SHORT REPORT

Three novel PAX6 mutations in patients with aniridia

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Aims: To describe mutations in the PAX6 gene in five patients with aniridia from three unrelated families.
Methods: The PAX6 gene was analysed using single stranded conformational polymorphism analysis and direct sequencing.
Results: In one family, three individuals from two generations had aniridia, whereas in each of the other families only one member was affected. The first patient had the heterozygous Q221X (1023C → T) nonsense mutation in exon 8. The same mutation was found in his mother and sister. Another patient had a heterozygous Q297X (1252C → T) mutation in exon 10. The third patient carried a heterozygous IVS5+2T → C mutation leading to aberrant splicing of mRNA.
Conclusions: These findings provide further examples of haploinsufficiency of PAX6 in aniridia.

Aniridia is a panocular disorder denominated after noticeable iris hypoplasia. Complications include cataracts, corneal vascularisation, and glaucoma, and affected patients often have severe visual impairment. The features may range from almost complete absence of the iris, through enlargement and irregularity of the pupil mimicking a coloboma, to small defects in the anterior layer noticeable only on transillumination. Aniridia may occur as an isolated feature or in combination with various syndromes. The disease can be inherited as an autosomal dominant trait and has an incidence of about 1/80 000.[1] About one third of all cases of aniridia are sporadic.

Mutations of the PAX6 gene, located on chromosome 11p13, are responsible for about 80% of cases of aniridia in humans.[2] In addition, PAX6 mutations are also found in patients with a variety of clinically distinct autosomal dominantly inherited congenital eye malformations,[3] such as heterogeneous anterior segment malformations related to Peter's anomaly and Rieger's anomaly,[4] keratitis,[5] and foveal hypoplasia.[6] Loss of function of the mutant allele is found in almost all mutations and more than 80% of exonic substitutions result in nonsense codons. About 30% of identified PAX6 mutations are deletion or insertion events, 28% are C → T changes at CpG dinucleotides, and 20% are splicing errors.[3] The PAX6 mutation database (http://pax6.hgu.mrc.ac.uk) contains more than 200 different mutations.

Table 1 Summary of six PAX6 mutations in patients with aniridia

Patient	Sex	Status	Exon	DNA outcome	Protein outcome	Domain
DU	Male	Familial	8	1023C→T	Q221X	HD
NU	Female	Familial	8	1023C→T	Q221X	HD
IU	Female	Familial	8	1023C→T	Q221X	HD
LG	Male	Sporadic	10	1252C→T	Q297X	PST
DL	Male	Sporadic	Intron 5	IVS5+2T→C	Unknown	PD

HD, homeodomain; PD, paired domain; PST, proline/serine/threonine rich domain.

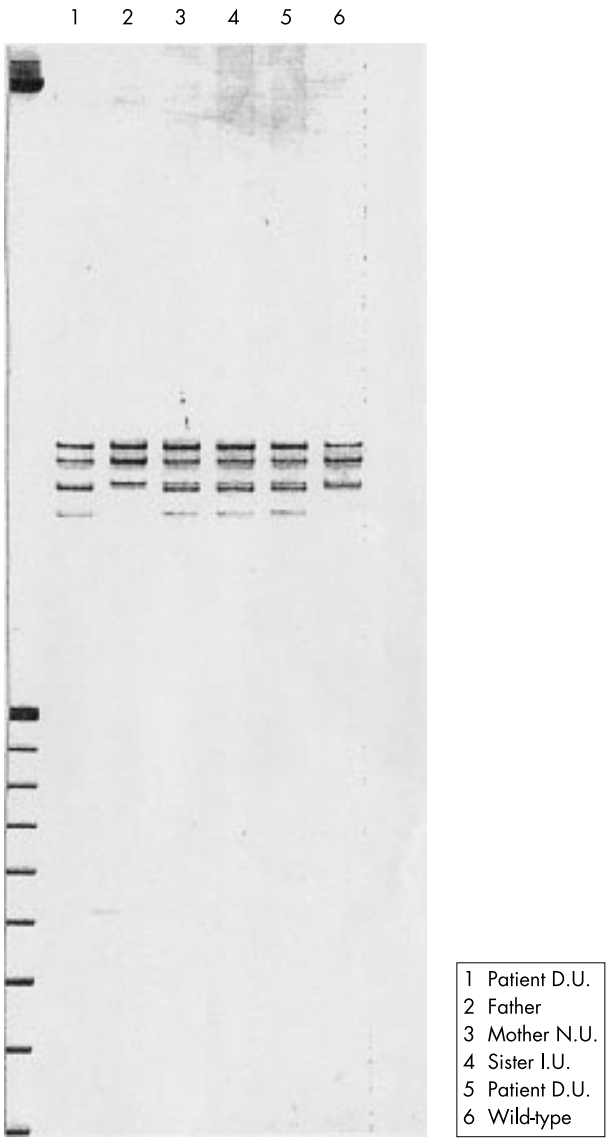

Figure 1 Single stranded conformational polymorphism analysis of exon 8 of the PAX6 gene from patient DU (lanes 1 and 5, mutation 1023C → T), father (lane 2, normal), mother NU (lane 3, mutation 1023C → T), sister IU (lane 4, mutation 1023C → T), and wild type (lane 6).

