Molecular analysis of Cypriot families with aniridia reveals a novel *PAX6* mutation

ANDREAS SYRIMIS[1], NAYIA NICOLAOU[1], ANGELOS ALEXANDROU[2], IOANNIS PAPAEVRIPIDOU[2], MICHAEL NICOLAOU[1], ELENI LOUKIANOU[3], CAROLINA SISMANI[2], STAVROS MALAS[4], VIOLETTA CHRISTOPHIDOU-ANASTASIADOU[1,5] and GEORGE A. TANTELES[1]

Departments of [1]Clinical Genetics, and [2]Cytogenetics and Genomics, The Cyprus Institute of Neurology and Genetics, 2370 Nicosia; [3]Department of Ophthalmology, Nicosia General Hospital, 2029 Nicosia; [4]Department of Developmental and Functional Genetics, The Cyprus Institute of Neurology and Genetics, 2370 Nicosia; [5]Department of Clinical Genetics, Archbishop Makarios III Hospital, 2012 Nicosia, Cyprus

Received January 9, 2018; Accepted May 21, 2018

DOI: 10.3892/mmr.2018.9126

Abstract. The present study investigated the clinical and mutational spectrum of aniridia in a cohort of 17 affected individuals from six families from Cyprus. Each proband was initially evaluated for copy number variants at the *PAX6* locus and subsequently underwent *PAX6* mutation screening. Sequence analysis of *FOXC1* and *PITX2* was performed in patients who did not carry a *PAX6* mutation. The most common clinical features in the group of aniridia patients associated with aniridia were nystagmus, cataracts and glaucoma. *PAX6* pathogenic mutations were identified in five out of six families (a diagnostic yield of 84%). Previously reported pathogenic mutations in *PAX6* were identified in four families, which comprise p.R203*, p.R240* and p.R317*. In addition, a novel pathogenic variant (p.E220Gfs*23) was identified in a single family. No pathogenic mutations were detected in *PAX6*, *FOXC1* or *PITX2* in the only patient with a sporadic form of aniridia-like phenotype, confirming the genetic heterogeneity associated with this disease. To the best of our knowledge this is the first report on the mutational spectrum of *PAX6* in aniridia patients of Cypriot ancestry. Mutational screening of *PAX6* serves a crucial role in distinguishing isolated from syndromic forms of aniridia, and it may therefore eliminate the need for renal ultrasound scan surveillance, delineate the phenotype and improve genetic counseling.

Introduction

Aniridia (MIM #106210) is a congenital disorder of complete or partial iris hypoplasia (1,2). The prevalence of aniridia ranges from 1:50,000 to 1:100,000 live births (3). Aniridia can occur as an isolated or a syndromic form (4). Approximately two thirds of all cases are familial following an autosomal dominant mode of inheritance with high penetrance, while the remaining cases are sporadic (3,5).

Classic aniridia is a panocular condition caused by *PAX6* heterozygous mutations and it affects the iris, cornea, lens, retina and optic nerve. It can be accompanied by foveal hypoplasia, strabismus and optic nerve hypoplasia, generally leading to impaired visual acuity, while late-onset manifestations can include nystagmus, glaucoma, cataract and corneal pannus (5,6). Patients may also display non-ocular sensory and neurological abnormalities, such as reduced olfaction and hearing difficulties (7), and a range of neuroanatomical abnormalities, including hypoplasia of the anterior commissure, the pineal glands and the optic chiasm (8,9). *PAX6* point mutations are responsible for classic aniridia in approximately 90% of patients (10,11). *PAX6* maps to chromosomal region 11p13 and encodes a transcription factor, which plays a crucial role in early ocular morphogenesis. It is also involved in the development of the central nervous system, gut and pancreas (12,13). Furthermore, deletions at 11p13 involving *PAX6* or the regulatory region upstream of *PAX6* leaving its coding region intact are thought to be rare causes of classic aniridia (14). A small number of patients develop aniridia as part of the WAGR syndrome (Wilms tumor, Aniridia, Genital anomalies, mental Retardation) caused by a contiguous gene deletion encompassing *PAX6* and *WT1* (4). Finally, about 10% of cases display aniridia-like phenotypes that result from mutations in other genes, such as *FOXC1* and *PITX2* (15-17). The purpose of this study was to analyze the *PAX6* gene in a group of aniridia patients of Cypriot ancestry and describe their clinical features. We identified three previously reported *PAX6* mutations, in addition to a novel frameshift variant that was identified in one of our familial cases.

