Molecular analysis of the PAX6 gene for congenital aniridia in the Korean population: Identification of four novel mutations

Shin Hae Park,1 Man Soo Kim,1 Hyojin Chae,2 Yonggoo Kim,2 Myungshin Kim2

1Seoul St. Mary’s Hospital, Department of Ophthalmology and Visual Science, College of Medicine, The Catholic University of Korea, Seoul, Korea; 2Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea

Purpose: To analyze the paired box gene 6 (PAX6) in Korean patients with congenital aniridia.

Methods: Genomic DNA was isolated from peripheral blood leukocytes of 22 aniridia patients in 18 unrelated families. Polymerase chain reaction was performed for all 14 exons of PAX6 followed by bidirectional sequencing.

Results: Fourteen different kinds of mutations were detected in 16 of 18 unrelated families (mutation detection rate: 88.9%), including four novel mutations; c.658G>T (p.Glu220*), c.464delG (p.Ser155Thrfs*52), c.879dupTGTA (p.Glu31Cysfs*26), and c.642A>C (p.Arg214Ser), among which the former three mutations induce premature termination of PAX6 protein translation. Approximately 92.9% of identified mutations lead to the premature termination of the protein resulting from 7 nonsense mutations (50.0%), 3 splicing errors (21.4%), 2 deletions (14.3%), and 1 insertion (7.1%).

Conclusions: Most of the mutations identified in Korean aniridia patients lead to the premature truncation of the PAX6 protein, supporting that PAX6 protein haploinsufficiency causes the classic aniridia phenotype. We also found four novel PAX6 mutations associated with aniridia.

Congenital aniridia (OMIM 106210) is a rare ocular malformation that affects the development of multiple ocular structures and is caused by a mutation in the paired box gene 6 (PAX6) located on chromosome 11p13 [1-3]. Iris hypoplasia is the most obvious sign, but a broad spectrum of disorders can manifest [2-4]. Many patients have corneal opacities, cataracts, nystagmus, and foveal and optic nerve hypoplasia. Aniridia typically causes severe visual impairment; the major causative factor of this condition is foveal hypoplasia [5].

The incidence of congenital aniridia ranges from 1:64,000 to 1:96,000 [5]. In two-thirds of the cases, it is inherited in an autosomal dominant fashion with almost complete penetrance and variable expressivity; and the remaining one-third of the cases are sporadic [1,6,7]. Some sporadic cases have a risk of developing Wilms tumor as a part of WAGR (Wilms tumor, aniridia, genitourinary abnormalities, and mental retardation; OMIM 194072), which is caused by deletion of both PAX6 and Wilms’ tumor gene (WT1) in the 11p13 region.

PAX6 was isolated as a candidate gene for aniridia by positional cloning in 1991 [8]. Heterozygous mutations are found in about 40%–80% of all non-syndromic aniridia patients [1,9-11]. Numerous PAX6 mutations have been detected in aniridia patients (Online Human PAX6 Allelic Database), and premature termination of the PAX6 protein is the most frequent type of mutation [11].

Although about 60 cases of congenital aniridia have been reported in Korea since the first report in 1977, little is known about the molecular characterization of congenital aniridia in Koreans [12-15]. Here, we analyzed PAX6 in 22 Korean aniridic patients and identified the genetic aberrations and genotype-phenotype correlations.

METHODS

This study was approved by the Ethics Committee of Seoul St. Mary’s Hospital, The Catholic University of Korea (KC11RISI0722). Informed consent was obtained from the patients.

We evaluated 22 patients in 18 unrelated aniridia families in Seoul St. Mary’s Hospital. The age, gender, visual acuity, family history, and previous ocular history of the patients were recorded. Thorough ocular examinations were performed, including best-corrected visual acuity (BCVA), intraocular pressure (IOP), and refractive measurement and slit lamp biomicroscopy of the anterior segment and fundus. After receiving informed consent, blood samples were collected from all patients for DNA extraction and PAX6 analysis.