Abbreviations: PCR, polymerase chain reaction; SSCP, single stranded conformational polymorphism; WAGR, Wilms's tumour, aniridia, genitourinary abnormalities, and mental retardation

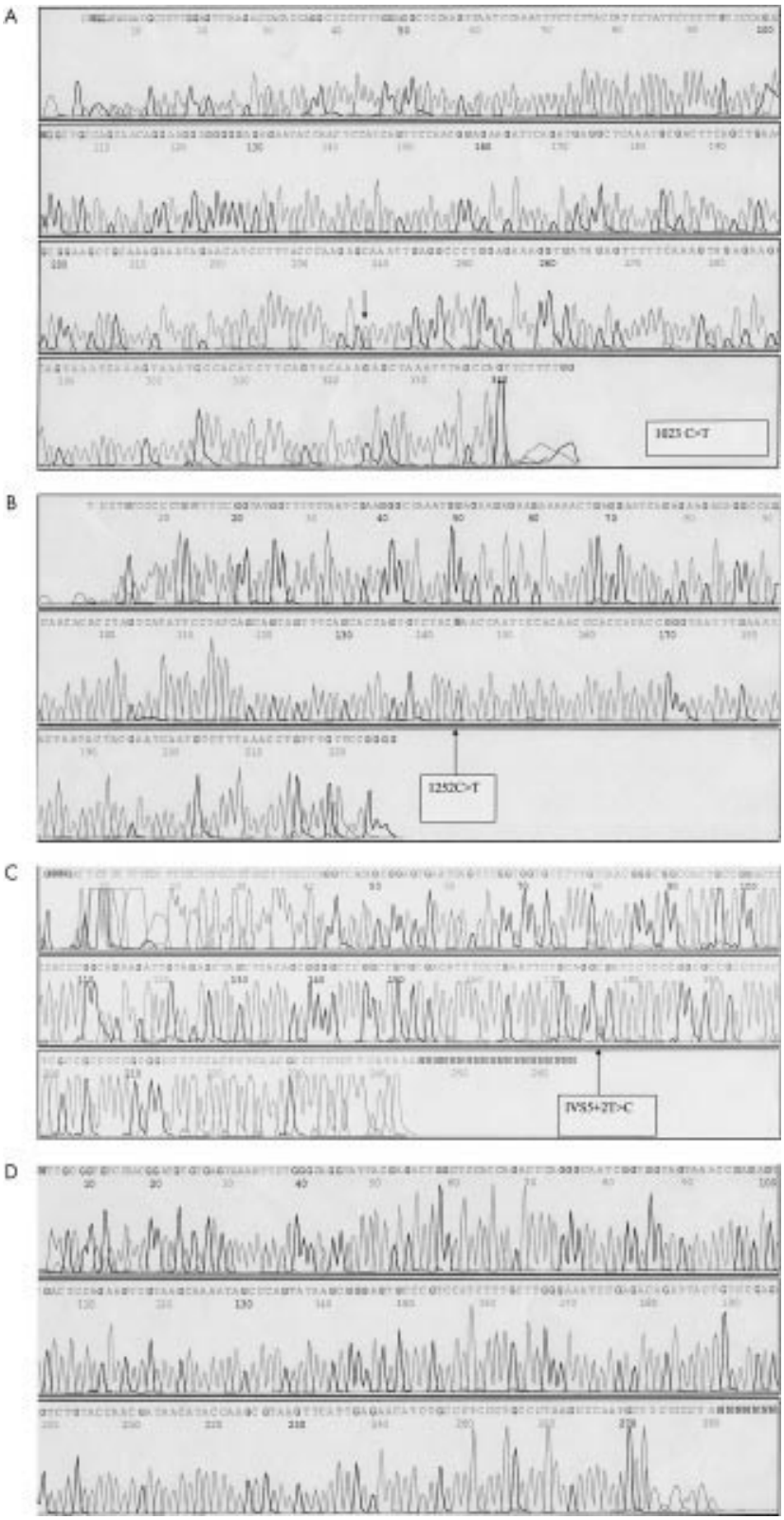

Figure 2 (A) Detection of nonsense mutation 1023C → T (Q221X) in patient DU by single stranded conformational polymorphism (SSCP) analysis and direct sequencing. (B) Detection of nonsense mutation 1252C → T (Q297X) in patient LG by SSCP analysis and direct sequencing. (C) Detection of intron mutation IVS5+2T → C in patient DL by SSCP analysis and direct sequencing. (D) SSCP analysis and direct sequencing of a normal control sample.

