CLINICAL QUIZ (p207) ANSWER

What Is the Diagnosis?

Bilateral Aniridia (OMIN #106210): Aniridia refers to partial or even complete iris hypoplasia (Figure 1a); it may sometimes be associated with foveal hypoplasia (Figure 1b) and presents with nystagmus early after birth. Aniridia is a panocular condition that is also associated with other ocular abnormalities of later onset. The incidence rate of aniridia is approximately 1:40,000-100,000 worldwide without racial or sexual bias.

Aniridia is usually suspected by paediatricians at an early stage following concern over the infant's vision by the parents. The findings should be confirmed by ophthalmologists[1] by slit lamp examination where iris and pupillary abnormalities can be identified with associated corneal opacification/vascularisation, cataract or glaucoma. Foveal hypoplasia as well as other rare optic nerve malformations can be shown with slit lamp fundoscopy but infants require binocular indirect ophthalmoscopy and may even need to be examined under anaesthesia. Foveal hypoplasia may also be confirmed using optical coherence tomography, but it may be difficult to perform in the presence of nystagmus.[2] High-frequency ultrasound biomicroscopy can be used to diagnose iris hypoplasia or aplasia in infants with corneal opacity or congenital glaucoma.[3] Molecular diagnosis is always helpful in supporting the clinical findings and provides further necessary information for clinical management (Figure 2).