Genomic DNA was isolated from peripheral blood leukocytes with the QIAmp DNA Mini Kit (Qiagen, Hamburg, Germany). The DNA was quantified spectrophotometrically using a ND-1000 (Nanodrop Technologies Inc., Wilmington, DE). All 14 exons (including an alternatively spliced exon 5a) of PAX6 were amplified using the primers as previously described (Table 1) [10]. For all amplicons, the genomic DNA was denatured at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 1 min,
and final extension at 72 for 5 min. The PCR products were examined by agarose gel (1.5%) electrophoresis followed by staining the gel in ethidium bromide (0.5 μg/ml), which then was visualized under ultraviolet (UV) light in a gel documentation system (Gel Doc 1000; Biorad, Hercules, CA). PCR amplicons were bidirectionally sequenced with the Big Dye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). RefSeq ID: NM_000280.3 was used for cDNA nucleotide numbering.

### RESULTS

**Ocular phenotypes:** Table 2 shows the ocular phenotypes of 22 patients in 18 unrelated families tested. The male/female ratio was 0.59. The percentage of sporadic cases was 36.4%. The mean age of the patients was 19.4±14.7 years. Total aniridia was demonstrated in 20 patients and partial aniridia in 2 patients (patients 6-1 and 17). Glaucoma, cataracts, keratopathy above grade II, and foveal hypoplasia were also observed in addition to iris aplasia in the patients, as detailed below. Nephroblastoma did not develop during the follow-up period.

Glaucoma was observed in 7 of 22 patients (31.8%). Six patients could maintain IOP within the normal range with topical anti-glaucoma medications, but one female (patient 12) required surgical treatment with Ahmed valve implantation in her left eye at 12 years old.

Cataracts were seen in 18 of 22 patients (81.8%), and 6 had received cataract surgery. Congenital corneal opacity was observed in two children (patients 2 and 14), who required penetrating keratoplasty. A fundus examination was performed in 20 patients who did not have severe corneal or lens opacity. Foveal hypoplasia, defined as the absence of a foveal reflex, was found in 17 patients (85%).

**Genetic analysis of PAX6:** The patients’ molecular findings are summarized in Table 3. Fourteen different mutations were detected in 16 of 18 unrelated families (88.9%). We found four novel mutations, including c.87_90dupTGTA, c.464delG, c.642A>C, and c.658G>T, in addition to 10 known mutations: c.11–2A>G, c.19G>T, c.301delG, c.317T>A, c.524–2A>G, c.607C>T (n=3), c.718C>T, c.901C>T, c.949C>T (n=2), and c.1183+2T>C [9,16-26].