Materials and methods

Patients. A total of 17 affected individuals from six families were evaluated with a complete ophthalmological

Correspondence to: Dr George A. Tanteles, Department of Clinical Genetics, The Cyprus Institute of Neurology and Genetics, 6 International Airport Avenue, 2370 Nicosia, Cyprus
E-mail: gtanteles@cing.ac.cy

Key words: aniridia, *PAX6*

examination and then referred to the Clinical Genetics Clinic at the Cyprus Institute of Neurology and Genetics to investigate for *PAX6* mutations. Informed consent was obtained by study participants or their guardians if they were younger than 18 year olds.

Array-comparative genomic hybridization (CGH). Array CGH analysis or multiplex ligation-dependent probe amplification assay (MLPA) were initially performed to investigate for whole gene deletions involving *PAX6* and/or *WT1* or deletions downstream of *PAX6*. Array-CGH was performed using the Cytochip ISCA array (version 1.0; BlueGnome, Cambridge, UK) with 180,000 oligos in a 4x180 k format. Fluorescent ratios were calculated using the Blue Fuse Multi Software (version 4.2; BlueGnome). MLPA was conducted using the SALSA probemix P219-B3 (MRC-Holland, Amsterdam, Netherlands). Fragment separation by capillary electrophoresis was performed on an ABI 3130xl genetic analyzer. MLPA analysis was performed using the Coffalyser.Net Software (version 1.4; MRC-Holland).

Mutation screening. Genomic DNA was isolated from peripheral blood using the QIAamp DNA Blood Midi Kit (Qiagen, Inc., Valencia, CA, USA). All coding exons of *PAX6*, *FOXC1* and *PITX2* were amplified by PCR using primers designed with Primer3 (http://frodo.wi.mit.edu/) and they are available upon request. Sequencing products were analyzed by a 3130xl Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The cDNA sequence of the most common human PAX6 transcript (NM_000280) is used for the variant nomenclature. Direct sequencing of *PITX2* and *FOXC1* was performed in patients who did not carry any *PAX6* mutations only.

Results

Seventeen patients from six families were recruited for this study, including eight males and nine females. One patient was a sporadic case while the remaining were familial ones. Patients from family 1 presented with isolated bilateral aniridia. The proband of family 2 presented with bilateral aniridia, cataracts, glaucoma of the left eye, nystagmus and reduced visual acuity. She also had pseudophakia of the left eye. Fundoscopy revealed small, hypoplastic discs, macular hypoplasia, mild foveal hypoplasia and optic nerve hypoplasia. Her mother had aniridia with loss of vision in the right eye and developed retinal detachment in the left. The proband of family 3 had bilateral aniridia, nystagmus, cataracts, glaucoma and photophobia. She also had a history of osteoporosis and hypercholesterolemia. Her mother had relapsing remitting multiple sclerosis in addition to aniridia. The proband of family 4 had bilateral aniridia, atrial septal defect and thyroid nodules. Patients from family 5 exhibited bilateral aniridia and cataracts. Finally, our sporadic patient from family 6 had bilateral aniridia, nystagmus, cataracts and glaucoma. He had a history of Peters anomaly and he showed markedly abnormal anterior segment. Non-ocular abnormalities included antenatal bilateral hydronephrosis, vesicoureteric obstruction, bilateral megaureter, bipolar disorder and hypertension. His parents were thought to be distantly related. All probands were negative for deletions spanning *PAX6* and/or *WT1*, excluding WAGR syndrome.

