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Novel c.300_301delinsT Mutation in *PITX2* in a Korean Family with Axenfeld-Rieger Syndrome

Jae Won Yun, M.D.[1], Hyun-Kyung Cho, M.D.[2], Soo-Young Oh, M.D.[3], Chang-Seok Ki, M.D.[1], and Changwon Kee, M.D.[3]

Departments of Laboratory Medicine and Genetics[1], Ophthalmology[2], and Obstetrics and Gynecology[3], Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Axenfeld-Rieger syndrome (ARS) is characterized by anomalies of the anterior segment of the eye and systemic abnormalities. Mutations in the *FOXC1* and *PITX2* genes are underlying causes of ARS, but there has been few reports on genetically confirmed ARS in Korea. We identified a novel *PITX2* mutation (c.300_301delinsT) in 2 Korean patients from a family with ARS. We expand the spectrum of *PITX2* mutations and, to the best of our knowledge, this is the first confirmed family of *PITX2*-related ARS in Korea.

Key Words: Axenfeld-Rieger syndrome, Homeobox protein PITX2, FOXC1 protein

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Corresponding author: Chang-Seok Ki
Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 135-710, Korea
Tel: +82-2-3410-2709
Fax: +82-2-3410-2719
E-mail: changski@skku.edu

Co-Corresponding author: Changwon Kee
Department of Ophthalmology, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 135-710, Korea
Tel: +82-2-3410-3569
Fax: +82-2-3410-0074
E-mail: ckee@skku.edu

INTRODUCTION

Axenfeld-Rieger syndrome (ARS) is an autosomal dominant disease that manifests as anomalies of the anterior segment of the eye and systemic abnormalities [1]. In the eye, this condition is characterized by varying degrees of anterior segment dysgenesis and is associated with a high risk of glaucoma. Other associated systemic issues include cardiovascular outflow tract malformations, craniofacial abnormalities, and pituitary abnormalities that can cause severe endocrinological sequelae [2].

Genetically, mutations in *FOXC1* on chromosome 6p25 and *PITX2* on chromosome 4q25 have been identified in ARS patients [3, 4]. The prevalence of *FOXC1* or *PITX2* mutations in the affected probands ranges from 40% to 70% [5-7]. *FOXC1* mutations are associated with only eye involvement, while *PITX2* mutations often result in systemic abnormalities in addition to eye issues. Although the causative mutations have yet to be identified, 13q14 and 16q24 are known to contain loci of interest. A pediatric patient who was compound heterozygous for mutations in *CYP1B1* showed characteristic anterior chamber anomalies and had an umbilical hernia [8-10].

PITX2, a member of the paired class of homeodomain tran-

scription factors, is expressed in the corneal endothelium, stroma, iris, ciliary body, and sclera, which all originate from the neural crest. This transcription factor is known to affect the development of the periocular mesenchyme [8, 11]. A coding region frameshift as well as nonsense and missense mutations are thought to compromise the ability of *PITX2* to bind to DNA. Copy number losses due to large chromosome rearrangements, small intragenic deletions, and a rare duplication have also been reported [12]. Here, we report a Korean family with clinical features of ARS carrying a novel c.300_301delinsT mutation in the *PITX2* gene.

CASE REPORT

1. Case 1 (proband)

A 4-yr-old girl was referred to our clinic because of uncontrolled intraocular pressure (IOP) under maximal tolerable medical therapy and corneal edema in both eyes. Her best-corrected visual acuity was 20/800 in both eyes. IOP as measured by Goldmann tonometry was 22 mm Hg (reference range: ≤21 mm Hg) in the right eye and 23 mm Hg (reference range: ≤21 mm Hg) in the left eye. The horizontal and vertical corneal diameter of both eyes was 9.5 mm and 10.0 mm, respectively. Posterior embryotoxon and a prominent Schwalbe's line were observed in both eyes. Iridocorneal adhesion and corectopia were also seen, and anterior insertion of the iris into the trabecular meshwork with prominent iris processes was observed by gonioscopic examination in both eyes. The optic disc was difficult to inspect because of corneal edema; the cup-to-disc (CD) ratio appeared to be 0.8 in both eyes. The patient had apparent microdontia.

