

A Clinical and Genetic Review of Aniridia

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Aniridia is a congenital pan-ocular, bilateral disorder. The term aniridia is a misleading misnomer, since at least a rudimentary iris is always present. Varied forms range from almost total absence to only mild hypoplasia of the iris. It is inherent in a number of syndromes, including Wilms tumor Aniridia-Genital anomalies-retardation (WAGR). Aniridia has been shown to be associated with mutations in the *PAX6* gene, located on chromosome 11p13, telomeric to the Wilms' tumor predisposition gene (*WT1*). The pair box gene 6 (*PAX6*) situated at 11p13 has been confirmed to be the leading gene associated with aniridia. The *PAX6* mutation is present in individuals worldwide and has been studied in Indian, Malaysian, Chinese and Mexican families. Several categories of *PAX6* mutations include: nonsense mutations, splicing mutations, frameshift mutations (deletion or insertion), in-frame insertion or deletion, missense mutations and run-on mutations. A novel *de novo* frameshift mutation in *PAX6* most possibly occurred in the paternal gamete. Mutation in *PAX6* brings about amino acid substitution for instance proline to glutamine. Deletion of 11p13 involves the *PAX6* (aniridia) locus and the adjacent *WT1* (Wilms tumor) locus. Haploinsufficiency at the *PAX6* locus brings on aniridia, a pan-ocular eye condition characterized by iris hypoplasia and various other anterior and posterior eye defects, subtle hypogonadotropic hypogonadism and borderline Growth Hormone (GH) deficiency. Aniridia may also be affiliated with retinal tears and detachments. Electroretinograms (ERGs) done in aniridia illustrate definite retinal dysfunction. Other clinical aspects related to aniridia are ptosis with reduced levator function and anterior polar cataracts. The *PAX6* gene mutation was also associated with early-onset diabetes mellitus and aniridia. Aniridia combined with zonular cataract and polydactyly was also described in a patient with Bardet-Biedl syndrome. Aniridia with sensorineural deafness (cochlear) and aplasia of the patella was also reported; an autosomal-dominant inheritance with 100% penetrance. The WAGR syndrome is associated with obesity. Because of all the associations with other diseases as mentioned above, ophthalmologists should pay more attention to aniridia as a sign of a systematic disease. As aniridia is a global disease and has been reported in all five continents and several countries with different ethnic groups, herein we reviewed this important disorder.

Keywords: Aniridia; Genetic; PAX6; Congenital; Disease

1. Context

Aniridia (OMIM 106210) is a rare congenital disorder characterized by the complete or partial absence of the iris. The incidence of this pathology is about 1 in 64000 - 96000 live births (1) some defects of the cornea, lens, retina, optic nerve, and/or the anterior chamber angle is accompanied by aniridia (2). Other ocular abnormalities, such as corneal opacification, cataract, glaucoma, fovea and optic nerve hypoplasia, nystagmus, and ectopia lentis, may accompany aniridia (3-5). The inheritance of an autosomal first characteristic with high penetrance and variable expressivity is recognized in two thirds of the cases of congenital aniridia (6, 7). The location of aniridia gene on chromosome 11p13 was validated by linkage analysis and positional cloning. Pair box gene 6 (*PAX6*) located at 11p13, is the major gene associated with aniridia (8-10). Some related syndromes with aniridia are Wilms tumor, bilateral sporadic aniridia, genitourinary abnormalities and mental retardation (WAGR) syndrome. Furthermore, *PAX6* mutations alter corneal cytokeratin expression, cell adhesion and glycoconjugate expression. These muta-

tions result in stem-cell deficiency, which in turn leads to a fragile cornea and Aniridia-Associated Keratopathy (AAK). The angle differentiation abnormality can result in glaucoma. Progressive angle closure glaucoma develops as a result of peripheral anterior synechia. The lens capsule is fragile and there is cataract formation. The iris is partially or totally deficient. The optic nerve and fovea are hypoplastic, and the retina may be prone to detachment (11). Here, we review the data regarding the mechanisms and the mutations that are related to aniridia as a universal disease, which is reported in all five continents and several countries with different ethnicities.

