Prenatal diagnosis for isolated aniridia: A case report and simplified diagnostic approach for ophthalmologists

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Approximately 60%–90% of the isolated aniridia (IA) in India is reported to be sporadic (simplex) in nature, with much lesser contribution by autosomal dominant inheritance. The Indian genomic profile for IA indicates the commonest mutations to be single nucleotide variations in *PAX6*, whereas copy number variants, especially deletions, are rare. Deletions involving *PAX6* along with another gene are even rarer. Our paper highlights an unreported Indian scenario of prenatal genetic counseling for sporadic IA due to *PAX6* and *ELP4* exon deletions and expands the mutation spectrum associated with IA in India.

Key words: Congenital aniridia, next-generation sequencing, PAX6 protein, prenatal genetic counseling

Aniridia is a congenital panocular disorder presenting in isolation or as a syndrome, inherited either as an autosomal-dominant trait or occurring sporadically.[1] If the specific mutation in the proband is known, preimplantation-genetic-diagnosis (PGD) or prenatal diagnosis (PND) can be offered.[1]

Case Report

A nonconsanguineous couple presented in the ninth week of pregnancy desiring prenatal genetic counseling. Their 6-year-old daughter had isolated aniridia (IA) (bilateral complete aniridia, nystagmus, and nonprogressive cataracts), without evidence of glaucoma or keratopathy. She had mild scholastic difficulties too. The parents' ophthalmic evaluation was normal. The couple had reservations about bearing another affected child. Despite outlining a <1% risk of recurrence for sporadic IA,[1,2] normal lifespan and relative lack of morbidity even if born affected; the couple was determined to prevent a recurrence of IA.

Proband's old records revealed normal karyotype and negative multiplex-ligation-probe-dependent-amplification for *PAX6-WT1*-deletion. Next-generation sequencing (NGS)-based clinical-exome sequencing (CES) was ordered in the proband. It revealed large heterozygous- pathogenic deletions in *PAX6* (exons 12-14) and *ELP4* (exons 8-12); confirmed by quantitative-PCR (qPCR) [Supplemental Files 1 and 2]. Testing the parents for the above family-specific mutation and germline mosaicism was declined stating financial restraints. Although the parents were clinically normal and the pedigree was unremarkable [Fig. 1], the proband's IA was most-probably sporadic. The couple conveyed their decision to discontinue the pregnancy if the subsequent prenatal test results returned unfavorably. Amniocentesis and fetal-DNA testing by qPCR for the above specific deletions returned negative, indicating an unaffected fetus. The couple delivered a healthy girl at term.

The genomic diagnosis had collateral benefits for the proband. Her six-monthly renal sonograms were discontinued since the deletions spared *WT1*-region. *PAX6-ELP4* involvement prompted brainstem-evoked-response audiometry (BERA) to rule out auditory-processing-defects, which returned normal. Importance of early detection for any underlying auditory deficits, especially in the setting of preexisting visual impairment and learning difficulties, was highlighted. The need for annual hearing and ophthalmic assessment was reiterated.

Discussion

Mutation in *PAX6*, a crucial gene for ocular morphogenesis, is responsible for 90% of the cases of congenital aniridia.[1] Other candidate genes include *PITX2, PITX3, FOXC1, ELP4*; some yet unidentified.[1] Indian studies have shown sporadic IA to be commoner (60-90%) than the inherited type.[3,4] The commonest IA mutations in India involve single nucleotide variants (SNV) of *PAX6* followed by copy number variants (CNV) in *PAX6*;

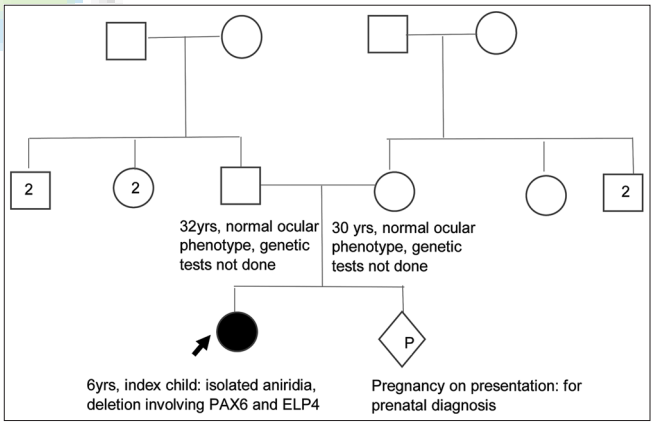

Figure 1: Pedigree chart of the proband. Note the index case was the only affected case in the family