Sequencing results. Four different heterozygous *PAX6* mutations were identified in five out of six families with aniridia, yielding a diagnostic rate of approximately 84%. Three stop-gain mutations identified in four familial cases (p.R203*, p.R240* and p.R317*) have been reported elsewhere, while the frameshift mutation c.659delA (p.E220Gfs*23) is novel and was found to occur *de novo* in the affected father (Fig. 1). Mutations were located in exons 8, 9 and 11. All mutations are summarized in Table I.

Direct sequencing of the coding exons and flanking intronic sites of *PITX2* and *FOXC1* in the proband of family 5 revealed no pathogenic mutations.

Discussion

In this study, we analyzed 17 patients from six different Cypriot families with aniridia and identified five different *PAX6* pathogenic variants in five out of six probands, yielding a diagnostic rate of 84% that is comparable to other studies in other populations (18-21). This is the first study on *PAX6* molecular analysis in aniridia patients of Cypriot ancestry. Although, deletions spanning the 3'regulatory region of *PAX6* or the *PAX6* coding region are less common than *PAX6* mutations in aniridia patients, we have performed array-CGH or MLPA first as it was recommended by Hingorani *et al* due to the clinical importance of detecting *WT1* deletions, which requires surveillance for Wilms tumor (7). None of our patients carried a deletion and therefore *PAX6* mutation screening was subsequently performed.

To date, 472 unique *PAX6* variants have been recorded in the Human *PAX6* Allelic Variant Database (http://lsdb.hgu.mrc.ac.uk/home.php?select_db=PAX6). Over 50% of these variants comprise frameshift or stop-gain variants resulting in a premature termination codon, which usually leads to the degradation of the truncated mRNA via nonsense-mediated decay. Therefore, these mutations result in 50% reduction in protein levels supporting haploinsufficiency as the main mechanism underlying aniridia (22,23). All of the identified variants in our study, as well, are predicted to lead to nonsense-mediated decay. Three of the identified *PAX6* variants in our study account for 16% of patients included in the *PAX6* mutation database (http://lsdb.hgu.mrc.ac.uk/home.php?select_db=PAX6). The first identified mutation (p.R203*) is currently reported in 40 patients, the second one (p.R240*) is currently reported in 51 patients and the third one (p.R317*) is currently reported in 41 patients. These are the most recurrent mutations found in *PAX6* in aniridia patients to date.

The PAX6 protein consists of a paired domain at the N-terminus, a homeodomain and a proline-serine-threonine rich transactivation (PST) domain at the C-terminus (24-26). In our study, mutations were found in exons encoding the linker domain (p.R203* and p.E220Gfs*23), the homeodomain (p.R240*) and the PST domain (p.R317*). No correlation between the location of the mutation and the associated phenotypes was observed. Even though all patients had truncating mutations, not all patients had cataract, glaucoma, nystagmus or foveal hypoplasia. In addition,

Table I. Summary of *PAX6* mutational spectrum in Cypriot families.

Protein change	Times reported in LOVD	Exon	Protein domain	Predicted effect
p.R203*	40	8	Linker domain	NMD
p.E220Gfs*23	0	8	Linker domain	NMD
p.R240*	51	9	Homeodomain	NMD
p.R317*	41	11	PST domain	NMD

LOVD, Leiden Open (Source) Variation Database; PST, proline-serine-threonine rich transactivation; NMD, nonsense-mediated decay.