2. Case 2 (mother)

The 35-yr-old mother of the patient had 20/20 best-corrected visual acuity in both eyes. IOP as measured by Goldmann tonometry was 14 mm Hg (reference range: ≤21 mm Hg) in both eyes. Her cornea was clear, but focal iris atrophy was observed in both eyes. A posterior embryotoxon, which is a prominent Schwalbe's line, and anterior insertion of the iris into the trabecular meshwork with prominent iris processes were observed by gonioscopic examination in both eyes. Fundus examination revealed a normal appearing optic disc with a CD ratio of 0.3 in the right eye and 0.4 in the left eye. This patient also had redundant periumbilical skin and noticeable microdontia. She had dentures due to microdontia.

3. Molecular genetic analysis of *PITX2*

Sequencing analyses for *FOXC1* and *PITX2* in the proband and a family study for *PITX2* mutations were performed. The purpose of the study and the procedures to be used were explained to all patients, and informed consent was obtained. Genomic DNA was isolated from peripheral blood leukocytes using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA). All exons with flanking intronic regions were amplified using the PCR with primers designed by the authors (available on request). Sequencing was performed with the ABI Prism 3100xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the BigDye Terminator Cycle Sequencing-Ready Reaction Kit (Applied Biosystems). Sequences were analyzed using Sequencher software (version 4.10.1, Gene Codes Corp., Ann Arbor, MI, USA) and compared with the reference sequence for

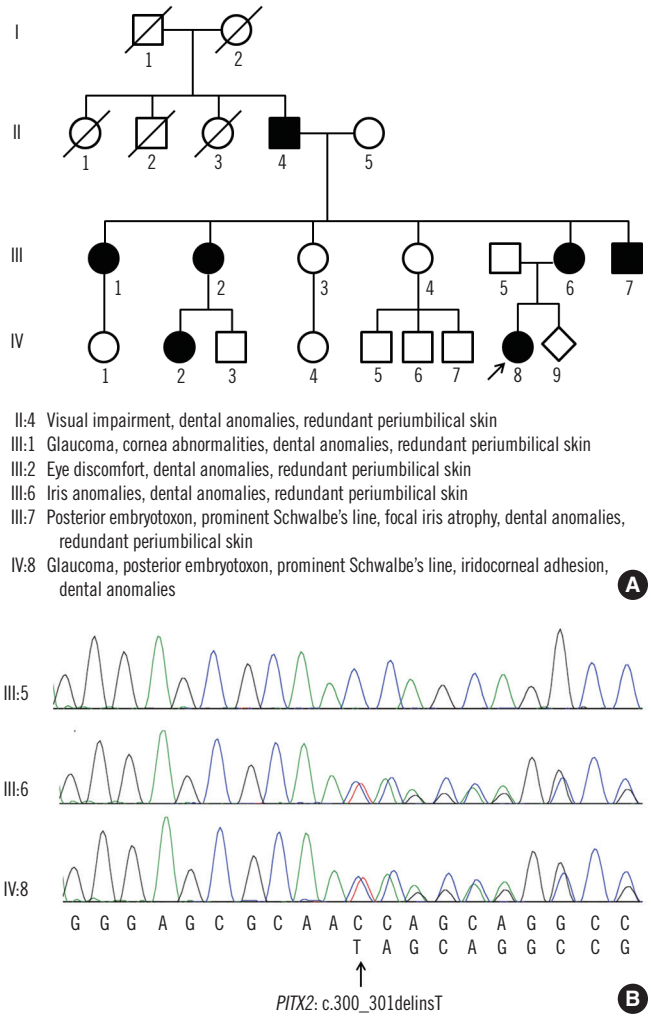

Fig. 1. Pedigree of the patient based on the clinical features (A). Direct sequencing results of the *PITX2* gene. III:5 is the father of the patient, III:6 is the mother, and V:8 is the patient (B).

PITX2 (NM_000325.5) and *FOXC1* (NM_001453.2).

The patient was found to be heterozygous for a 2-bp deletion and an insertion of T in the *PITX2* gene (c.300_301delinsT), a frameshift mutation predicted to result in premature termination at the 54th amino acid of the PITX2 protein (p.Gln101Serfs*54). Analysis of the parents revealed that this variant was inherited from the mother: the father had a wild-type sequence, while the mother had the same variant as her daughter (Fig. 1). When we tested 104 unaffected ethnically matched controls, none had the mutation. In addition, no other mutations were found in the *PITX2* gene of the patient or her parents. Neither mutation nor unknown variation was found in the *FOXC1* analysis.