The types of mutations were as follows: 11 single nucleotide substitutions, including 1 missense mutation.
| Case No | Age/gender | Inheritance | BCVA (OD/OS) | Nystagmus | Keratopathy | Cataract | Glaucoma | Macular hypoplasia | Comments |
|---------|------------|-------------|--------------|-----------|-------------|----------|----------|-------------------|----------|
| 1-1     | 27/F       | Familial    | 0.1/0.1      | +         | Grade II    | +        | -        | +                 |          |
| 1-2     | 3Mon/F     | Familial    | 0.2/0.16     | +         | Grade IV    | -        | -        |                  | uncheckable |
| 2       | 34/M       | Sporadic    | 0.25/0.16    | +         | Grade I     | -        | -        |                  |          |
| 3       | 15/M       | Sporadic    | 0.04/0.04    | +         | Grade II    | -        | -        |                  |          |
| 4       | 1/F        | Sporadic    | 0.03/0.03    | +         | Grade IV    | +        | +, eyedrops |                  |          |
| 5       | 24/M       | Familial    | 0.04/0.04    | +         | Grade II    | +        | +, eyedrops | +                |          |
| 6-1     | 31/F       | Sporadic    | HM/0.02      | +         | Grade IV    | +        | +, eyedrops | +                |          |
| 6-2     | 1/M        | Familial    | F&F (+)      | +         | Grade II    | +        | +, eyedrops | +                |          |
| 7-1     | 8/F        | Familial    | 0.1/FC 30 cm | +         | Grade III   | +        | -        | +                |          |
| 7-2     | 40/M       | Familial    | FC 30 cm /0.1| +         | Grade IV    | +        | +, eyedrops | +                |          |
| 8-1     | 48/F       | Familial    | 0.02/0.02    | +         | Grade IV    | +        | -        | +                |          |
| 8-2     | 15/F       | Familial    | 0.160.06     | +         | Grade I     | +        | +, surgery | +                |          |
| 9       | 21/M       | Sporadic    | 0.32/0.2     | +         | Grade IV    | +        | +, eyedrops | uncheckable | Corneal opacity |
| 10      | 3/M        | Sporadic    | FC10 cm/LP-  | +         | Grade IV    | +        | +, eyedrops | uncheckable | Corneal opacity |
| 11      | 30/M       | Familial    | 0.1/FC 30 cm | +         | Grade IV    | +        | -        | +                |          |
| 12      | 15/F       | Familial    | FC50 cm/0.1  | +         | Grade I     | +        | -        | +                |          |
| 13      | 8/M        | Sporadic    | 0.16/0.2     | +         | Grade II    | +        | -        | +                |          |
| 14      | 4/M        | Familial    | 0.16/0.16    | +         | Grade III   | +        | +, eyedrops | +                |          |
| 15      | 30/M       | Familial    | 0.02/0.1     | +         | Grade III   | +        | +, eyedrops | +                |          |
| 16      | 48/F       | Familial    | 0.04/0.04    | +         | Grade IV    | +        | -        | +                |          |
| 17      | 16/M       | Familial    | 0.06/0.06    | +         | Grade I     | +        | -        | +                |          |
| 18      | 8Mon/M     | Sporadic    | F&F (+)      | +         | Grade II    | +        | -        | -                |          |

M: Male; F: Female; F&F: Fix and follow; PD: paired domain; LNK: linker domain; HD: Homeodomain; PST: proline-, serine-, and threonine-rich transregulatory domain. Keratopathy was graded as follows: grade 0, clear; grade 1, peripheral muddling with ingrowth of neovascular tissue not exceeding 1 mm from the limbal arch; grade II, peripheral neovascularization in at least the peripheral half of the cornea, corneal clouding, and subepithelial fibrosis; grade III, involvement of the central cornea.
(7.1%), 7 nonsense mutations (50.0%), and 3 intronic mutations that lead to splicing errors (21.4%), in addition to 3 indel mutations that resulted in frameshifts (21.4%).

The DNA-binding domains (DBDs) of the PAX6 protein were composed of the 128 amino acid paired domain (PD) and the 61 amino acid homeodomain (HD) separated by a linker region (LNK). The proline-, serine-, and threonine-rich transregulatory (PST) domain in the COOH-terminal region was composed of 152 amino acids. In this study, 5 kinds of mutations (35.7%) occurred in the PD, and 3 kinds of mutations (21.4%) occurred in the LNK, HD, and PST domains. Relatively fewer mutations have been detected in the PST domain considering its long size.

Four novel mutations were detected in this study (Table 3, Figure 1). Patient 3 showed the c.87_90dupTGTA† mutation in exon 5 within the PD, which resulted in premature termination due to the frame-shift. Patients 6–1 (mother) and 6–2 (son) possessed a 1-bp deletion, c.464delG (p.Ser155Thrfs*52), in exon 7, which causes a frameshift and premature termination of translation in the LNK domain of the PAX6 protein. Patient 10 was 3 year-old male and had a novel missense mutation, c.642A>C (p.Arg214Ser), in exon 8 within the HD. This mutation was predicted to be not tolerable by SIFT analysis and possibly damaging by PolyPhen analysis. This child had a severe clinical manifestation of aniridia with marked corneal opacity and increased IOP in both eyes. This mutation was not detected in his unaffected mother. Patient 11 showed a novel nonsense mutation, c.658G>T (p.Glu220*), in exon 8, which results in premature termination within the LNK domain.