2. Evidence Acquisition

To review the current key characteristics of aniridia as a rare congenital disorder, several databases such as PubMed and Google scholar, using the following keywords were investigated: aniridia, genetic and congenital disorder, and *PAX6*. Herein, the qualitative results

emanated from the reviewed articles are presented and discussed.

3. Results

In general, aniridia occurs in isolation, but in sporadic cases deletions of 11p13 involving both *PAX6* and *WT1* gene, which is located 700 kb telemetrically from *PAX6*, give rise to Wilm's tumor, bilateral sporadic aniridia, genitourinary abnormalities and mental retardation (WAGR) syndrome (12). The *PAX6* mutations with a variety of ocular malformations include: nonsense mutations, splicing mutations, frame shift mutations (deletion or insertion), in-frame insertion or deletion, missense mutations and run-on mutations. Many mutations of *PAX6* have been identified by separate groups, and are recorded in the *PAX6* allelic variant database (13). According to the study by Tzoulaki et al. (14), genotype-phenotype correlations of *PAX6* mutations, and the distribution of aniridia-associated *PAX6* mutations are as follows: 38.9% nonsense mutations, of which the majority (59%) are C-T changes at the CpG dinucleotides, in exons 8, 9, 10 and 11; 13.2% splice mutations; 25.3% frameshift deletions or insertions; 6.2% in-frame deletions or insertions; 11.7% missense mutations; and 4.7% run-on mutations (14). Premature translational termination on one of the alleles in these mutations produces haploid insufficiency of *PAX6* with decreased expression of the protein product (15). In the study of Shimo et al. aniridia with a heterozygous *PAX6* mutation was reported in a female patient. *Pax6* is a hormone-stimulating test that revealed a slightly impaired pituitary function, including subtle hypogonadotropic hypogonadism and borderline growth hormone (GH) deficiency (16). Two novel mutations of the *PAX6* gene causing different phenotypes were detected in a cohort of Chinese patients in a study by Zhang et al. (17). The *PAX6* gene mutations were studied in a cohort of patients with different clinical phenotypes including aniridia, coloboma of iris and choroid, or anterior segment malformations with Peters' anomaly. Sequencing of the *PAX6* gene, revealed three intragenic mutations, including a novel heterozygous splicing-site mutation, c.357-3C4G (p.Ser119fsX), in patients of the aniridia group. A strange missense mutation, c.643T4C (p.S216P), was detected in the anterior seg-

ment of the malformation group. The p.S216P mutation located in the homeodomain region of the *PAX6* caused the phenotype of Peters' anomaly in the A6 family, with various expressions. Zhang et al. (17) through multiplex ligation-dependent probe amplification (MLPA) analysis detected a large deletion including the whole *PAX6* gene and DKFZ p686k1684 gene in one sporadic patient from the aniridia group. Bandah et al. (18) identified aniridia in one family with an Ashkenazi-Jewish origin. Aniridia accompanied with congenital cataract, nystagmus and glaucoma had affected the index patient and her daughter. A heterozygous *PAX6* frameshift mutation in exon 6 (c.577_578insG, insG@Gly72) was identified in the affected individuals and not in any of the unaffected family members, including the parents of the index patient. Microsatellite analysis revealed that the index patient inherited the disease haplotype from her unaffected father. In a family with autosomal dominant aniridia, Bandah et al. (18) also found a novel *de novo* frameshift mutation in *PAX6*, which presumably occurred in the paternal gamete. In a study by Yan et al., *PAX6* was analyzed in a Chinese pedigree of nystagmus, cataract and iris anomalies. This mutation in *PAX6* caused an amino acid substitution of proline to glutamine at position 118 (p.P118Q) of the paired domain of the *PAX6* protein (19) (Figure 1).

