion of lens. In cases where details could not be appreciated were possibly due to greater density of cataract and involvement of all the zones of lens in cataract. Following surgery majority of our patients developed visual acuity of 6/9-6/12 with two of them having visual acuity of 6/6. One patient developed 6/18 vision which may have been due to central nuclear nature of cataract which did not allow any light to pass through thereby producing stimulus deprivation amblyopia.

Genetic studies have contributed to the idea that genes involved in early onset cataract are also implicated in age-related cataract. In particular, mutations in some genes *(MIP* and *yC-crystalliri)* result in progressive cataracts,[29,30] whilst familial adult onset pulverulent cataracts has been linked to the CAAR locus.[31] It may be suggested that mutations in certain genes may have a detrimental effect on eye lens development resulting in congenital cataract.

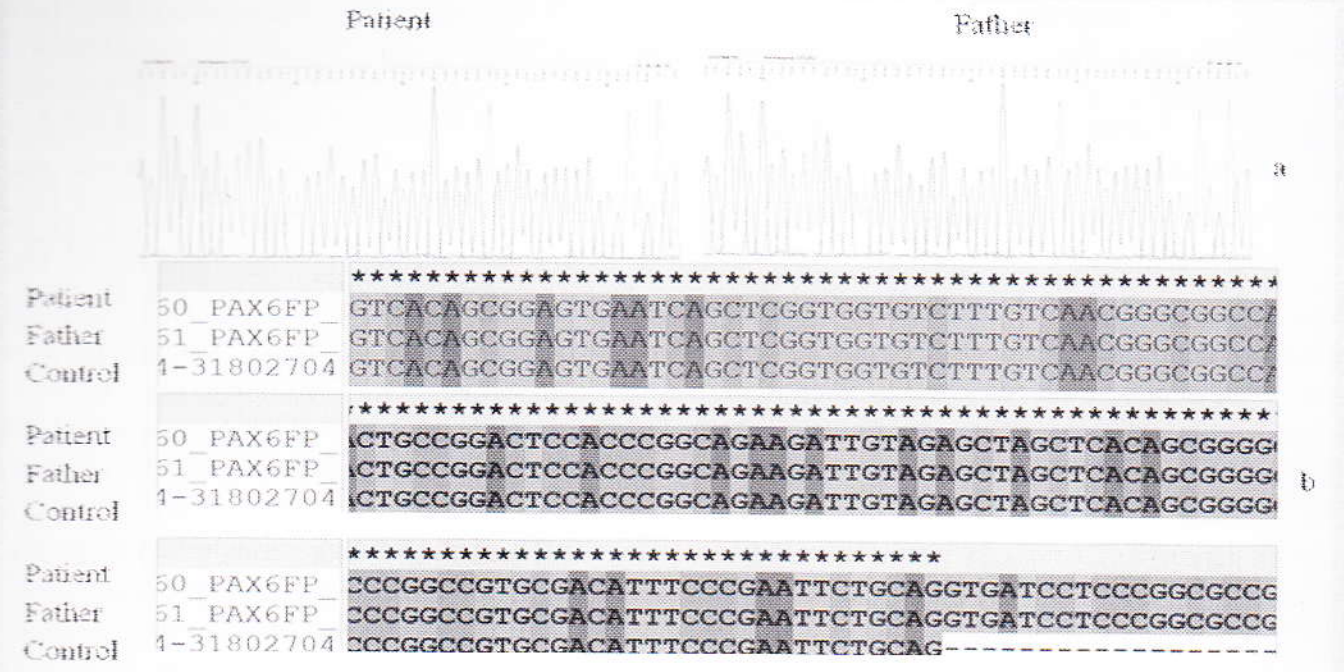
PAX 6 gene encodes paired box gene 6, one of many human homologs of the gene found in *Drosophila Melanogaster* named " prd ". In addition to the hallmark feature of this gene family, a conserved paired box domain, the encoded protein also contains a homeo box domain. Both domains are known to bind DNA, and function as regulators of gene transcription. This gene is expressed in the developing nervous system, and in developing eyes. Mutations in this gene are known to cause ocular disorders such as aniridia and Peter's

anomaly. Alternatively spliced transcript variants encoding either the same or different isoform have been found for this gene. There are around 40 mutations known to cause congenital cataracts, of which only 1 is caused by mutations in the pathogenesis of the independent occurrence of ADCC (G18W), the mutation interfering with the target gene PAX6 binding, and reduce its transcriptional activation function located in the 11p13. A novel PAX6 gene mutation was identified in a Chinese aniridia family.This mutation may also contribute to congenital cataracts in these aniridia patients.[132-135].