Single nucleotide variation (SNV) c.766–12C>T (rs667773) was detected in 3 patients in 2 probands (7–1, 7–2, 10) who represented c.524–2A>G and c.642A>C, respectively.

**Genotype-phenotype correlation:** Ophthalmic and genetic findings exhibited inter- and intrafamilial phenotypic variabilities of the disease. Patient 1–2 had bilateral congenital corneal opacities presumed to be accompanying Peters anomaly, which were not observed in her mother (Patient 1–1) with the same splicing error mutation (c.11–2A>G). In the family with the c.464delG mutation (Patients 6–1 and 6–2), one showed complete aniridia, and the other showed partial aniridia. Patient 8–2 had juvenile onset glaucoma in both eyes, which was not detected in Patients 8–1 and 9 with the same nonsense mutation (c.607C>T).

We did not find any phenotypic differences according to the location of the identified genotype. Nineteen patients had total aniridia irrespective of the domain of identified mutations, except for Patient 6–1 with the LNK domain frameshift mutation. The ocular phenotypes in patients with three truncating mutations in the PST domains, which retained the intact DNA-binding domains, were comparable to that with mutations within the PD and HD domains.
DISCUSSION

In this report, we described PAX6 mutations in 22 Korean aniridia patients from 18 unrelated families. The mutation detection rate was 88.9% (16/18). The mutation spectrum of PAX6 in aniridia was highly biased, as 92.9% of identified mutations included 7 nonsense mutations (50.0%), 3 splicing errors (21.4%), 2 deletions (14.3%), and 1 insertion (7.1%), leading to the premature truncation of the protein and one missense mutation inducing an amino acid change in the HD domain. Interestingly, 4 novel mutations were identified in this study, including 3 mutations (c.87_90dupTGTA, c.464delG, and c.658G>T) leading to the premature termination of the PAX6 protein and one missense mutation (c.642A>C).

The phenotype of aniridia could be explained by the haploinsufficiency of the PAX6 protein, in which the mutated PAX6 protein does not have any transcriptional activity and the remaining single normal copy of PAX6 is not enough to produce a sufficient threshold level of biologically active PAX6 protein to initiate the transcription of its target genes [11,27,28]. A critical dose of PAX6 protein is required to initiate the transcription of its downstream target genes for normal eye development [28]. Nonsense-mediated decay, which is the process in which mRNAs containing premature termination codons are degraded before they produce large amounts of truncated proteins, is relevant to the pathomechanism of aniridia because the major mutations detected in aniridia are truncations. Haploinsufficiency in the mutants in the COOH-terminal half of the PAX6 protein could be explained by dominant-negative effects, which could be caused by competition for DNA-binding between truncated PAX6 proteins and wild-type PAX6 proteins. Some truncated mutants have 3–5 fold higher affinities to various DNA binding sites when compared with the wild-type PAX6 [27].