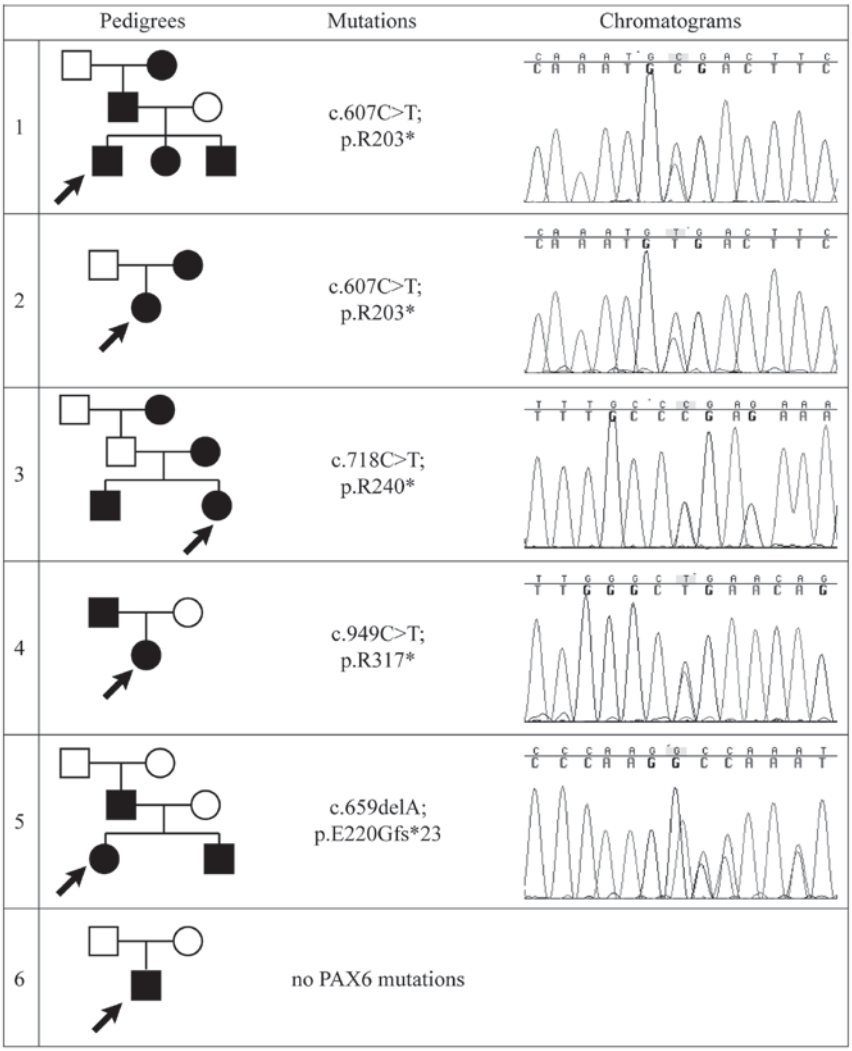

Figure 1. Pedigrees of the participating families and their corresponding chromatograms showing the identified mutations in *PAX6*.

the impairment of visual acuity varied between patients carrying the same *PAX6* mutation, as previously observed in other studies (27). The commonest additional ocular features seen in our group of patients were nystagmus, cataracts and glaucoma.

However, no copy number variants or mutations were detected in *PAX6*, *FOXC1* or *PITX2* in the proband of family 6 with a sporadic form of classical aniridia. Although, classical aniridia is primarily caused by *PAX6* mutations, its phenotypic presentation may overlap with aniridia-like phenotypes. The abnormal anterior segment seen in our patient prompted us to directly sequence *FOXC1* and *PITX2*, because mutations in these genes are more commonly associated with anterior segment dysgenesis even though they can also cause isolated aniridia. *FOXC1* mutations are more commonly associated with isolated ocular, heart and/or hearing defects and *PITX2* are more commonly associated with ocular, dental and umbilical anomalies. However, both genes account for approximately 40% of cases with Axenfeld-Rieger syndrome only (28,29). Therefore, our molecular findings confirm the genetic heterogeneity that underlies aniridia-like phenotypes as other recent reports have suggested (19,30).

In conclusion, a high diagnostic yield (84%) was obtained in this study, which was the first one to be conducted in Cyprus for aniridia patients. We have identified a novel frameshift mutation in one of our families thus expanding the number of *PAX6* mutations that cause aniridia. Mutation screening of *PAX6* plays a crucial role in determining whether the affected individual has isolated aniridia or WAGR syndrome. The identification of a *PAX6* mutation eliminates the need for surveillance by renal ultrasound and improves genetic counseling as well as the accuracy of prognosis and recurrence risk.