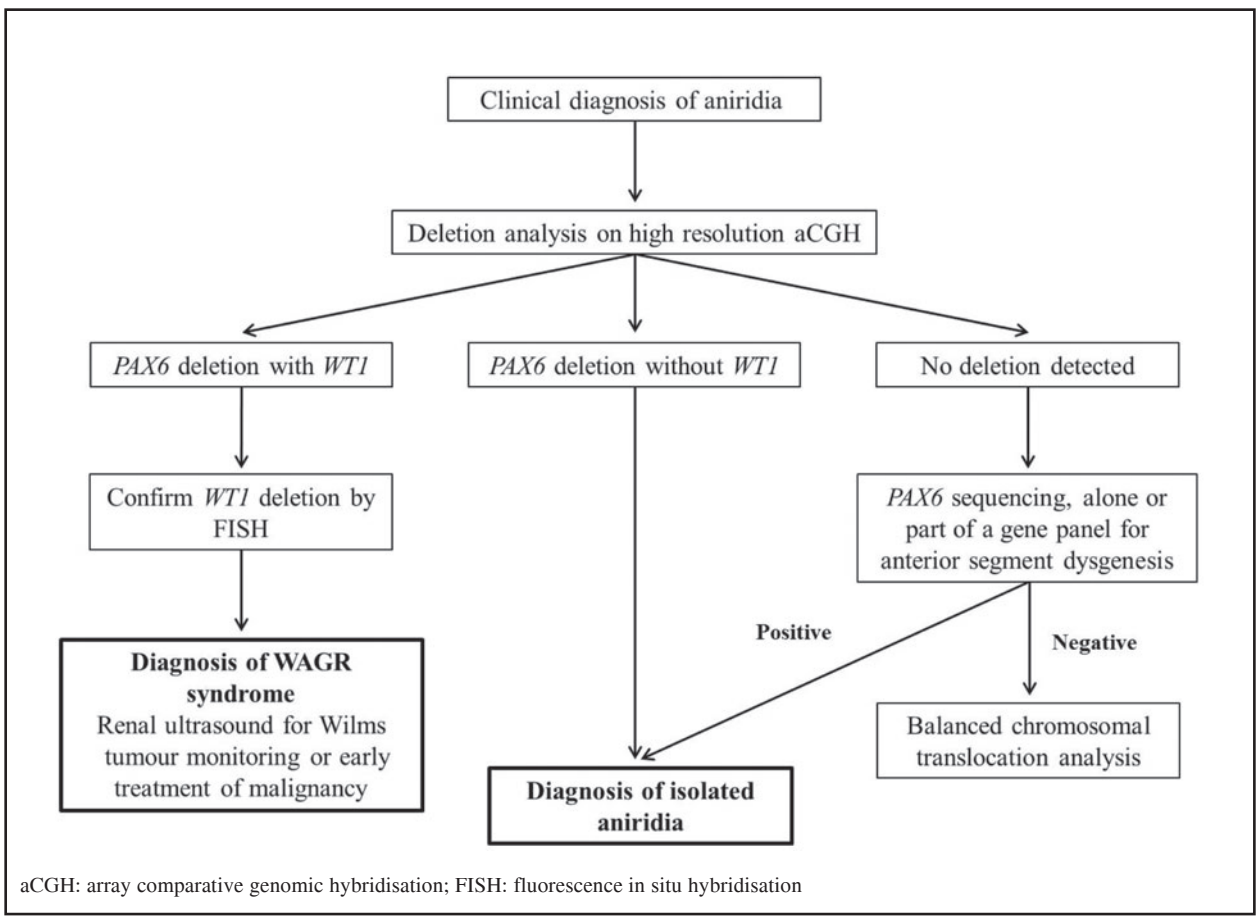

Figure 2 Diagnostic flowchart for patients with aniridia.

How Is the Diagnosis of PAX6 Mutation Established in This Patient?

Aniridia is associated with various genetic syndromes and chromosomal anomalies and it is important to rule out WAGR syndrome (Wilms tumour-aniridia-genital anomalies-retardation syndrome, OMIN#194072) which is associated with increased risk of Wilms tumour.[1] In our patient, array comparative genomic hybridisation (aCGH) was performed (Tsan Yuk Hospital, Hong Kong) and ruled out deletion encompassing the *WT1* and *PAX6* genes located in chromosome 11p13. Secondly, a gene panel of 14 genes related to anterior segment dysgenesis *(DCDC1*, *ELP4*, *FOXE3*, *PAX6*, *WT1*, *LAMB2*, *PITX2*, *PITX3*, *FOXC2*, *FOXC1*, *CYP1B1*, *COL4A1*, *BMP4*, and *B3GALTL)* was performed using Next Generation Sequencing (NGS) (Casey Eye Institute, USA). The result showed that a mutation (c.718C>T, CGA>TGA) in exon 10 of the *PAX6* gene resulting in a substitution of arginine (CGA) to a termination codon (TGA) (Figure 3). This mutation has been reported by Glaser et al in 1992[4] and is one of the most frequent mutation leading to aniridia reported.[5] The finding is confirmed using Sanger sequencing (Figure 4).

Molecular Genetics of Aniridia

PAX6-related aniridia is autosomal dominant in inheritance. It can occur *de novo* (30%) or in individuals with positive family history (70%).[6] Offspring of individuals with aniridia and *PAX6* mutation will have 50% chance of inheriting the condition.[7] Mutation can occur in the sequence or the regulating region of the *PAX6* gene located in the short arm of chromosome 11.[8] The *PAX6* gene encodes the paired box protein Pax-6 (PAX6) which is also known as aniridia type II protein.[9] This protein is a transcription factor and is important in regulating organogenesis by changing expression of other genes.[10] The *PAX6* gene is expressed in the developing organs and is active in early ocular morphogenesis. It is shown to have multiple roles in the development of eye, i.e. cornea, iris, lens and retina.[11] The *PAX6* gene targets itself as well as genes encoding other development regulators, cell adhesion molecules and structural proteins such as corneal keratins and lens crystallins.[10,11] Level of *PAX6* gene activity has been shown to have correlation to the severity of the aniridia phenotype.[4] Mutations of *PAX6* gene result in premature translational termination on one of the two copies of the *PAX6* gene causing haploinsufficiency with decreased expression of the protein product.[12] With the various involvement of *PAX6* gene in the development of the eye, this resulting under expression is likely to cause hypoplasia in the eye. The PAX6 protein is responsible for regulating other growth regulator genes and *FGF8* is one of them. *FGF8* is part of the FGF signaling pathway which regulates cranial growth and pattern. Studies have shown that mutation in the *PAX6* gene cause under-expression of *FGF8* and abnormal FGF signaling will lead to abnormal cranial development such as craniosynostosis and cleft palate.[13] This explains the cleft palate found in our patient.