DISCUSSION

We found a novel *PITX2* gene mutation (c.300_301delinsT) that appears to result in ARS through haploinsufficiency either due to a frameshift-mediated decay of mutant mRNA or production of mutant *PITX2* protein, in a Korean family affected by autosomal dominant ARS. The spectrum of Axenfeld-Rieger malformations demonstrates locus heterogeneity with the involvement of 2 major genes, *PITX2* and *FOXC1* [13]. This is the first report of a *PITX2* gene mutation associated with ARS in a Korean individual. To our knowledge, 7 cases of ARS have been reported in Korea in addition to the present 2 cases. In one of those cases, the diagnosis of ARS was confirmed by a molecular method, which showed a novel *FOXC1* mutation [14-17]. The clinical features, including ocular and extraocular symptoms and molecular features, are described in Table 1.

The *PITX2* gene is located on 4q25, and mutations have been identified in 10-60% of probands with ARS [8, 18]. Most commonly, coding region frameshifts and nonsense and missense mutations are present, which are thought to affect DNA binding. A small number of large chromosome rearrangements, small

Table 1. Clinical and molecular features of Axenfeld-Rieger syndrome patients in South Korea

Case No.	Sex/Age (yr)	Ocular symptoms	Extraocular symptoms	Gene	Mutation	Reference
1	M/20	Iridocorneal adhesion Iris nevus Ectropion uveae Corectopia Prominent Schwalbe's line	Microdontia Hypodontia Maxillary and mandibular hypoplasia Umbilical protrusion	NT		Cho et al. [14]
2	F/13	Glaucoma Posterior embryotoxon Prominent Schwalbe's line	PDA MR Hypertelorism Broad forehead Abnormal palpebral fissure Flat nasal bridge Hypodontia	NT		Lee et al. [16]
3	F/4	Corectopia Iris hypoplasia Sclerocornea Posterior embryotoxon Superior oblique anomaly	Maxillary hypoplasia Hypodontia	NT		Park et al. [17]
4-7	M/40 M/12 F/11 F/10	Glaucoma Iridocorneal adhesion Iris hypoplasia	Hypertelorism Telecantus Broad flat nose	*F0XC1*	c.317delA	Kim et al. [15]
8	F/4	Glaucoma Posterior embryotoxon Prominent Schwalbe's line Iridocorneal adhesion Corectopia	Microdontia	*PTIX2*	c.300_301delinsT	This report
9	F/35	Posterior embryotoxon Prominent Schwalbe's line Iris atrophy Corectopia	Redundant periumbilical skin Microdontia	*PTIX2*	c.300_301delinsT	This report

Abbreviations: NT, not tested; PDA, patent ductus ateriosus; MR, mitral regurgitation.

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intragenic deletions, and a rare duplication resulting in copy number loss have also been described [12]. All individuals who inherit the ARS-associated *PITX2* or *FOXC1* alleles exhibit ocular symptoms, with full penetrance [2]. Compared to *FOXC1* mutations, *PITX2* mutations are more commonly associated with the extraocular systemic abnormalities of ARS. In our case, the patient and her mother had extraocular abnormalities such as dental anomalies with or without redundant periumbilical skin. On the basis of the systemic findings, we predicted a *PITX2* mutation rather than a *FOXC1* mutation, but sequenced both genes. Although gain-of-function mutations in *PITX2* have been suggested to cause ARS, most intragenic *PITX2* mutations are loss-of-function mutations that yield a protein defective in DNA binding or one that is unable to transactivate downstream genes, or both [12]. *FOXC1* transcriptional activity is negatively regulated by *PITX2*, which explains how *FOXC1* duplication and *PITX2* deletion result in similar phenotypes [19].

Phenotypes of the novel *PITX2* mutation (c.300_301delinsT) in this family were variable. Although only the patient and her parents were fully genetically evaluated, ocular or extraocular manifestations of ARS were present in seven of the family members (Fig. 1). The patient showed severe glaucoma, Peters' anomaly, and visual impairment and therefore, requires a trabeculectomy and corneal transplantation. However, although her mother shared the *PITX2* gene mutation, her ocular symptoms were mild and could be controlled with eye drops. In ARS, varying degrees of anterior segment dysgenesis may be present, and careful examination of the features of the anterior segment is necessary to establish the diagnosis for each family member. Other systemic symptoms including cardiovascular outflow tract malformations, craniofacial abnormalities, and pituitary abnormalities were not typical in this family. However, dental anomalies and redundant periumbilical skin were observed.