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DOI: 10.4103/ijo.IJO_2208_20

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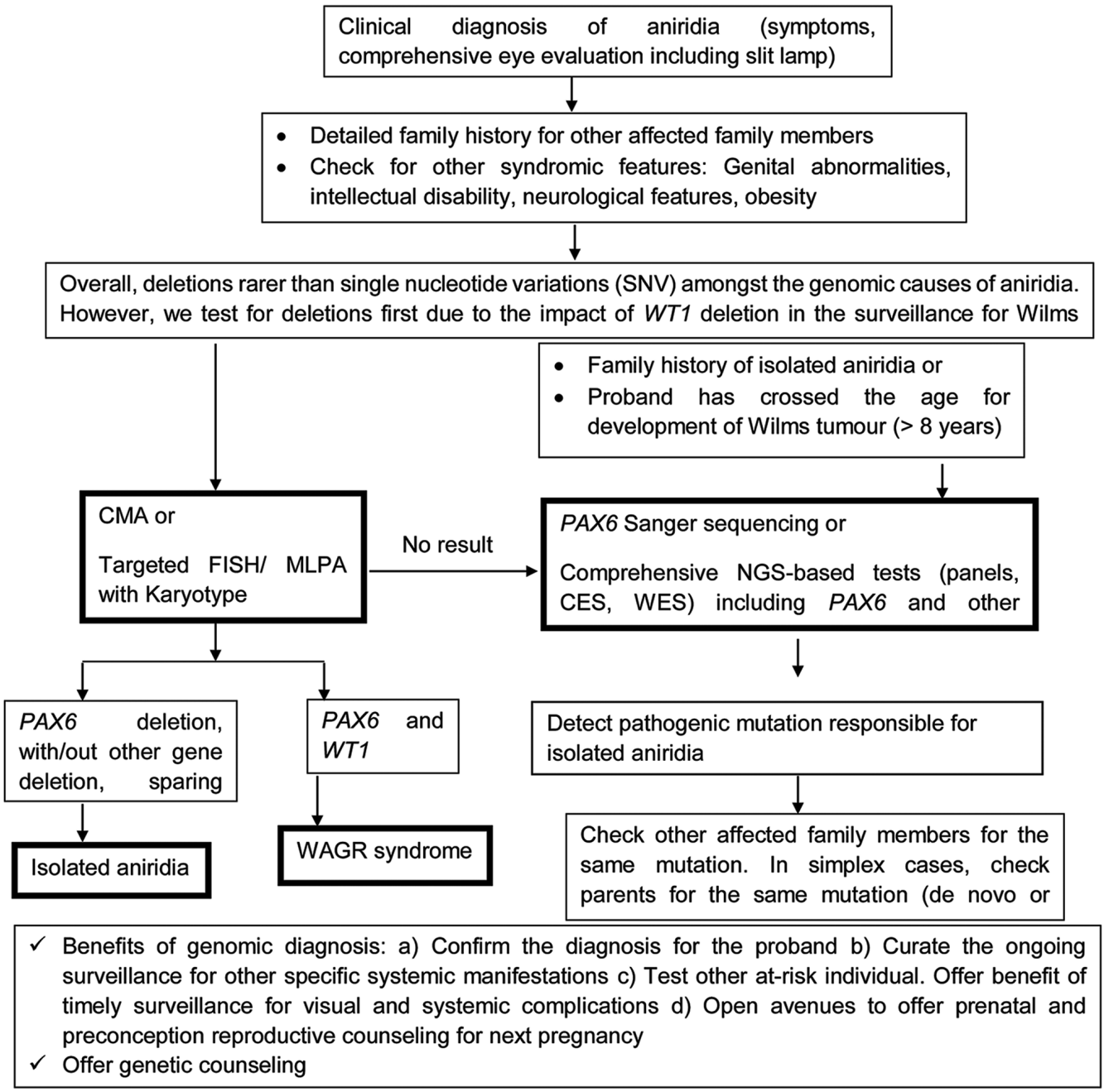
Received: 09-Jul-2020 Revision: 13-Sep-2020
Accepted: 10-Dec-2020 Published: 01-Apr-2021

Cite this article as: Bajaj S, Koradia DR, Kothari M. Prenatal diagnosis for isolated aniridia: A case report and simplified diagnostic approach for ophthalmologists. Indian J Ophthalmol Case Rep 2021;1:302-4.



