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Septo-optic dysplasia and WS1 in the proband of a WS1 family segregating for a novel mutation in PAX3 exon 7

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Abstract

A four generation family (UoM1) was ascertained with Waardenburg syndrome type 1 (WS1). The proband exhibited both WS1 and septo-optic dysplasia. A G to C transversion was identified in PAX3 exon 7 in four subjects affected with WS1 in this family including the proband. This glutamine to histidine missense mutation at position 391 may also affect splicing. There are over 50 mutations characterised in PAX3 in WS1 patients; however, this is the first example of a WS1 mutation in exon 7 of PAX3.

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Waardenburg syndrome (WS) is an autosomal dominant disorder characterised by pigmentary and facial anomalies, deafness, and other clinical traits.[1-3] There are four clinical subtypes of WS, WS1 (OMIM 193500), WS2 (OMIM 193510), WS3 (OMIM 148820), and WS4 (OMIM 277580). Mutations in WS1 and WS2 have been identified in PAX3 and MITF, respectively.[1 4-21] We report a WS1 family (UoM1) in which the proband has both WS1 and septo-optic dysplasia (SOD). SOD is a sporadic developmental malformation of the anterior midline structures of the brain encompassing a spectrum of optic nerve hypoplasia, hypoplasia of the chiasm and primitive optic ventricles, absence or hypoplasia of the septum pellucidum and infundibulum, partial or complete absence of the corpus callosum, and neuroendocrine deficiencies.[22 23] There are no published reports of WS1 and SOD occurring in the same person and no mutations reported to be associated with SOD. We screened for mutations in PAX3 and MITF in this four generation WS1 family and also in six unrelated subjects with isolated SOD.

Methods

The family (fig 1A) was ascertained at the University of Michigan Pediatric Genetics Clinic. Patients with SOD were ascertained through the University of Michigan Pediatric Endocrinology Clinic. The father of the proband has Waardenburg syndrome type I (fig 1A). He was examined by MRI for SOD and was found to be normal. DNA was isolated from blood using the PUREGENE kit (Gentra Systems). The proband of UoM1, 33 unrelated WS1, WS2, and WS4 patients, 60 random subjects without WS, and six unrelated subjects with SOD were screened for mutations. The polymerase chain reaction (PCR) was performed for the eight exons of PAX3 and the nine exons of MITF as described previously[1 12 13 17 19 21] followed by single strand conformation polymorphism (SSCP) analysis on MDE™ 0.5 × Hydrolink^{R} gel (AT Biochem). Electrophoresis was performed at 8 W at room temperature with and without 10% glycerol. Relevant DNA fragments were cloned into a pGEM^{R}-T Vector System (Promega) and sequenced using Sequenase Version 2.0 (Amersham). Cycle sequencing reactions were performed following the manufacturer's protocol using the Δ*Taq* Cycle Sequencing kit (Amersham). A mutant allele specific primer (TF195: CTAGAAACACGGGACTGACG), the normal upstream exon 7 primer (TF141: AGAAAACATGATGGTTGACAATC), and a PAX3 exon 4 primer pair as a control were used for allele specific PCR amplification.

Results and discussion

In the proband of UoM1 a SSCP variant was detected in PAX3 exon 7. The SSCP variant was not observed in the 33 unrelated WS1, WS2, and WS4 patients nor in 60 random subjects. Affected members of UoM1 have a G to C transversion in PAX3 exon 7 (fig 1B). Four WS1 patients in the family (II.2, III.3, III.2, IV.2), including the proband with WS1 and SOD, amplified both the mutant allele specific fragment (270 bp) and the control fragment (242 bp) (fig 1C). Only the control fragment PCR amplified in the unaffected mother (III.4) and in 60 random subjects. Thus, the G to C transversion in PAX3 exon 7 is not a common polymorphism and is probably the basis of WS1 in this family. Although more than 50 mutations have been identified in PAX3 in WS1 patients, the Q391H substitution is the first mutation in PAX3 exon 7 (fig 2). This substitution has several potential effects on the expression of PAX3. First, glutamine 391 is replaced with histidine (Q391H). Second, since Q391H changes an important nucleotide in a 5' splice site, it is reasonable to hypothesise that the Q391H PAX3 mutation could cripple normal PAX3 pre-mRNA splicing resulting in aberrantly spliced transcripts.[24 25] An identically positioned G to C transversion at the end of exon 1 in the β globin gene was shown to inhibit almost completely correct splicing in cell free extracts.[26] A drastically reduced amount of Q391H substituted PAX3 protein together with products altered in the transactivation domain could