The clinical manifestations associated with aniridia express variable phenotypes. We did not find any phenotypic differences according to the location of the identified genotypes. Our results also exhibited interfamilial (Patients 8–2 and 9) and intrafamilial (Patients 1–1 and 1–2, Patients 6–1 and 6–2, and Patients 8–1 and 8–2) phenotypic variabilities of the disease. Atchaneeyasakul et al. reported that the total aniridia phenotype was associated with mutations at the COOH-terminus, whereas partial aniridia patients carried mutations that resulted in a loss of the homeodomain with or without a loss of the paired domain [29]. However, our results are not consistent with that finding. Total aniridia was observed in 19 patients irrespective of the domain of identified mutations, except for patient 6–1 with the frameshift mutation within the LNK. The reason for variable phenotypes among individuals with the same mutation is unclear. The variable expressivity could be explained by subtle differences in PAX6 protein levels and the ratio of mutant to wild type PAX6 protein or the
interactions with other factors [27]. Mutations behind the HD could potentially lead to more severe phenotypes than truncating mutations within the DNA-binding domains, such as the PD and HD [11,28]. Generally, the PAX6 missense mutation occurs less frequently in aniridia and has a tendency to be associated with milder phenotypes [18,30]. A PAX6 protein with an amino acid substitution could still retain some residual activity and result in partial haploinsufficiency. Some missense mutations might have the potential to impair the proper folding of the PAX6 protein and compromise the normal three-dimensional structure. In our 3-year-old male (Patient 10) carrying the novel missense mutation c.642A>C (p.Arg214Ser), other factors than the mutated PAX6 protein might contribute to his severe phenotype.

According to the PAX6 mutation database, the most frequent PAX6 mutations in aniridia are c.607C>T, c.718C>T, c.949C>T, and c.1267dupT (Online Human PAX6 Allele Database) [9,11]. The former three mutations were also identified in this study. The distribution of the identified mutations was as follows: 35.7% in the PD, 21.4% in the LNK, 21.4% in the HD, and 21.4% in the PST domains. Definite mutational hot spots were not observed in our study. In two patients, a PAX6 mutation was not identified with direct DNA sequencing throughout the whole gene. Exon deletions and deletions of control regions can be the cause of isolated aniridia, so that tests used to identify gene copy number, such as quantitative PCR, multiplex ligation-dependent probe amplification (MLPA), and array comparative genomic hybridization, may be helpful to clarify such cases.

c.766-12C>T (rs667773) was found in 2 probands. This SNV has been considered as probably a neutral polymorphism and defined as variation in 1000 Genomes. Various mutations were detected with this SNV including c.949C>T, c.277G>A, c.607C>T, c.1267dupT in the PAX6 mutation database (Online Human PAX6 Allelic Database), and we cannot find any cosegregation pattern.

Generally, it is recommended to perform several analyses in aniridia to obtain the maximum detection yield, as aniridia could be caused by different types of genetic aberrations. Chromosomal rearrangement and deletion can be detected by karyotype analysis especially in the cases of WAGR or the aniridia patients presenting other malformations [24]. Fluorescence in situ hybridization and MLPA can detect cryptic deletion of PAX6 effectively [24,31]. Detection of PAX6 mutations was performed using direct sequencing method combined with or without mutation detection screening tools such as DHPLC (denaturing high performance liquid chromatography) or SSCP (single-strand conformation polymorphism). Mutation detection rate by direct sequencing of PAX6 was variable as follows; 47% (18/38) in Chinese [32], 49% (34/70) in Caucasian [31], 30% (9/30) in Mexican [9], and 67% (4/6) in Thai patients [29]. To our best knowledge, our PAX6 mutation detection rate of 88.9% is the one of highest rates by single test alone. One of the estimated reason of our high mutation detection rate is the characteristics of patients included in this study. Most of patients had clinically definite non-syndromic aniridia with total absence of iris (20/22). The other possible reason to improve detection rate is that we performed bidirectional sequencing in all samples because the mutations in aniridia patients were distributed throughout the whole exon and intron of PAX6. Based on our result, the bidirectional DNA sequencing including whole exon and intron-exon boundary of PAX6 could be recommended as the first screening test for the molecular confirmation of aniridia, especially when it is not combined with other systemic abnormalities such as renal tumor, genitourinary abnormalities, and mental retardation.

In conclusion, most of the mutations identified in Korean aniridia patients lead to the premature truncation of the PAX6 protein, supporting that haploinsufficiency of the PAX6 protein causes the classic aniridia phenotype. Also, we found four novel PAX6 mutations associated with aniridia.