Abbreviations: CMA: Chromosomal microarray, FISH: Fluorescent insitu hybridization, MLPA: Multiplex ligation probe dependent amplification, NGS: Next-generation sequencing, CES: Clinical exome sequencing, WES: Whole exome sequencing

Figure 2: Flowchart for the genetic approach to a case with aniridia

deletions being rarer than duplications.[3,4] Deletion of *PAX6* and *ELP4* in the same patient, as described here, is the first to be reported in an Indian patient.

ELP4 has crucial regulatory action on *PAX6*.[1] Disruption of *ELP4* alone can induce classical aniridia, even in absence of *PAX6*-mutation.[1] The traditional use of specific genetic tests targeting only *PAX6* (deletion studies, Sanger-sequencing) could be responsible for under-reporting of *ELP4* in IA. Use of chromosomal-microarray (CMA), NGS-based testing options, and targeted tools to detect *ELP4*-deletions could increase our knowledge about *ELP4* involvement in IA.[1] Incomplete genomic diagnosis can especially have an impact on prenatal counseling.

With an expansive understanding of genetics, ophthalmologists would be increasingly expected to incorporate these principles in practice.[5,6] The newer genetic tests; CMA, Sanger-sequencing, and NGS-based options (CES, whole-exome-sequencing, panels, whole-genome-sequencing)

cost in the range of 10,000.00₹–1,50,000.00₹. The onus of choosing the most appropriate test rests on the ordering physician. Ordering the correct test has an impact in terms of the expenses incurred by patient as well as time taken to reach the correct diagnosis. Lack of information about these newer genetic tests,[7] prohibitive costs and accessibility could be some of the factors resulting in an underutilization of genomics in Indian ophthalmology, with the exception of premier teaching institutes. We suggest a simplified approach toward genetic diagnosis of IA [Fig. 2].[1,5]

Precise molecular diagnosis in cases of congenital aniridia has many benefits: a) Diagnosis confirmation b) Curates ongoing surveillance c) Enables testing other at-risk individuals, offering timely surveillance for visual and systemic complications d) Opens avenues to prenatal and preconception reproductive counseling e) With the usher of gene-therapy, genome-editing, CRISPR-CAS9; precise genomic diagnosis can be potentially helpful, most of the futuristic therapies being genotype-specific.[5] For example, ataluren, a drug under-trial phase, is known to improve vision in aniridia, specifically for the types with nonsense-mutations in *PAX6*.[1]

In our case, the parents' outlook toward the recurrence of aniridia and the value of PND can be considered with differing medical perspectives. Given the relative lack of morbidity and normal lifespan; pregnancy-termination in IA is not the obvious medical advice.[1] The purpose of seeking PND could sometimes be to prepare oneself, mentally and financially, for a child needing special care.[8] Amanda *et al*. highlight such a family of father-son duo affected with IA. Even though PND detected the next pregnancy to be affected with IA, the couple gave birth to the baby.[8] PGD, a technique which aids the prospective parents to select the healthy embryo pre-implantation, is an option for those who wish to have an unaffected child, and yet avoid invasive tests and the 'emotional labour' of terminating an affected pregnancy.[9] Literature documents two families who availed of PGD for IA. However, the challenges of in-vitro-fertilization and costs involved in PGD may be unacceptable to some families. Sometimes, severity of the proband's illness, unpredictability of the phenotype of the affected fetus,[1] inaccessibility of visual-rehabilitation services, out-of-pocket expenses,[10] challenged socio-educational background, and perception of parents' own quality of life due to visual-impairment in their child,[11] may make them consider discontinuing a pregnancy with unfavorable PND results.[9] It is thus recommended to follow nondirective genetic counseling that allows couples to take their own informed decision in such grey-zones.[1,7]

Conclusion

We intend to highlight the benefits of timely and appropriate prenatal genetic counseling while underlining its 'nondirective' nature, and report the first Indian IA with *PAX6* and *ELP4* deletion.

Acknowledgements

We would like to thank the parents of the child for their approval and kind support toward publishing this case.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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