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CLINICAL UTILITY GENE CARD

Clinical utility gene card for: Aniridia

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)
Aniridia (Aniridia II).

1.2 OMIM# of the disease
106210.

1.3 Name of the analysed genes or DNA/chromosome segments:
PAX6, *ELP4*.

1.4 OMIM# of the gene(s)
607108 (*PAX6*), 606985 (*ELP4*).

1.5 Mutational spectrum
Approximately two-thirds of aniridia cases are familial with autosomal dominant inheritance, the remainder are sporadic.[1] Aniridia occurs either as an isolated ocular abnormality or as part of the Wilms tumour-aniridia-genital anomalies-retardation (WAGR) syndrome (see Clinical Utility Gene Card for WAGR syndrome[2]). Loss of function of one copy of the *PAX6* gene (NCBI reference sequence NM_001310160.1 and NM_001310161.1) occurs in around 90% of aniridia cases, with intragenic variants accounting for two-thirds and chromosomal rearrangements for one-third of patients. Over 94% of all intragenic point variants result in either premature termination codons (mainly through nonsense, splice and frame-shift insertions or deletions), C-terminal extensions (CTE) or missense changes.[1] To date (January 2016), 403 unique *PAX6* gene variants have been submitted to the online Human *PAX6* Mutation Database (http://pax6/hgu.mrc.ac.uk/). These variants are scattered throughout the *PAX6* gene, generally disrupting transcription or translation. To date, of the 696 public entries in the *PAX6* Mutation Database, over 100 different premature termination codons are recorded, with ~20% accounted for by just four common nonsense variants of arginine (R) residues: c.607C>T, p.R203* (36 reports); c.718C>T, p.R240* (48 reports); c.781C>T, p.R261* (19 reports); and c.949C>T, p.R317* (35 reports). The CTE variants are associated with more severe aniridic phenotypes,[3,4] the *c.1267dupT* has been reported 21 times.

One case of aniridia was reported to have been caused by a heterozygous point variant in the ultra-conserved PAX6 *cis*-regulatory element (SIMO) that resides 150 kb downstream from *PAX6* in intron 9 of the *ELP4* gene (NCBI reference sequence NM_001288726.1).[5] Deletion of regulatory elements, or their separation from the PAX6 transcription machinery through inversion or translocation breakpoints, may manifest with a classical aniridic phenotype. There have been a few reports on aniridia-like phenotypes secondary to variants in *FOXC1*.[6,7]

1.6 Analytical methods
Array comparative genomic hybridisation or multiplex ligation-dependent probe amplification assay (MLPA) should be performed initially to detect deletions or duplications, then bi-directional fluorescent Sanger sequencing of coding and intron–exon boundaries of *PAX6* should follow. Some molecular service labs offer fluorescence *in situ* hybridisation to identify rearrangements that may disrupt *PAX6* without copy number change.[8,9]

1.7 Analytical validation
Parallel bi-directional fluorescent Sanger sequencing of known controls is required to validate procedures. Diagnostic testing must be carried out within a laboratory environment working to standards compliant with the ISO 15189.

1.8 Estimated frequency of the disease (Incidence at birth ('birth prevalence') or population prevalence)
If known to be variable between ethnic groups, please report):

The prevalence of aniridia in the general population is between 1 in 40 000–100 000 with no known predilection for a particular race or gender.

1.9 Diagnostic setting:

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

Comment: Not applicable.

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2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical sensitivity
(**proportion of positive tests if the genotype is present**)

The analytical sensitivity and specificity of bi-directional Sanger sequencing is estimated to be >98% for the detection of nucleotide base changes, small deletions and insertions in the regions analysed. Analytical sensitivity and specificity of MLPA testing is essentially 100% with appropriate testing; very rarely sub-microscopic deletions may reduce analytical sensitivity of MLPA. Patients who appear not to have variants on testing described above, may have deep intronic or other variants in the regulatory elements missed through exonic analysis.

2.2 Analytical specificity
(**proportion of negative tests if the genotype is not present**)

See above. Estimated analytical specificity of >98% given current testing methodologies, based on false positives that may arise due to misinterpretation of rare polymorphic variants that rarely occur in Sanger sequencing.

2.3 Clinical sensitivity
(**proportion of positive tests if the disease is present**)

The clinical sensitivity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

Aniridia presents in infancy, typically characterised by complete or partial iris hypoplasia and foveal hypoplasia, resulting in reduced visual acuity and nystagmus. There are progressive features including cataract, glaucoma and corneal abnormalities. The clinical sensitivity is >98%. Although rare phenocopies do exist and include dominant alleles of *FOXC1, PITX2* and *PITX3* which can cause diagnostic difficulties.[6,7,10,11] Phenotypically subtle *PAX6* variants have been documented,[12–14] including those that segregate with nystagmus, foveal hypoplasia and autosomal dominant keratitis in the absence of iris abnormalities supporting the concept of gene dosage effects, variable expressivity and gonadal mosaicism.[15] Deep phenotyping studies have revealed smaller corpus callosum area on brain volumetry following magnetic resonance imaging, and subtle hearing difficulties associated with interhemispheric transfer problems.[16] The clinical examination alone in atypical phenotypes would not lead to a *PAX6*-related molecular diagnosis, highlighting the importance of genetic testing.