What Are the Management Issues for the Patient and Other Family Members?

Management of aniridia whether in isolated form or part of WAGR syndrome is focused around the eyes. During childhood, patients should have regular eye examinations and spectacles should be used to correct refractive errors.[6] Photochromic or tinted lens would also be beneficial in reducing sensitivity to light stimulants due to large pupillary aperture. The eye examinations should also include intraocular pressure measurement, optic disc examination and visual field assessment (when the child is old enough to understand and perform the test). For glaucoma, topical treatment is usually chosen before surgery which is reserved for nonresponsive to treatment. Glaucoma surgery may include trabeculectomy, drainage tube surgery or cyclodiode laser treatment.[14] Since it is of later onset in aniridia, glaucoma in infancy is rare and is usually treated with early surgery which includes goniotomy and trabeculotomy. Corneal problems in aniridia are related to limbal stem cell deficiency. Mild cases usually treated with lubricants, mucolytics and may be lacrimal punctal occlusion.[14, 15] Surgery on the cornea is usually undertaken only when corneal opacification causes significant visual impairment since it carries a poor prognosis. Corneal surgery includes limbal stem cell transplantation, anterior lamellar keratoplasty, penetrating keratoplasty and keratoprosthesis (reserved for cases whose traditional corneal transplantation has failed repeatedly).[15] Although cataract extraction can improve visual acuity in patients with opaque lens, other aniridic ocular changes may limit visual acuity improvement. Cataract surgeries in aniridic patients are also technically difficult and with high risks of developing complications due to