ACKNOWLEDGMENTS
This work was supported by “Laboratory reagent development and evaluation for clinical application (10024719)” under the Industrial Source Technology Development Programs of the Ministry of Knowledge Economy (MKE) of Korea.

REFERENCES
1. Prosser J, van Heyningen V. PAX 6 mutations reviewed. Hum Mutat 1998; 11:93-108. [PMID: 9482572]
2. Hill RE, Janson IM. Molecular genetics of the PAX gene family. Curr Opin Cell Biol 1992; 4:967-72. [PMID: 1485966]
3. Macdonald R, Wilson SW. PAX proteins and eye development. Curr Opin Neurobiol 1996; 6:49-56. [PMID: 8794051]
4. Holland EJ, Djalilian AR, Schwartz GS. Management aniridic keratopathy with keratolimbal allograft: a limbal stem cell transplantation technique. Ophthalmology 2003; 110:125-30. [PMID: 12511357]
5. Tremblay F, Gupta SK, De Becker I, Guernsey DL, Neumann PE. Effects of PAX 6 mutations on retinal function: an electroretinographic study. Am J Ophthalmol 1998; 126:211-8. [PMID: 9727515]
6. Nelson LB, Spaeth GL, Nowinski TS, Margo CE, Jackson L. Aniridia a reviews. Surv Ophthalmol 1984; 28:621-42. [PMID: 6330922]
7. Shaw MW, Falls HF, Neel JV. Congenital aniridia. Am J Hum Genet 1960; 12:389-415. [PMID: 17948455]
8. Ton CC, Hirvonen H, Miwa H, Weil MM, Monaghan P, Jordan T, van Heyningen V, Hastie ND, Meijers-Heijboer H, Drechsler M, Roder-Pokora B, Collins F, Swaroop A, Strong L, Saunders G. Positional cloning and characterization of a paired box- and homeobox-containing gene from the aniridia region. Cell 1991; 67:1059-74. [PMID: 1684738]
9. Villarroel CE, Villanueva-Mendoza C, Orozco L, Alcántara-Ortigoza MA, Jiménez DF, Ordaz JC, González-del Angel A. Molecular analysis of the PAX6 gene in Mexican patients
with congenital aniridia: report of four novel mutations. Mol Vis 2008; 14:1650-8. [PMID: 18776953]

10. Glaser T, Walton DS, Maas RL. Genomic structure, evolutionary conservation and aniridia mutations in the human PAX6 gene. Nat Genet 1992; 2:232-9. [PMID: 1345175]

11. Kokotas H, Petersen MB. Clinical and molecular aspects of aniridia. Clin Genet 2010; 77:409-20. [PMID: 20132240]

12. Park YG, Suh DH, Lee HS. 4 Cases of Congenital Aniridia. J Korean Ophthalmol Soc 1977; 18:419-22.

13. Ahn SK, Kang JS, Shyn KH. A case of congenital aniridia. J Korean Ophthalmol Soc 1989; 30:81-94.

14. Kim JH, Hwang BS, Lee JH, Cha SC. PAX6 Mutations and Clinical Features of Congenital Aniridia. J Korean Ophthalmol Soc 2008; 49:1794-800.

15. Park SH, Park YG, Lee MY, Kim MS. Clinical Features of Korean Patients with congenital Aniridia. Korean J Ophthalmol Soc 2010; 24:291-6. [PMID: 1917456]

16. Churchill AJ, Hanson IM, Markham AF. Prenatal diagnosis of aniridia. Ophthalmology 2000; 107:1153-6.  [PMID: 10857836]

17. Hever AM, Williamson KA, van Heyningen V. Developmental malformations of the eye: the role of PAX6, SOX2 and OTX2. Clin Genet 2006; 69:459-70. [PMID: 16712695]

18. Hingorani M, Williamson KA, Moore AT, van Heyningen V. Detailed ophthalmologic evaluation of 43 individuals with PAX6 mutations. Invest Ophthalmol Vis Sci 2009; 50:2581-90. [PMID: 19218613]