"Mutations are found throughout the gene, so that extensive investigation is required in each case"

The PAX6 gene encodes a transcriptional regulator containing two DNA binding domains, a paired domain and a paired-type domain, a link domain, and a proline/serine/threonine rich domain.[7] The human PAX6 gene consists of 14 exons, the initiation codon is in exon 4 and the termination codon in exon 13. The protein is 422 amino acids in length and a second PAX6 isoform, derived from alternative splicing, has a short insertion in the paired domain.[8] Mutations are found throughout the gene, so that extensive investigation is required in each case.[3]

MATERIAL AND METHODS

DNA samples

We analysed PAX6 mutations in five patients from three families with aniridia after informed consent. These were the only patients with this clinical condition and therefore no affected patient was excluded from investigation. Genomic DNA was prepared by a standard procedure from isolated leucocytes.

PCR-SSCP assay and sequencing

Mutations for PAX6 were detected by single stranded conformational polymorphism (SSCP) analysis and direct sequencing. Polymerase chain reaction (PCR) primers used for the amplification of the 14 exons of PAX6 were synthesised according to the sequence reported by Glaser and colleagues[7] and Love *et al*.[9] The PCR conditions and SSCP analysis were as described by Tadokoro *et al*.[10]

RESULTS

Table 1 summarises the mutations detected in the patients with aniridia from three unrelated families. All patients had complete aniridia. All mutations are new and probably lead to the formation of a truncated PAX6 protein.

Patient DU carries a heterozygous C → T transition (1023C → T) that changes codon 221 (CAA for glutamine) into a stop codon (TAA) (Q221X), predicting a functional "null allele". The same mutation was also detected in the patient's mother and sister, but not in his father (figs 1, 2A).

Patient LG had a heterozygous C → T transition (1252C → T) in exon 10 that alters codon 297 (CAA for glutamine) into a stop codon (TAA) (Q297X) (fig 2B). The mutation was undetectable in the parents, brother, and sister, suggesting that it represents a de novo mutation.

In patient DL, the mutation IVS5+2T → C was detected (fig 2B). This mutation introduces a novel restriction site for the restriction enzyme Cac8 I. Genotyping of DNA samples from the patient's parents and sister showed no mutation. Thus, the patient probably carries a de novo mutation.

DISCUSSION

Aniridia is a human eye malformation caused by heterozygous null mutations of PAX6, a paired box transcription factor, or microdeletions of chromosome 11p13 that encompass PAX6 and are associated with WAGR syndrome (Wilms's tumour, aniridia, genitourinary abnormalities, and mental retardation). The inheritance is autosomal dominant with high penetrance but variable expressivity.[3] PAX6 mutations have been found in about 80% of patients with aniridia (both sporadic and familial).[11] Normal eye development is highly susceptible to the degree of PAX6 expression; haploinsufficiency causes aniridia, and overexpression also leads to developmental defects (microphthalmia) of the eye.[12 13]

The PAX6 gene exhibits a very high sequence conservation throughout evolution and as yet undiscovered missense mutations might be associated with unidentified phenotypes.[3] PAX6 is involved in the development of the Rathke pouch and early anterior pituitary gland, and its expression controls the established boundaries of somatotrope, lactotrope, and thyrotrope cell types.[14] The PAX6 gene is also expressed during the early stages of pancreatic development in the mouse.[15] In addition, PAX6 was found to be a key regulator of pancreatic islet hormone gene transcription and pivotal for normal islet development.[16] Moreover, it transactivates the glucagon and insulin promoters.

The human PAX6 gene is involved in ocular morphogenesis, and PAX6 mutations have been detected in various types of ocular anomalies, such as aniridia, corneal dystrophy, congenital cataract, and foveal hypoplasia. The gene encodes a transcriptional regulator and produces two alternative splice isoforms that have distinct DNA binding specificities.[17 18] These splice variants are found only in vertebrates.[8 19]

Take home messages

- Three patients from one family had the same heterozygous Q221X (1023C → T) nonsense mutation in exon 8
- Another patient had a heterozygous Q297X (1252C → T) mutation in exon 10, which leads to a prematurely truncated protein, and the third patient carried a heterozygous IVS5+2T → C mutation, which leads to aberrant mRNA splicing
- These findings provide further examples of haploinsufficiency of PAX6 in aniridia
- The sole detection of PAX6 mutations in these patients excludes a high risk for the development of Wilms's tumours/WAGR syndrome

"The detection of PAX6 mutations facilitates comprehensive genetic counselling"

PAX6 mutations are found in a high proportion of patients with aniridia and new technology such as chip sequencing will facilitate high throughput and efficient mutation detection. The Wilms's tumour susceptibility gene (WT1), which is adjacent to the PAX6 gene, can also be deleted, resulting in a predisposition to Wilms's tumour as part of the WAGR syndrome. The sole detection of PAX6 mutations in our patients thus excludes a high risk for the development of Wilms's tumours/WAGR syndrome. Last but not least, the detection of PAX6 mutations facilitates comprehensive genetic counselling.

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