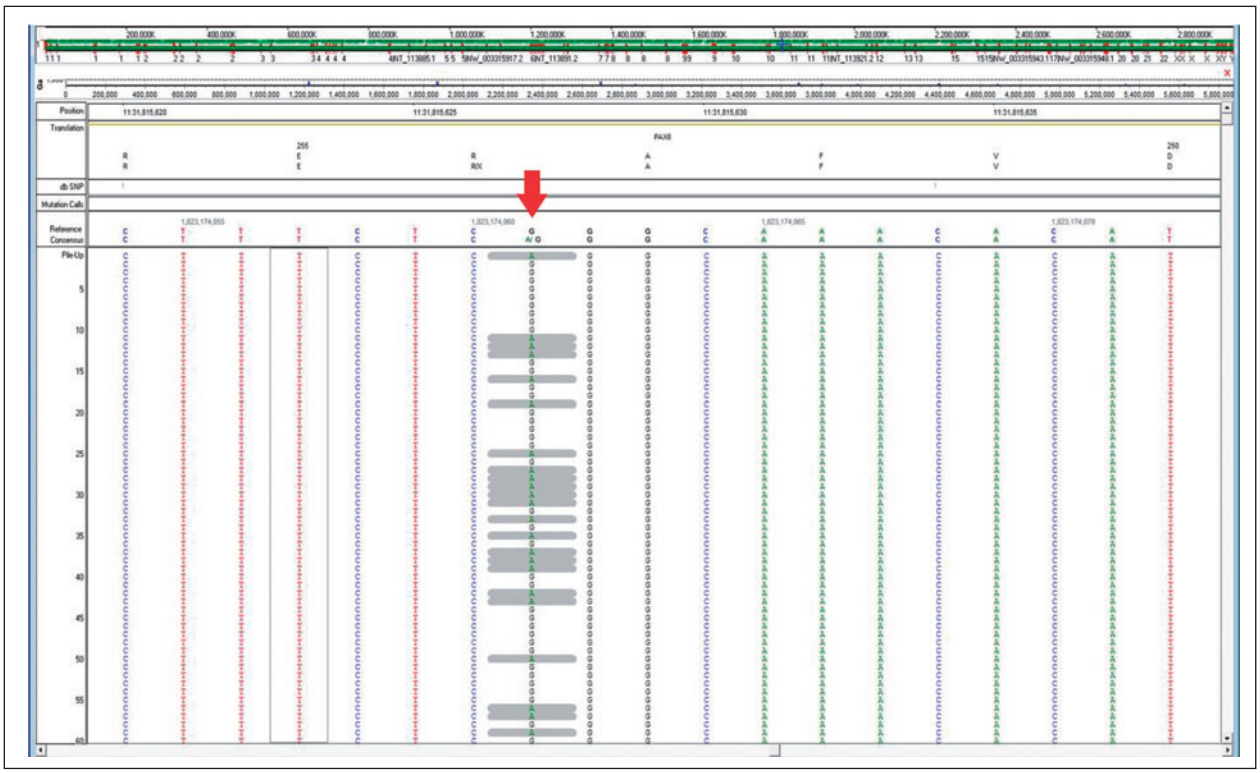

Figure 3 *PAX6* sequencing result by Next Generation Sequencing (NGS). Arrow indicates point of nucleotide change. The sequence show the reverse stands of DNA, therefore a change of C to T would be shown as G to A.

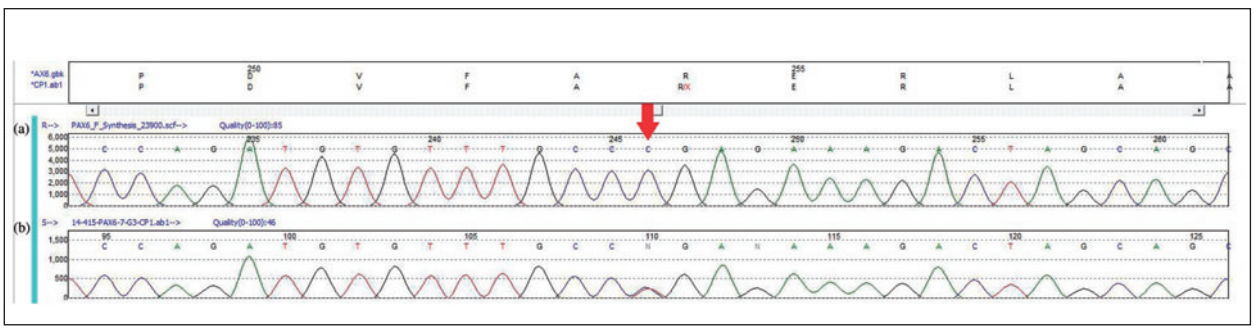

Figure 4 Confirmatory Sanger sequencing of the PAX6 gene. Arrow indicates point of nucleotide substitution. The first graph (a) indicates the sequence of the reference strand which is downloaded from the National Center for Biotechnology Information (NCBI). The second graph (b) is the strand sequenced from the patient's sample as a template. Note the overlapping wave at the point of substitution, indicating the cytosine (C) has been substitute to thymine (T) on one the two alleles.

zonular weakness and abnormal lens capsules.[16] Multiple intraocular surgeries may trigger fibrotic membrane to grow from the root of the iris tissue behind the cornea and lens, causing displacement or entrapment of the intraocular lens and opacification of cornea.[17] This is called aniridic fibrosis syndrome and early removal of the remnant membrane by surgery is recommended to salvage the eye. In case with WAGR syndrome, where *WT1* deletion is present, regular renal screening with ultrasound should be carried out to detect the development of Wilms tumour. The follow up should be carried out in presence of both paediatricians and oncologist every 3 months until the patient turns 8 years of age. Educational and social support would also be recommended for both types of aniridia.

Since the mutation of isolated aniridia can be passed onto the next generation, molecular testing should be followed by genetic counselling. In isolated aniridia, cases with family history and *de novo* have a 50% chance of passing on the mutation to their offspring since isolated aniridia is autosomal dominantly inherited. Whereas in WAGR syndrome, abnormally in genitourinary systems are found in male and can be pleiotropic when *WT1* is deleted especially in cases with large deletions. Therefore the probability of reproductive transmission of *PAX6* and *WT1* deletion found in WAGR is relatively low and most are originated from *de novo* mutation, meaning siblings are rarely affected. However smaller deletions with milder syndromes can be passed on vertically and siblings may have a 50% chance of inheriting the mutation. Prenatal diagnosis can be offered in the form of chorionic villus sampling (10-12 weeks gestation) or amniocentesis (15-18 weeks gestation) to detect any genetic anomaly.

Acknowledgements

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