In summary, we found a novel c.300_301delinsT mutation in *PITX2* in a Korean family with ARS. We suggest that this *PITX2* mutation causes typical ARS. Our results extend the spectrum of *PITX2* mutations and highlight the role of *PITX2* in the development and progression of ARS.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Fitch N and Kaback M. The Axenfeld syndrome and the Rieger syndrome. J Med Genet 1978;15:30-4.
2. Chang TC, Summers CG, Schimmenti LA, Grajewski AL. Axenfeld-Rieger syndrome: new perspectives. Br J Ophthalmol 2012;96:318-22.
3. Semina EV, Reiter R, Leysens NJ, Alward WL, Small KW, Datson NA, et al. Cloning and characterization of a novel bicoid-related homeobox transcription factor gene, RIEG, involved in Rieger syndrome. Nat Genet 1996;14:392-9.
4. Mirzayans F, Gould DB, Héon E, Billingsley GD, Cheung JC, Mears AJ, et al. Axenfeld-Rieger syndrome resulting from mutation of the FKHL7 gene on chromosome 6p25. Eur J Hum Genet 2000;8:71-4.
5. Strungaru MH, Dinu I, Walter MA. Genotype-phenotype correlations in Axenfeld-Rieger malformation and glaucoma patients with *FOXC1* and *PITX2* mutations. Invest Ophthalmol Vis Sci 2007;48:228-37.
6. Weisschuh N, Dressler P, Schuettauf F, Wolf C, Wissinger B, Gramer E. Novel mutations of *FOXC1* and *PITX2* in patients with Axenfeld-Rieger malformations. Invest Ophthalmol Vis Sci 2006;47:3846-52.
7. Weisschuh N, De Baere E, Wissinger B, Tümer Z. Clinical utility gene card for: Axenfeld-Rieger syndrome. Eur J Hum Genet 2011;19.
8. Lines MA, Kozlowski K, Kulak SC, Allingham RR, Héon E, Ritch R, et al. Characterization and prevalence of *PITX2* microdeletions and mutations in Axenfeld-Rieger malformations. Invest Ophthalmol Vis Sci 2004;45:828-33.
9. Phillips JC, del Bono EA, Haines JL, Pralea AM, Cohen JS, Greff LJ, et al. A second locus for Rieger syndrome maps to chromosome 13q14. Am J Hum Genet 1996;59:613-9.
10. Tanwar M, Dada T, Dada R. Axenfeld-Rieger syndrome associated with congenital glaucoma and cytochrome P4501B1 gene mutations. Case Rep Med 2010;2010. pii: 212656.doi: 10.1155/2010/212656.
11. Diehl AG, Zareparsi S, Qian M, Khanna R, Angeles R, Gage PJ. Extraocular muscle morphogenesis and gene expression are regulated by Pitx2 gene dose. Invest Ophthalmol Vis Sci 2006;47:1785-93.
12. Tümer Z and Bach-Holm D. Axenfeld-Rieger syndrome and spectrum of *PITX2* and *FOXC1* mutations. Eur J Hum Genet 2009;17:1527-39.
13. Gould DB, Mears AJ, Pearce WG, Walter MA. Autosomal dominant Axenfeld-Rieger anomaly maps to 6p25. Am J Hum Genet 1997;61:765-8.
14. Cho HJ and Cho YW. A case of Axenfeld-Rieger syndrome. J Korean Ophthalmol Soc 2000;41:258-63.
15. Kim GN, Ki CS, Seo SW, Yoo JM, Han YS, Chung IY, et al. A novel forkhead box C1 gene mutation in a Korean family with Axenfeld-Rieger syndrome. Mol Vis 2013;19:935-43.
16. Lee JH, Chae SH, Huh J, Kang IS, Lee HJ, Yang JH, et al. A case of Axenfeld-Rieger syndrome with severe mitral regurgitation. J Korean Pediatr Cardiol Soc 2006;10:216-9.
17. Park SW, Kim HG, Heo H, Park YG. Anomalous scleral insertion of superior oblique in Axenfeld-Rieger syndrome. Korean J Ophthalmol 2009;23:62-4.
18. Borges AS, Susanna R Jr, Carani JC, Betinjane AJ, Alward WL, Stone EM, et al. Genetic analysis of *PITX2* and *FOXC1* in Rieger Syndrome patients from Brazil. J Glaucoma 2002;11:51-6.
19. Berry FB, Lines MA, Oas JM, Footz T, Underhill DA, Gage PJ, et al. Functional interactions between *FOXC1* and *PITX2* underlie the sensitivity to *FOXC1* gene dose in Axenfeld-Rieger syndrome and anterior segment dysgenesis. Hum Mol Genet 2006;15:905-19.