Acknowledgements

The authors would like to thank Dr Irene Savvidou and Neophyta Pantelidou (both working at the Archbishop Makarios III Hospital, Cyprus) for their help gathering the clinical information and all of the families who participated in the study.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AS performed mutation screening of the patients, analyzed the results and prepared the manuscript. NN also performed mutation screening of the patients and was a major contributor in writing and editing the manuscript. AA and IP performed array-CGH or MLPA in the participants. MN supervised and assisted AS with the Sanger sequencing and the interpretation of the results. EL was the ophthalmologist who contributed clinical data regarding the patients. CS supervised the performance of the array-CGH and MLPA, contributed to the interpretation of the results and provided the sequencing facility. SM contributed to the design of this project, the analysis and interpretation of the data and he ensured that the questions related to the accuracy of this work were appropriately investigated and resolved. VCA and GAT were the pediatricians and clinical geneticists who examined the families and requested genetic testing, they each contributed to the conception of the research project and ensured that questions related to the study were adequately and accurately resolved. GAT was also the principal investigator of the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Participants of this study underwent *PAX6* screening as part of the diagnostic workup that was performed at the Cyprus Institute of Neurology and Genetics to facilitate the clinical diagnosis, an application to the Cyprus National Bioethics Committee was not necessary for this. Written informed consent for participation in the study was obtained from all participants or their legal guardians prior to their inclusion.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Nelson LB, Spaeth GL, Nowinski TS, Margo CE and Jackson L: Aniridia. A review. Surv Ophthalmol 28: 621-642, 1984.
2. Hingorani M and Moore A: Aniridia. In: GeneReviews® [Internet]. Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Ledbetter N, Mefford HC, Smith RJH and Stephens K (eds). University of Washington Seattle, WA, 1993.
3. Grønskov K, Olsen JH, Sand A, Pedersen W, Carlsen N, Bak Jylling AM, Lyngbye T, Brøndum-Nielsen K and Rosenberg T: Population-based risk estimates of Wilms tumor in sporadic aniridia. A comprehensive mutation screening procedure of PAX6 identifies 80% of mutations in aniridia. Hum Genet 109: 11-18, 2001.
4. Fischbach BV, Trout KL, Lewis J, Luis CA and Sika M: WAGR syndrome: A clinical review of 54 cases. Pediatrics 116: 984-988, 2005.
5. Valenzuela A and Cline RA: Ocular and nonocular findings in patients with aniridia. Can J Ophthalmol 39: 632-638, 2004.
6. Lee H, Khan R and O'Keefe M: Aniridia: Current pathology and management. Acta Ophthalmol 86: 708-715, 2008.
7. Hingorani M, Hanson I and van Heyningen V: Aniridia. Eur J Hum Genet 20: 1011-1017, 2012.
8. Sisodiya SM, Free SL, Williamson KA, Mitchell TN, Willis C, Stevens JM, Kendall BE, Shorvon SD, Hanson IM, Moore AT and van Heyningen V: PAX6 haploinsufficiency causes cerebral malformation and olfactory dysfunction in humans. Nat Genet 28: 214-216, 2001.
9. Grant MK, Bobilev AM, Pierce JE, DeWitte J and Lauderdale JD: Structural brain abnormalities in 12 persons with aniridia. Version 2. F1000Res 6: 255, 2017.
10. Jordan T, Hanson I, Zaletayev D, Hodgson S, Prosser J, Seawright A, Hastie N and van Heyningen V: The human PAX6 gene is mutated in two patients with aniridia. Nat Genet 1: 328-332, 1992.
11. Robinson DO, Howarth RJ, Williamson KA, van Heyningen V, Beal SJ and 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 146A: 558-569, 2008.
12. Simpson TI and Price DJ: Pax6; a pleiotropic player in development. Bioessays 24: 1041-1051, 2002.
13. van Heyningen V and Williamson KA: PAX6 in sensory development. Hum Mol Genet 11: 1161-1167, 2002.
14. Crolla JA and van Heyningen V: Frequent chromosome aberrations revealed by molecular cytogenetic studies in patients with aniridia. Am J Hum Genet 71: 1138-1149, 2002.
15. Perveen R, Lloyd IC, Clayton-Smith J, Churchill A, van Heyningen V, Hanson I, Taylor D, McKeown C, Super M, Kerr B, *et al*: Phenotypic variability and asymmetry of Rieger syndrome associated with PITX2 mutations. Invest Ophthalmol Vis Sci 41: 2456-2460, 2000.
16. Khan AO, Aldahmesh MA and Al-Amri A: Heterozygous FOXC1 mutation (M161K) associated with congenital glaucoma and aniridia in an infant and a milder phenotype in her mother. Ophthalmic Genet 29: 67-71, 2008.
17. Ito YA, Footz TK, Berry FB, Mirzayans F, Yu M, Khan AO and Walter MA: Severe molecular defects of a novel FOXC1 W152G mutation result in aniridia. Invest Ophthalmol Vis Sci 50: 3573-3579, 2009.
18. Bobilev AM, McDougal ME, Taylor WL, Geisert EE, Netland PA and Lauderdale JD: Assessment of PAX6 alleles in 66 families with aniridia. Clin Genet 89: 669-677, 2016.