Glaser T et al.*, 1994**[35] found that PAX6 is located on human chromosome 11p13, and mutations in this gene lead to a variety of hereditary ocular malformations of the anterior and posterior segment, including aniridia,[36] coloboma of the iris,[37] keratitis,[138] congenital cataracts,[35] Peter's anomaly,[39] and optic nerve defects.[40] ***Fucheng Cai et al.*, 2010**[41] identified a novel deletion mutation of *PAX6* in a Chinese family with aniridia and congenital cataract. This finding expands the mutation spectrum of *PAX6* and is useful and valuable for genetic counseling and prenatal diagnosis in families with aniridia accompanied with congenital cataract. ***Dansault et al. [42] reported 14 affected members carrying a p.S74G mutation in exon 6 of PAX6 gene. All of them were suffering from diverse congenital ocular abnormalities including congenital cataracts, diverse neurological manifestations and variable cognitive impairments. Recently, ***Chien et al.*** [43] had identified a p.R317X PAX6 mutation in a patient (familial case) suffering from cataract, aniridia, nystagmus and was developmentally delayed. ***Manel Chograni et al.***[44] reported no mutation in the four genes of congenital cataract and its flanking regions. Only variations that did not segregate with the studied phenotypes (ARCC associated to Mental retardation (MR), ARCC associated with MR and microcephaly) were reported.He detected three intronic variations in PAX6 gene: IVS4 -274insG (intron 4), IVS12 -174G>A (intron12) in the four studied families and IVS4 -195G>A (intron 4) in two families.