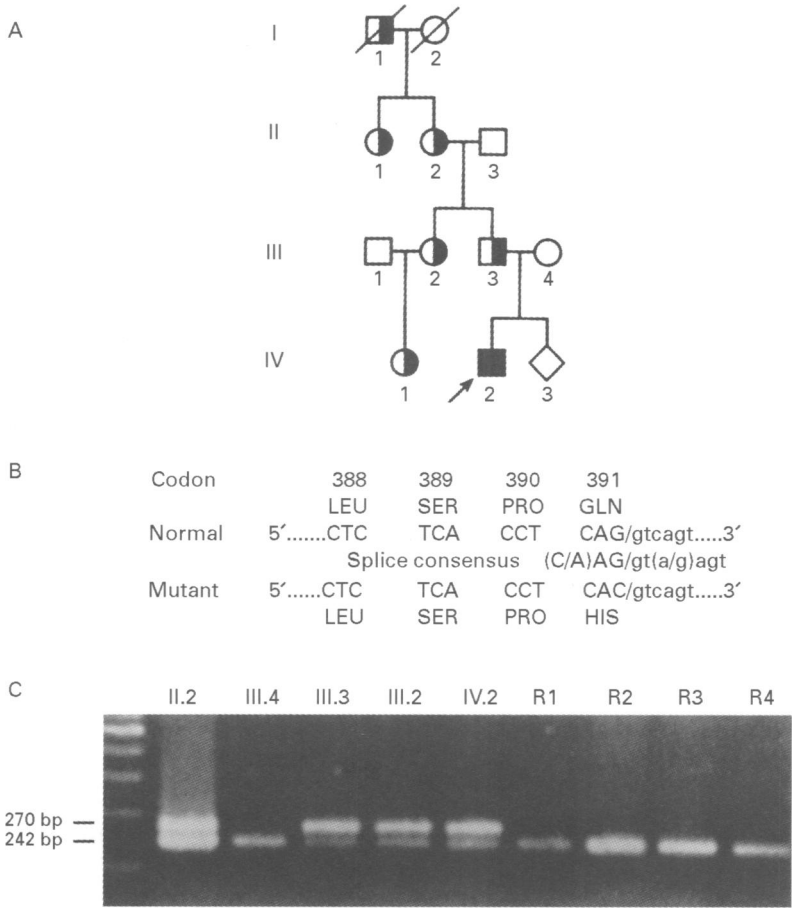

Figure 1 (A) Pedigree of UoM1. The right half of the symbols is filled in to represent the WS phenotype and the left half is filled in to represent SOD. (B) Codons 388 to 391 of PAX3 exon 7 showing the Q391H mutation in family UoM1. (C) PCR amplification of DNA from WS patients (II.2, III.2, III.3, IV.2) show both the 270 and 242 base pair fragments while amplification of DNA from the unaffected mother (III.4) and the four random samples (R) show only the 242 base pair control PCR product.

theoretically be produced. No other sequence variations in PAX3 and MITF were detected in the proband and no PAX3 or MITF sequence variants were detected in any of six unrelated subjects with SOD. A connection between WS1 and SOD could not be established in this study. There are over 50 mutations reported in PAX3 and Q391H is the first example of a PAX3 exon 7 mutation associated with WS1.

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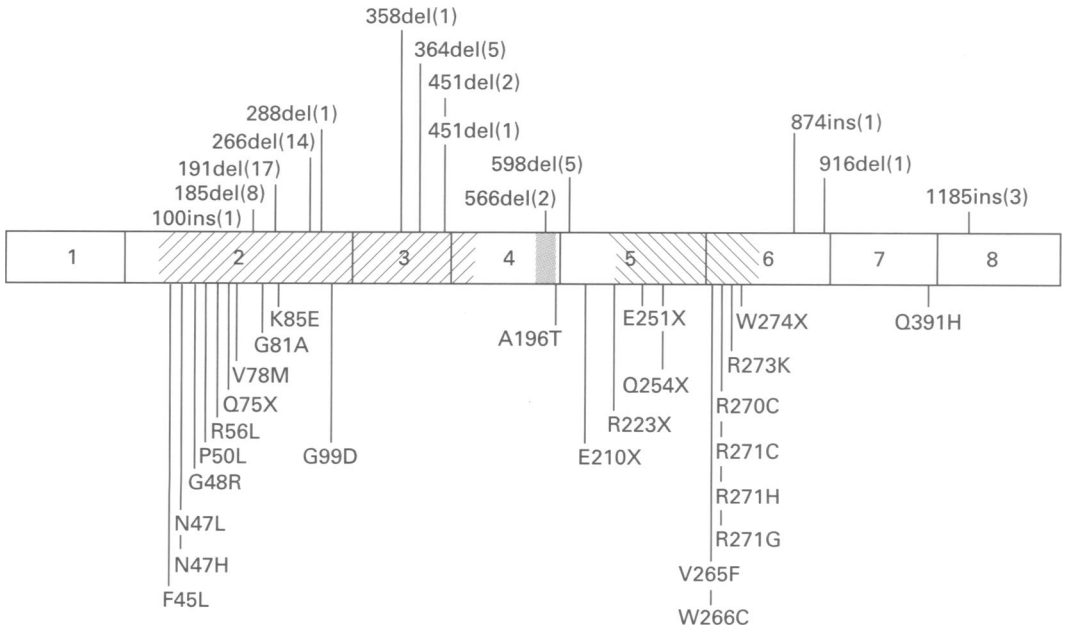

Figure 2 Diagram of the PAX3 gene with published locations of mutations in relation to the eight exons and the DNA binding domains. The mutations above the gene diagram represent deletions and insertions designated by the nucleotide position and those below represent missense and nonsense mutations designated by the amino acid sequence. The paired domain is designated with right diagonal lines within exons 2, 3, and a portion of 4, the octapeptide domain is designated with closed circles within exon 4, and the homeodomain is designated with left diagonal lines within portions of exons 5 and 6.

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