19. Primignani P, Allegrini D, Manfredini E, Romitti L, Mauri L, Patrosso MC, Veniani E, Franzoni A, Del Longo A, Gesu GP, *et al*: Screening of PAX6 gene in Italian congenital aniridia patients revealed four novel mutations. Ophthalmic Genet 37: 307-313, 2016.
20. Pérez-Solórzano S, Chacón-Camacho OF, Astiazarán MC, Ledesma-Gil G and Zenteno JC: PAX6 allelic heterogeneity in Mexican congenital aniridia patients: Expanding the mutational spectrum with seven novel pathogenic variants. Clin Exp Ophthalmol 45: 875-883, 2017.
21. Vasilyeva TA, Voskresenskaya AA, Käsmann-Kellner B, Khlebnikova OV, Pozdeyeva NA, Bayazutdinova GM, Kutsev SI, Ginter EK, Semina EV, Marakhonov AV and Zinchenko RA: Molecular analysis of patients with aniridia in Russian Federation broadens the spectrum of PAX6 mutations. Clin Genet 92: 639-644, 2017.
22. Vincent MC, Pujo AL, Olivier D and Calvas P: Screening for PAX6 gene mutations is consistent with haploinsufficiency as the main mechanism leading to various ocular defects. Eur J Hum Genet 11: 163-169, 2003.
23. Bhuvanagiri M, Schlitter AM, Hentze MW and Kulozik AE: NMD: RNA biology meets human genetic medicine. Biochem J 430: 365-377, 2010.
24. Glaser T, Jepeal L, Edwards JG, Young SR, Favor J and Maas RL: PAX6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central nervous system defects. Nat Genet 7: 463-471, 1994.
25. Wilson DS, Guenther B, Desplan C and Kuriyan J: High resolution crystal structure of a paired (Pax) class cooperative homeodomain dimer on DNA. Cell 82: 709-719, 1995.
26. Xu HE, Rould MA, Xu W, Epstein JA, Maas RL and Pabo CO: Crystal structure of the human Pax6 paired domain-DNA complex reveals specific roles for the linker region and carboxy-terminal subdomain in DNA binding. Genes Dev 13: 1263-1275, 1999.
27. Yokoi T, Nishina S, Fukami M, Ogata T, Hosono K, Hotta Y and Azuma N: Genotype-phenotype correlation of PAX6 gene mutations in aniridia. Hum Genome Var 3: 15052, 2016.
28. Reis LM, Tyler RC, Volkmann Kloss BA, Schilter KF, Levin AV, Lowry RB, Zwijnenburg PJ, Stroh E, Broeckel U, Murray JC and Semina EV: PITX2 and FOXC1 spectrum of mutations in ocular syndromes. Eur J Hum Genet 20: 1224-1233, 2012.
29. Samant M, Chauhan BK, Lathrop KL and Nischal KK: Congenital aniridia: Etiology, manifestations and management. Exp Rev Ophthalmol 11: 135-144, 2016.
30. Ansari M, Rainger J, Hanson IM, Williamson KA, Sharkey F, Harewood L, Sandilands A, Clayton-Smith J, Dollfus H, Bitoun P, *et al*: Genetic analysis of 'PAX6-negative' individuals with aniridia or gillespie syndrome. PLoS One 11: e0153757, 2016.