19. Neethirajan G, Krishnadas SR, Vijayalakshmi P, Shashikant S, Sundaresan P. PAX6 gene variations associated with aniridia in south India. BMC Med Genet 2004; 5:9. [PMID: 15086958]

20. Gupta SK, De Becker I, Tremblay F, Guernsey DL, Neumann PE. Genotype/phenotype correlations in aniridia. Am J Ophthalmol 1998; 126:203-10. [PMID: 9727514]

21. Maekawa M, Iwayama Y, Nakamura K, Sato M, Toyota T, Ohnishi T, Yamada K, Miyachi T, Tsuji M, Hattori E, Maekawa N, Osumi N, Mori N, Yoshikawa T. A novel missense mutation (Leu46Val) of PAX6 found in an autistic patient. Neurosci Lett 2009; 462:267-71. [PMID: 19607881]

22. Chien YH, Huang HP, Hwu WL, Chien YH, Chang TC, Lee NC. Eye anomalies and neurological manifestations in patients with PAX6 mutations. Mol Vis 2009; 15:2139-45. [PMID: 19898691]

23. Redeker EJ, de Visser AS, Bergen AA, Mannens MM. Multiplex ligation-dependent probe amplification (MLPA) enhances the molecular diagnosis of aniridia and related disorders. Mol Vis 2008; 14:836-40. [PMID: 18483559]

24. Robinson DO, Howarth RJ, Williamson KA, van Heyningen V, Beal SJ, Crolla JA. Genetic analysis of chromosome 11p13 and the PAX6 gene in a series of 125 cases referred with aniridia. Am J Med Genet A 2008; 146A:558-69. [PMID: 18241071]

25. Li PC, Yao Q, Ren X, Zhang MC, Li H, Liu JY, Sheng SY, Wang Q, Liu MG. Analysis of PAX6 gene in a Chinese family with congenital aniridia. Zhonghua Yan Ke Za Zhi 2009; 45:931-4. [PMID: 20137456]

26. Vincent MC, Pujo AL, Olivier D, Calvas P. Screening for PAX6 gene mutations is consistent with haploinsufficiency as the main mechanism leading to various ocular defects. Eur J Hum Genet 2003; 11:163-9. [PMID: 12634864]

27. Singh S, Tang HK, Lee JY, Saunders GF. Truncation mutations in the transactivation region of PAX6 result in dominant-negative mutants. J Biol Chem 1998; 273:21531-41. [PMID: 9705283]

28. Cvekl A, Sax CM, Bresnick EH, Piatigorsky J. A complex array of positive and negative elements regulates the chicken alpha A-crystallin gene: involvement of Pax-6, USF, CREB and/or CREM, and AP-1 proteins. Mol Cell Biol 1994; 14:7363-76. [PMID: 7935450]

29. Atchaneeyasakul LO, Trinavarat A, Dulayajinda D, Kumpornsin K, Tungnoppakhun W, Yenchitsomanus PT, Limwongse C. Novel and de-novo truncating PAX6 mutations and ocular phenotypes in Thai aniridia patients. Ophthalmic Genet 2006; 27:21-7. [PMID: 16543198]

30. Grønskov K, Rosenberg T, Sand A, Brandum-Nielsen K. Mutational analysis of PAX6: 16 novel mutations including 5 missense mutations with a mild aniridia phenotype. Eur J Hum Genet 1999; 7:724-86. [PMID: 10234503]

31. Redeker EJ, de Visser AS, Bergen AA, Mannens MM. Multiplex ligation-dependent probe amplification (MLPA) enhances the molecular diagnosis of aniridia and related disorders. Mol Vis 2008; 14:836-40. [PMID: 18483559]

32. Zhang X, Wang P, Li S, Xiao X, Guo X, Zhang Q. Mutation spectrum of PAX6 in Chinese patients with aniridia. Mol Vis 2011; 17:2139-47. [PMID: 21850189]