Short report

A new PAX6 mutation in familial aniridia

Isabel Hanson, Alison Brown, Veronica van Heyningen

Abstract

Aniridia (lack of iris) is caused by loss of function mutations in one copy of the PAX6 gene. Here we present a new PAX6 splice mutation in a family with autosomal dominant aniridia. The mutation is a single nucleotide change which, although occurring within an exon, affects the splice junction consensus and results in skipping of that exon.

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Aniridia is a sight threatening congenital malformation of the eye which is chiefly characterised by severe iris hypoplasia.¹ A candidate aniridia gene, PAX6, was isolated from chromosomal region 11p13 by positional cloning and was shown to be expressed in the developing eye.² PAX6 is a member of the Pax family of developmental genes and contains two conserved DNA binding domains, a paired box and a paired type homeobox.^{3,4} Intragenic mutations of the PAX6 gene have now been shown in 23 aniridia patients.⁵⁻¹¹ These mutations would be predicted to disrupt the function of one copy of the gene and are consistent with the hypothesis that aniridia is caused by haploid insufficiency of PAX6.

Mutation analysis was carried out by nested

PCR of reverse transcribed RNA from patient lymphoblastoid cell lines.⁷ Nested RT-PCR of the PAX6 homeodomain and proline-serine-threonine (PST) rich domain in patient II.2 (fig 1) showed a shorter PCR product in addition to a product of wild type size. The smaller fragment was purified and directly sequenced⁷ and was found to be deleted for 151 bp which corresponded precisely to exon 12 (data not shown). RT-PCR analysis of the other members of the family showed that the presence of the shorter product segregated with the disease (fig 1).

To determine the genomic basis of this exon skipping event, the intron-exon boundaries of

Figure 1 RT-PCR analysis of the 3' half of the PAX6 open reading frame in a family with autosomal dominant aniridia. The structure of the pedigree is shown at the top, with filled symbols indicating the affected subjects and the empty circle indicating the unaffected mother. PCR products were resolved on a 1% agarose, 1.5% NuSieve gel in 0.5 × TBE with ϕ X174/HaeIII size markers (M). The wild type product size is 711 bp; all three aniridia patients have an additional shorter band.

Figure 2 (A) Sequencing of the splice junction at the 3' end of exon 12 in II.2 (affected) and I.2 (normal). To prepare template for sequencing, 50 to 100 ng genomic DNA were amplified using Cetus Amplitaq with the PAX6 primers D152 (exon 12, 5'-CCCACATATGCAGACACACAT-3', nt 1484-1504 in reference 2) and C401 (exon 13, reference 7). PCR conditions were (94°C, one minute) × 1; (94°C, 30 seconds; 58°C, 30 seconds; 72°C, one minute) × 35; (72°C, 10 minutes) × 1. The sequence shown in the figure begins near the 3' end of exon 12 and crosses the exon/intron boundary, which is represented by a horizontal bar. Patient II.2 is heterozygous at the last nucleotide of exon 12. (B) Diagnosis of the mutation by MaeII digestion of genomic PCR products encompassing the 3' end of exon 12. For each family member the genomic PCR product (D152/C401) is shown undigested (-) and digested with MaeII (+). The wild type product 850 bp is not cut but the mutant product, with a single MaeII site at the position of nucleotide substitution, is cut once to give products of 60 bp (not visible) and 790 bp. Gel running conditions was as for fig 1.

MRC Human Genetics Unit,
Western General Hospital,
Crewe Road, Edinburgh EH4 2XU,
UK

I Hanson
A Brown
V van Heyningen

Correspondence to:
Dr Hanson.

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exon 12 were PCR amplified from the genomic DNA of II-2 and I-2. The splice acceptor sequence at the 5' boundary of exon 12 was shown by direct sequencing of PCR products to be normal (data not shown). However, direct sequencing of the 3' boundary of exon 12 showed that the last nucleotide of the exon was heterozygous (G wild type, C mutant) in the patient (fig 2A). The 3'-terminal residue is a G in 77% of exons and forms part of the consensus sequence CAG/GTAAGT (the last base of the exon is underlined) for recognition of the splice donor by complementary base pairing to U1 snRNA, a component of the spliceosome.¹² The wild type exon 12 splice donor (CAG/GTGAGC) already differs from the consensus at two positions (underlined); presumably the patient's mutation (CAC/GTGAGC) reduces the complementarity further so that the splice site is no longer recognised by the snRNA. Although pathological mutations involving substitution of the last base of an exon are rare, exon skipping is known to be a consequence of this type of mutation.¹²⁻¹⁴ The patient's mutation creates a site at the genomic level for the restriction endonuclease *MaeII* (ACGT). *MaeII* digestion of a genomic PCR product containing the splice donor confirmed the presence of the mutation in the affected members of the family but not the unaffected member (fig 2B).

Although the genomic mutation presented here is new, the consequence of the mutation, namely skipping of exon 12, is the same as that in patient "VIGMA" described by Hanson *et al.*⁷ Translation of the exon 12 deleted PAX6 mRNA would result in a protein product in which the C-terminal half of the PST rich domain was replaced by a nonsense peptide. GAL4 fusion ex-

periments have shown that the ability of the PAX6 PST rich region to behave as an activation domain is severely compromised if it is truncated at the C-terminal end.¹¹ This observation may explain the molecular basis of aniridia in the present family and in patient "VIGMA".⁷

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