In a study by Lin et al. (20) that investigated the paired box gene 6 (*PAX6*) in three patients, including two patients from two successive generations of one family and one sporadic patient with a heterozygous *PAX6* frameshift mutation, c.891del A (p.Gln297HisfsX68) in exon 10 was identified in the affected individuals and not in any of the unaffected family members, including the unaffected family members of the proband patient's generation. Lin et al. (20) detected one novel mutation, c.607C > T (Arg203X) in exon 8, in the unrelated sporadic patient. In another study, Chen et al. (21) reported on a Chinese family with autosomal dominant congenital aniridia. A novel heterozygous *PAX6* deletion, c.1251_1353del103 (p.Pro418Serfs*87), affecting exon 14 and the 3'-untranslated-region (3'-UTR) was identified in a family with congenital aniridia. Bioinformatics analysis showed that the deletion led to remarkable changes of the *PAX6* protein, including a frameshift, changes of protein sequence, and a

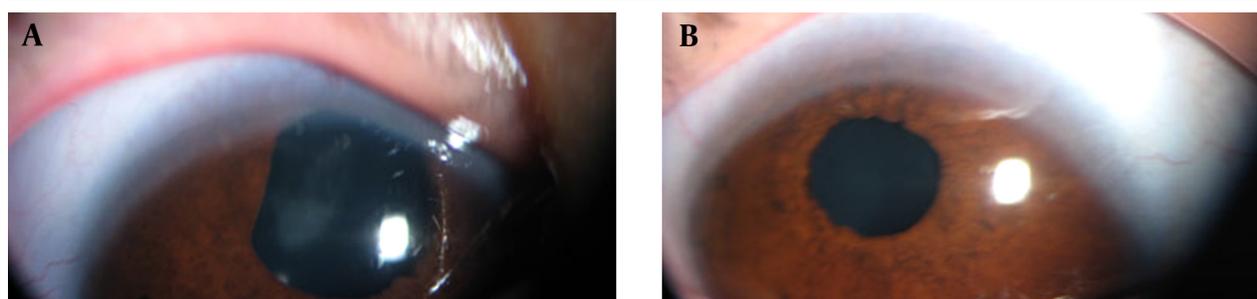


Figure 1. A and B Show Iris Anomalies, and Nasally and Superiorly Displaced Pupil and Cataract in Both Eyes of the Proband

COOH-terminal extension. The COOH-terminal extension might also affect microRNA binding sites in the 3'-UTR as predicted by the TargetScan (21). Ivanov et al. (22) by direct sequencing screened for the *PAX6* gene in three groups of patients including those affected by aniridia, those with diverse ocular manifestations, and those with Peters' anomaly. Two mutations were investigated by generating crystallographic representations of the amino acid changes. The L46P mutation was found in patients presenting bilateral microphthalmia, cataracts, and nystagmus. The S74G mutation was found in a large family that had congenital ocular abnormalities, diverse neurological manifestations and variable cognitive impairments. The 579delG deletion (V48fsX53) was induced in the affected members of the same family with bilateral aniridia associated with congenital cataract, foveal hypoplasia and nystagmus. A *de novo* previously known nucleotide change, g.972C > T (Q179X), in exon 8, leading to a stop codon and a heterozygous g.555C > A (C40X) recurrent nonsense mutation in exon 5. No mutations were found in patients with Peters' anomaly (22). Peter et al. (23) studied fourteen family members, eight affected and six unaffected controls, in a family with ptosis, cataract, iris hypoplasia and gradual corneal opacification occurring in relation to a *PAX6* mutation. Ptosis was seen in eight affected subjects with reduced levator function, anterior polar cataracts and corneal changes of variable severity, two of these patients had undergone penetrating keratoplasties. Five patients had iris hypoplasia. One patient had aphakic glaucoma and another had hypoplastic optic discs. Four of the six controls showed no ocular features of this syndrome, and two had isolated mild ptosis. In all eight patients, genetic analysis confirmed a pathogenic *PAX6* mutation in exon 12 (c1439delC), yet this was not found in any of the controls (23). Valenzuela and Cline (24) noted that aniridia is brought by mutation of *PAX6* or deletion of a regulatory region controlling its expression, or as part of the Wilms' tumor-Aniridia-Genital anomalies-Retardation (WAGR) syndrome, with a deletion of 11p13 involving the *PAX6* (aniridia) locus and the adjacent *WT1* (Wilms tumor) locus. Aniridia has also been reported in four Japanese families by Kondo-Saitoh et al. (7) PCR-SSCA (polymerase chain reaction-Single strand conformation polymorphism analysis) of the proband from one family demonstrated an extra-band in the PCR product for *PAX6* exon 8. Base-sequence analysis showed that the patient was a heterozygote for a C to T transition mutation at codon 203. Furthermore, DNAs from the patient and another affected member in the same family were cut with MaeIII into two fragments, while non-affected members in the family showed only one MaeIII fragment; the outcome confirmed the presence of mutation. In another family, PCR-SSCA revealed an extra-band in the PCR product for exon 9. Sequencing detected a C->T substitution at codon 240 in a patient; the mutation resulted in the loss of an *AvaI* site. The mutation in the patient was confirmed by *AvaI* cleavage analysis. The two