2.4 Clinical specificity
(**proportion of negative tests if the disease is not present**)

The clinical specificity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

Aniridia is a congenital disorder, a positive test in a patient without signs of this condition is unlikely, and hence the clinical specificity will be high, nearing 100%.

2.5 Positive clinical predictive value (life-time risk of developing the disease if the test is positive)

Estimated >99% for *PAX6* variants, as aniridia presents in infancy with high penetrance.

2.6 Negative clinical predictive value (probability of not developing the disease if the test is negative)

Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:

Nearly 100% if no aniridia.

Index case in that family had not been tested:

Nearly 100% if no aniridia, however the clinical phenotype can be variable with subtle signs and familial recurrence if parental mosaicism, partial or non-penetrance exists, therefore it is recommended that the family is tested.[17]

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected
(To be answered if in 1.9 'A' was marked)

3.1.1 Can a diagnosis be made other than through a genetic test?

No (continue with 3.1.4)	☐	
Yes	☒	
	Clinically	☒
	Imaging	☒
	Endoscopy	☐
	Biochemistry	☐
	Electrophysiology	☐
	Other (please describe)	

3.1.2 Describe the burden of alternative diagnostic methods to the patient

Patients with suspected aniridia can be diagnosed based on clinical examination, predominantly with the slit-lamp (hand-held for infants) to identify iris and pupillary abnormalities, corneal opacification and vascularisation, and cataract or glaucoma. Fundoscopy (indirect ophthalmoscopy for infants) may reveal foveal hypoplasia and associated optic nerve abnormalities, however, an examination under anaesthesia may be required. Optical coherence tomography (OCT) may be useful to document foveal hypoplasia, but this may be difficult in the presence of nystagmus and in young children, though hand-held OCT may help.[18] If severe corneal opacity or oedema exists due to congenital glaucoma, anterior segment ultrasound biomicroscopy may aid detection of any iris defects.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

Aniridia is a rare disorder, and patients will often require tertiary referral for accurate diagnosis. Clinical examination provides a cost-effective diagnosis, but high-resolution imaging can be costly.

3.1.4 Will disease management be influenced by the result of a genetic test?

No ☐	
Yes ☒	
Therapy (please describe)	Pharmacological approaches such as translational bypass therapy using ataluren are being developed to treat nonsense-mediated aniridia.[19]
Prognosis (please describe)	The genotype has been associated with disease severity; null variants predominantly cause classical aniridia with iris aplasia and progressive sight-threatening disease.[3] CTE variants are associated with more severe aniridic phenotypes,[4] whereas missense variants produce a variable spectrum ranging from mild iris defects and preserved visual acuity to severe features including optic nerve malformations, Peters anomaly and microphthalmia.[20,21]
Management (please describe)	Aniridia should be managed by specialists with expertise in this condition. Supportive measures for those with sight impairment include involvement of social services. Regular follow-up will be required to monitor progression of corneal disease, cataract and glaucoma with medical and surgical interventions where needed. Regular refraction and provision of tinted or photochromic lenses to reduce light sensitivity. Genetic counselling will be offered to the family. Audiological evaluation may help identify and support early school age children with aniridia-associated central auditory processing deficits.[15]

3.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history
(To be answered if in 1.9 'B' was marked)

3.2.1 Will the result of a genetic test influence lifestyle and prevention?

If the test result is positive (please describe)	The result may impact on career choices and inform family planning. Currently, no medical or lifestyle therapies exist to prevent sight deterioration or disease progression. If a deletion involves both *PAX6* and *WT1* leading to a risk of Wilms tumour, the influence on lifestyle will significantly change, please refer to the management section of the Clinical Utility Gene Card for WAGR syndrome.[2]
If the test result is negative (please describe)	The result will inform family planning.

3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?
Some aniridic patients may be 'legally blind' or experience progressive vision loss making professions that require perfect vision near impossible. Hence, a clinically confirmed diagnosis can help to provide guidance in career choice.

3.3 Genetic risk assessment in family members of a diseased person
(To be answered if in 1.9 'C' was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?
Yes.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
No. Parents of an index patient with a *de novo* gene variant should be examined for subtle aniridic changes, and be checked for parental mosaicism or non-penetrance.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Yes. Seventy percent of patients with isolated aniridia have an affected parent.[22] Risk to siblings is dependent on the genetic status of the proband's parents.[23]

3.4 Prenatal diagnosis
(To be answered if in 1.9 'D' was marked)

Prenatal diagnosis is offered to patients with a confirmed disease-causing *PAX6* variant or regulatory region deletion to enable them to be fully informed, however, acting upon the result in terms of termination is not normally advised as this condition can have a variable clinical phenotype. An individual with isolated aniridia has a 50% chance of passing the *PAX6* pathogenic variant to their offspring. If the parents appear unaffected but they have an affected child, rare germ line mosaicism can exist, this should be investigated in order to identify the risk to further siblings.

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?
Yes. Prenatal testing using foetal cells obtained by amniocentesis (~15–18 weeks gestation) or chorionic villus sampling (~10–12 weeks gestation) is possible for pregnancies at increased risk for isolated aniridia if the pathogenic variant or regulatory region deletion has been identified.[24]

4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe)

Yes. Understanding the genetic result provides information regarding recurrence risk, inheritance patterns, facilitates decision making through effective genetic counselling, enables patients to participate in research studies including clinical trials and join organisations such as the Aniridia Foundation International (AFI) for support and guidance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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