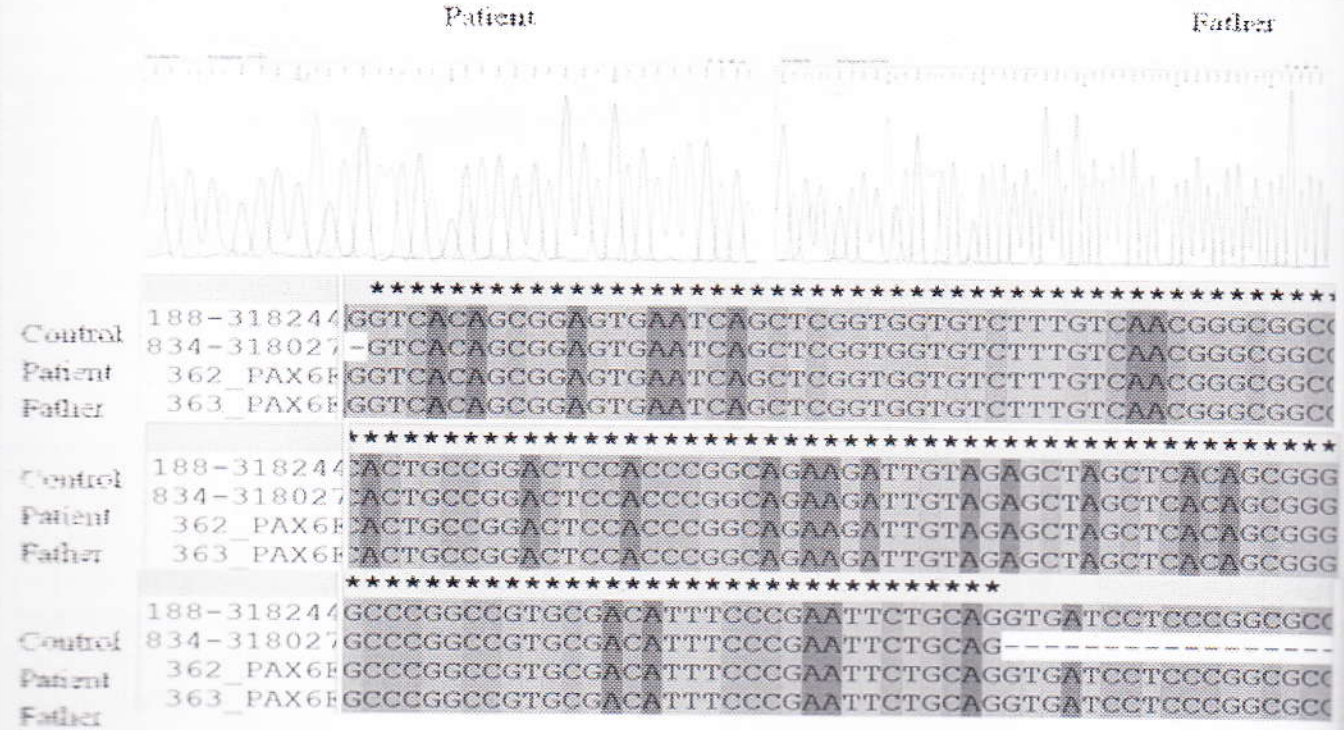
Manel Chograni et al.[45] identified a novel nonsense mutation (p.Q89X) in exon 6 of PAX6 gene in a Tunisian family with aniridia and congenital cataracts. Additionally, he highlighted the predicted pathogenic effect of the reported nonsense mutation, p.R240X, in a second Tunisian family with aniridia, congenital cataracts and variable ocular anomalies. These two mutations lead to truncated proteins and added to the large spectrum of nonsense mutations associated with aniridia. ***Li Wang et al.***[46] identified a novel missense mutation (c.1147A>T) in exon 12 of *PAX6* gene associated with autosomal dominant congenital aniridia and cataract in a Chinese family. It gives further evidence of genotype heterogeneity in congenital aniridia associated with *PAX6*. ***Noriyuki Azuma et al.***[47] ascertained a novel missense mutation in four pedigrees with Peter's anomaly, congenital cataract, Axenfeldt anomaly, and/or foveal hypoplasia, which, to our knowledge, is the first mutation identified in the splicevariant region. A TrA transition at the 20th nucleotide position of exon 5a results in a ValrAsp (GTCrGAC) substitution at the 7th codon of the alternative splice region. Tom Glaser ***et al.***[48] characterized two PAX6 mutations in a family segregating aniridia and a milder syndrome consisting of congenital cataracts and late onset corneal dystrophy.

Very little work has been performed to correlate an association between PAX 6 gene and congenital cataract. PAX6 plays an important role in development of eye. It is emphasized that it may play an important role in congenital cataract too. We could not demonstrate any polymorphisms of PAX 6 gene in our subjects possibly due to smaller sample size and non familial cases. But further work in this study is required and may possibly let us a peep into the role of PAX6 in the development of eye and its association with the congenital cataract in this part of the country.



a. Figure representing the chromatogram for family 360-361.
b. Multiple sequence alignment show that no change was observed

SEQUENCING RESULTS FOR FAMILY 360-361



a. Figure representing the chromatogram for family 362-363.
b. Multiple sequence alignment show that no change was observed

SEQUENCING RESULTS FOR FAMILY 362-363.

REFERENCES-

1. Apple DJ, Ram J, Foster A, Peng Q. Elimination of cataract blindness: a global perspective entering the new millennium. Surv Ophthalmol. 2000;45 Suppl 1:S1-196.

2. Gilbert C, Foster A. Childhood blindness in the context of VISION 2020 - The right to sight. Bull World Health Organ. 2001;79(3):227-32.

3. Foster A. Worldwide blindness, increasing but avoidable. Semin Ophthalmol. 1993; 8(3):166-70.

4. Rahi JS, Sripathi S, Gilbert C, Foster A. Childhood blindness in India: causes in 1318 blind school students in nine states. Eye (Lond). 1995;9(5):545-50.

5. Francis PJ, Berry V, Bhattacharya SS, Moore AT.Genetics of childhood cataract. Institute of Ophthalmology, London EC1V 9EL.

6. Basic and clinical science course (2011–2012). *Pediatric ophthalmology and Strabismus*. American Academy of Ophthalmology.