transition mutations observed in the two families also predict the conversion of arginine to a stop codon (R203X and R240X, respectively) around the homeodomain (HD), leading to the truncation of the *PAX6* protein within its glycine-rich region. No abnormal Single-Strand Conformation Polymorphism (SSCP) bands or abnormal restriction fragments were detected in patients from the other two families (7). In 2013, hereditary causes of the paired box 6 (*PAX6*) gene responsible for aniridia in two three-generation Chinese families were studied by Chen et al. (25). A heterozygous *PAX6* mutation in exon 5 (c.112delC, p.Arg38GlyfsX16) was identified in FAMILY-1, which was predicted to generate a frameshift and created a premature termination codon. A heterozygous *PAX6* mutation in exon 7 (c.362C > T, p.Ser121Leu) was recognized in FAMILY-2. Each mutation cosegregated with the affected individuals in the family and did not exist in unaffected family members and 200 unrelated normal controls. The *PAX6* messenger ribonucleic acid level was about 50% lower in patients with aniridia than in unaffected family members in FAMILY-1 (25). Phenotypes and identity of the genetic defect responsible for aniridia and congenital progressive cataract were reported in a three-generation Chinese family in 2012. Sequencing of the candidate gene detected a heterozygous c.307C > T transition in the coding region of *PAX6*, resulting in the substitution of a highly conserved arginine codon for a termination codon (p.R103X) (26). In a Malaysian family, a heterozygous G deletion (c.857delG) in exon 7 was determined using a PCR-HRM (high resolution melting) (27). In another aniridia study, sequencing of *PAX6* showed that a heterozygous duplication mutation, c.95_105dup11, generated a non-functional truncated protein at position Gly36 (p.G36X), which was found in affected individuals but not in any of the unaffected family members including the parents of the proband. Mutation of *PAX6* occurred *de novo* on a paternal chromosome by direct duplication, which presumably created replication slippage or unequal non-sister chromatids exchange during spermatogenesis (28). A novel *PAX6* duplication in exon 5 at position c.474dupC was identified by Goswami et al. This was a report on a duplication in a North Indian family with autosomal dominant aniridia (29). In a study by Godavova et al. (30), aniridia DNA analysis revealed the presence of p.Gln180X *PAX6* mutation in all of the affected individuals. The mutation led to shortened and therefore non-functional proteins. The 14 exons of the *PAX6* gene were analyzed exon-by-exon using SSCP in six aniridia families by Davis and Cowell (31). In each family band shifts were observed on the SSCP gels for only one exon and direct PCR-sequencing revealed mutations in each case. Two mutations involved C->T transitions in CGAarg codons in exons 9 and 11. Another C->T transition converted a CAG-glutamine to a TAG-stop in exon 7. Small insertions created frameshifts, which produced downstream stop codons in another two patients and an A->T mutation disrupted the splice donor site of exon 5 in the remaining