7. **Gilbert CE,** *Canovas R, Hagan M, et al. Causes of childhood blindness: results from West Africa, South India and Chile. Eye 1993;7:*184–*8.*

8. **Francis P**, *Berry V, Bhattacharya S, et al. Genetics of childhood cataract. J Med Genet2000;*37:481–*8.*

9. Gill D, Klose R, Munier FL, McFadden M, Priston M, Billingsley G, et al. Genetic heterogeneity of the Coppock-like cataract: a mutation in CRYBB2 on chromosome 22q11.2. Invest Ophthalmol Vis Sci. 2000;41(1):159-65.

10. Guleria K, Sperling K, Singh D, Varon R, Singh JR, Vanita V. A novel mutation in the connexin 46 (GJA3) gene associated with autosomal dominant congenital cataract in an Indian family. Mol Vis. 2007;13:1657-65.

11. Hejtmamcik JF, Smaoui N. Molecular genetics of cataract. Dev Ophthalmol. 2003;37:67-82.

12. Bhat SP. Crystallins, genes and cataract. Prog Drug Res. 2003;60:205-63.

13. Hansen L, Yao W, Eiberg H, Kjaer KW, Baggesen K, Hejtmancik 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.

14. Berry V, Francis P, Kaushal S, Moore A, Bhattacharya S. Missense mutations in MIP underlie autosomal dominant "polymorphic" and lamellar cataracts linked to 12q. Nat Genet. 2000;25(1):15-7.

15. Jakobs PM, Hess JF, FitzGerald PG, Kramer P, Weleber RG, Litt M. Autosomal-dominant congenital cataract associated with deletion mutation in the human beaded filament protein gene BFSP2. Am J Hum Genet. 2000;66(4):1432-6.

16. Semina EV, Ferrell RE, Mintz-Hittner HA, Bitoun P, Alward WL, Reiter RS, et al. A novel homeobox gene PITX3 is mutated in families with autosomal-dominant cataract and ASMD. Nat Genet. 1998;19(2):167-70.

17. Vanita V, Singh D, Robinson PN, Sperling K, Singh JR. A novel mutation in the DNA-binding domain of

MAF at 16q23-1 associated with autosomal dominant "cerulean cataract" in an Indian family. Am J Med Genet A. 2006;140(6):558-66.

18. Forshew T, Johnson CA, Khaliq S, Pasha S, Willis C, Abbasi R, et al. Locus heterogeneity in autosomal recessive congenital cataracts: linkage to 9q and germline HSF4 mutations. Hum Genet.2005;117(5):4529.

19.Jordan T, Hanson I, Zaletayev D, Hodgson S, Prosser J, Seawright A, Hastie N, van Heyningen V (August 1992). "The human PAX6 gene is mutated in two patients with aniridia". *Nat.Genet.* **1**(5):328-32.

20. "Genes and Mapped Phenotypes." National Center for Biotechnology Information. U.S. National Library of Medicine, 12 Apr. 2014. Web. 14 Apr. 2014.

21. "PAX6." Genetics Home Reference. U.S. National Library of Medicine, 7 Apr. 2014. Web. 14 Apr. 2014.

22. "PAX6 in Sensory Development." Human Molecular Genetics. Oxford Journals, 15 May 2002. Web. 14 Apr. 2014.

23. Shengxiu, Li, Dan Goldowitz, and Douglas J. Swanson. "The Requirement of Pax6 for Postnatal Eye Development: Evidence from Experimental Mouse Chimeras." Investigative Ophthalmology & Visual Science, 1 July 2007. Web. 14 Apr. 2014.

24. Xie, Q., and D. Ung. "Gene Regulation by PAX6: Structural-functional Correlations of Missense Mutants and Transcriptional Control of Trpm3/miR-204."

25. National Center for Biotechnology Information. U.S. National Library of Medicine, 6 Mar. 2014. Web. 14 Apr. 2014.

26. Walcher T, Xie Q, Sun J, Irmler M, Beckers J, Öztürk T, Niessing D, Stoykova A, Cvekl A, Ninkovic J, Götz M (March 2013). "Functional dissection of the paired domain of Pax6 reveals molecular mechanisms of coordinating neurogenesis and proliferation". *Development* **140** (5): 1123–36.