family members (31). Van et al. (32) diagnosed two female neonates that were postpartum with bilateral aniridia. The first patient had the familial form, induced by a point mutation in the paired box 6 (*PAX6*) gene. The second patient had sporadic aniridia caused by a *de novo* microdeletion involving both the *PAX6* gene as well as the Wilms tumor suppressor-1 (*WT1*) gene (32). In the study of Sonoda et al. (33), a variety of *PAX6* gene mutations were identified in patients with aniridia and/or allied ocular dysgenesis, such as keratopathy, Peters' anomaly, foveal hypoplasia, and nystagmus. A novel missense mutation in the *PAX6* gene was found in all affected individuals examined. This mutation predicted a proline to arginine change at codon 118 (P118R) in the paired domain of *PAX6* protein (33). In another study, Villarroel et al. (34) analyzed *PAX6* variants in a group of Mexican aniridia patients and identified eight different mutations: four (c.184_188dupGAGAC, c.361T > C, c.879dupC, and c.277G > A) were new and another four (c.969C > T, IVS6+1G > C, c.853delC, and IVS7-2A > G) were previously reported. A substitution at position 969 was observed in two patients. Most of the mutations predicted either truncation of the *PAX6* protein or conservative amino acid changes in the paired domain. They also detected two intronic non-pathogenic variations, IVS9-12C > T and IVS2+9G > A, that had been previously reported. Because the latter variation was considered potentially pathogenic, it was analyzed in 103 healthy Mexican newborns where they found an allelic frequency of 0.1116 for the A allele (34). Yasuda et al. (35) reported that diabetes was cosegregated with aniridia in one family, and a single nucleotide polymorphism in intron 9 of the *PAX6* gene was correlated with the disorder, suggesting that a mutation, possibly located in an uncharacterized portion of the *PAX6* gene, can explain both diabetes and aniridia in this family (35). *PAX6* is also involved in the development of the endocrine pancreas, and has been reported to be a genetic factor common to aniridia and glucose intolerance, although the latter is usually mild. A case of *PAX6* gene mutation with early-onset diabetes mellitus and aniridia was reported by Nishi et al. (36). Low insulin secretory capacity in her parents suggested that her insulin secretory defect is caused not only by *PAX6* mutation but other genetic factors inherited from her parents. Bremond-Gignac et al. (37) examined 33 probands, out of which, 27 were affected with congenital cataract while the remaining six were affected with aniridia (with or without cataract). The coding regions of *FOXE3*, *PAX6*, *PITX2*, and *PITX3* were examined by direct DNA sequencing of gene-specific PCR products. A novel dominant mutation at the stop codon of *FOXE3*, c.959G > C (p.X320Ser>X72), was identified in a patient with congenital cataract. Another novel *FOXE3* sequence change, c.571-579dup (p.Tyr191_Pro193dup), was identified in a patient with aniridia (37). Cheng et al. (38) assessed the mutations in *PAX6*. The aCGH analysis showed two copies of *PAX6* but a 566 kb hemizygous deletion of chromosome 11p13, including four annotated

genes; doublecortin domain containing 1 (*DCDC1*), DnaJ homolog subfamily C member 24 (*DNAJC24*), IMPI inner mitochondrial membrane (*IMMP1L*), and elongation factor protein 4 (*ELP4*) downstream of *PAX6* (38). Neethirajan et al. (39) studied one of the causes of aniridia. Haploinsufficiency at the *PAX6* locus causes aniridia, a panocular eye condition characterized by iris hypoplasia and a variety of other anterior and posterior eye defects leading to poor vision. Single-Strand Conformation Polymorphism band shifts, indicative of DNA base pair mutations, were observed in five of the patients. These new mutations were c.1174delTG (in exon 10), c.710delC (exon 6), c.406delTT (exon 5) and c.393insTCAGC (exon 5). The other nonsense mutation, a transition (c.1080C > T) in exon 9, was reported previously as a mutation hotspot for *PAX6* in other ethnic pedigrees (39). Yuan et al. (40) studied the role of the *PAX6* gene in hereditary aniridia in a northeastern Chinese population and identified two novel *PAX6* mutations. The first was a nine base pair (bp) deletion in exon 5 (c.483del9) that makes a *PAX6* protein with *de novo* in-frame deletions of aspartic acid, isoleucine, and serine at the amino acid codon positions 41-43 (40). Ramirez-Miranda and Zenteno (41) studied the *PAX6* gene in a group of patients with congenital aniridia from Mexican mestizo origin and found three novel intragenic deletions; a 15 bp deletion in exon 9 that removes the last two codons of the exon and the first nine bases of intron 10, including the conserved GT splicing donor signal; a 14 bp deletion in exon 6 that introduces a premature stop signal, 15 codons downstream, and a 4 bp deletion in exon seven, which introduces a stop signal 22 codons downstream, in three unrelated probands (41). Wang et al. (42) identified four novel mutations including c.141 + 1G > A, c.184 - 3C > G, c.542C > A (Ser181X), and c.562C > T (Gln188X) and one known mutation c.120C > A (Cys40X) in *PAX6* of five unrelated patients with aniridia. All five mutations are expected to generate null alleles of *PAX6*. Varied ocular phenotypes were observed in different patients within families (42). Similarly, aniridia was noted by Perveen et al. (43) in an individual from a family with *PITX2*. Other anterior segment dysgenesis including Peter's anomaly, Rieger syndrome and anomaly and anterior-segment mesenchymal dysgenesis may also be seen in patients with this mutation. Mirkinson and Mirkinson (44) reported another syndrome with aniridia and aplasia of the patella, an autosomal-dominant inheritance with 100% penetrance. In 2007, two siblings with 46XY disorder, congenital adrenal hypoplasia, aniridia, dysmorphic facial features, intrauterine growth retardation and skeletal abnormalities were identified by Coman et al. (45). Courteney-Harris and Mills (46) reported aniridia with sensorineural deafness (cochlear) in a father and daughter, with the second daughter only having deafness. Bremond-Gignac et al. (47) described WAGRO syndrome (WAGR + Obesity); this may be attributed to the deletion of an obesity gene. Aniridia combined with zonal cataract and polydactyly was also explained in a

patient with Bardet-Biedl syndrome by Verloes et al. (48). Electroretinograms (ERGs) performed by Tremblay et al. (49) for patients with aniridia, revealed definite retinal dysfunction, although its etiology is not yet clear. Aniridia may also be associated with retinal tears and detachments. Dowler et al. (50) described that retinal detachments occurred in four eyes of three children due to giant retinal tears. All the eyes were buphthalmic, and had no prior history of cataract extraction or posterior segment surgery. The patients were treated with vitreolensectomy and silicone oil injection. Two of the eyes recovered useful vision (51). To determine the value of Optical Coherence Tomography (OCT) as a diagnostic tool in the critical evaluation of phenotypic variability seen in an aniridia family with a novel *PAX6* mutation, anterior segment OCT demonstrated a range of iridocorneal angle abnormalities and corneal thickening in only three of the patients while ciliary body hypoplasia identified this in all four affected patients. Posterior segment OCT demonstrated dome-shaped, hypoplastic macular profiles in the two affected children. Novel outer retinal changes were also seen, suggestive of a phototoxic retinopathy not previously recognized in aniridia. Ocular disease segregated with a novel *PAX6* Q178X nonsense mutation with Western blot analysis suggesting that this led to haploinsufficiency of *PAX6* protein (51).

4. Conclusions

As aniridia is a complicated disorder that results in defective vision from multiple causes, ophthalmologists should consider aniridia in patients with unusual iris malformations. Corneal opacification often develops later in childhood and may lead to progressive deterioration of visual acuity. The corneal abnormality is due to a stem cell deficiency and therefore keratolimbal allograft stem cell transplantation may be a more effective treatment than corneal transplantation. Examination of family members may be the key in making the diagnosis. Any refractive error should be corrected, as this may improve vision. All aniridic patients should be screened regularly for glaucoma, as this condition may occur at any age and can result to permanent vision loss. Therefore, ophthalmologists should be aware of patients' complications and likewise should emphasize the importance of disease and consult about the outcomes of surgery. Systemic and ocular associations should be considered, as they may be present in combination with aniridia. All children with sporadic aniridia should undergo chromosomal deletion analysis to exclude the possibility of Wilms tumor formation. Positive results require consultation with an oncologist along with repeated abdominal ultrasonographic examinations. Haploinsufficiency at the *PAX6* locus causes aniridia, a panocular eye condition characterized by iris hypoplasia and various anterior and posterior eye defects leading to poor vision. All observed mutations support the notion that haploinsufficiency in *PAX6* results in aniridia and related eye abnormalities.

Conflict of interest

None declared.

Founding Source

None declared.

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