27. Strachan, Tom, Andrew P. Read. PAX genes. (1994) Current Opinion in Genetics and Development. 4: 427-438.

28. Zuker, Charles S. (1994) On the Evolution of Eyes: Would You Like It Simple or Compound? Science. 265: 742-743.

29. Francis PJ, Berry V, Bhattacharya SS, Moore AT. The genetics of childhood cataract. *J Med Genet.* 2000;37:481–4

30. Ren Z, Li A, Shastry BS, et al. A 5-base insertion in the _C-crystallin gene is associated with autosomal dominant variable zonular pulverulent cataract. *Hum Genet*. 2000;106:531–537.88.

31. Heon E, Paterson AD, Fraser M, et al. A progressive autosomal recessive cataract locus maps to chromosome 9q13–q22. *Am J Hum Genet*. 2001;68:772–777.

32. Wang KJ, Zhu SQ, Cheng J. Progress in pathogenic genes and their functions of congenital cataract. 2010;46(3):280-284

33. Cai F, Zhu J, Chen W, Ke T, Wang F, Tu X, Zhang Y, Jin R, Wu X. A novel PAX6 mutation in a large Chinese family with aniridia and congenital cataract. 2010;16:1141-1145.

34. Song S, Liu Y, Guo S, Zhang L, Zhang X, Wang S, Lu A, Li L. A novel PAX6 gene mutation in a Chinese family with aniridia. 2005;11:335-337.

35. Glaser T, Jepeal L, Edwards JG, Young SR, Favor J, Maas RL. PAX6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central nervous system defects. Nat Genet 1994; 7: 463–471. Erratum in: Nat Genet 1994;8:203.

36. Davis A, Cowell JK. Mutations in the PAX6 gene in patients with hereditary aniridia. Hum Mol Genet 1993; 2: 2093–2097.

37. Vincent MC, Gallai R, Olivier D, Speeg-Schatz C, Flament J, Calvas P et al. Variable phenotype related to a novel PAX6 mutation (IVS4þ5G4C) in a family presenting congenital nystagmus and foveal hypoplasia. Am J Ophthalmol 2004; 138: 1016–1021.

38. Mirzayans F, Pearce WG, MacDonald IM, Walter MA. Mutation of the PAX6 gene in patients with autosomal dominant keratitis. Am J Hum Genet 1995; 57: 539–548.

39. Hanson IM, Fletcher JM, Jordan T, Brown A, Taylor D, Adams RJ et al. Mutations at the PAX6 locus are found in heterogeneous anterior segment malformations including Peters' anomaly. Nat Genet 1994; 6: 168–173.

40. Azuma N, Yamaguchi Y, Handa H, Tadokoro K, Asaka A, Kawase E et al. Mutations of the PAX6 gene detected in patients with a variety of optic-nerve malformations. Am J Hum Genet 2003; 72: 1565–1570.

41. Fucheng Cai, et al., A novel *PAX6* mutation in a large Chinese family with aniridia and congenital cataract *Molecular Vision* 2010; 16:1141-1145.

42. Dansault A, et al., Three new PAX6 mutations including one causing an unusual ophthalmic phenotype associated with neurodevelopmental abnormalities. Mol Vis 2007, 13:511-23.

43. Chien YH, et al.,Eye anomalies and neurological manifestations in patients with PAX6 mutations. Mol Vis 2009, 15:2139-45.

44. Manel Chograni, et al., Absence of mutations in four genes encoding for congenital cataract and expressed in the human brain in Tunisian families with cataract and mental retardation BMC Ophthalmology 2011, 11:35.

45. Manel Chograni, et al., Molecular analysis of the PAX6 gene for aniridia and congenital cataracts in Tunisian families. Human Genome Variation (2014) 1, 14008.

46. L. Wang, et al., Identification of one novel mutant *PAX6* allele in Chinese congenital aniridia and cataract family Int J Clin Exp Med 2016;9(3):5848-5853

47. Nomyuki A., et al., Missense Mutation in the Alternative Splice Region of the PAX6 Gene in Eye Anomalies *Am. J. Hum. Genet. 65:656–663, 1999*

48. Tom glaser, et al.,PAX6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central nervous system defects. *Nature genetics*.1994;7:463-471.