Novel mutations of SOX10 suggest a dominant negative role in Waardenburg-Shah syndrome

Mai Har Sham, Vincent Chi Hang Lui, Benedict Ling Sze Chen, Ming Fu, Paul Kwong Hang Tam

EDITOR—Waardenburg syndrome (OMIM 193500) is a rare disorder (1 in 40 000 live births) characterised by distinctive facial features, pigmentary disturbance (white forelock, heterochromia iridis, white eyelashes, leucoderma), and cochlear deafness.1 Waardenburg-Shah syndrome combines the features of Waardenburg syndrome and Hirschsprung’s disease (also called Waardenburg-Hirschsprung disease, Waardenburg syndrome type IV, WS4) (OMIM 277580). In addition to having white forelock, eyebrows, and eyelashes, the patients present in the neonatal period with intestinal obstruction, characteristic of Hirschsprung’s disease.2–4 Mutations in three different genes have been identified in WS4 patients. These genes include the endothelin-B receptor gene (EDNRB),3 the gene for its ligand endothelin-3 (EDN3),4,5 and the SOX10 gene. It has been observed that heterozygous mutations of EDNRB or EDN3 are found in Hirschsprung’s disease alone6,11 and only homozygous mutations of either gene are found in WS4. Therefore, when resulting from EDN3 or EDNRB mutations, WS4 is inherited as an autosomal recessive trait.

Among the WS4 patients studied so far, 10 out of 37 have been reported to have had SOX10 mutations.8–16 Interestingly, when SOX10 mutations are involved, WS4 is inherited as an autosomal dominant condition. The presence of SOX10 mutations in the Waardenburg-Shah patients suggest that the SOX10 gene could be involved in regulatory and signalling pathways for the normal development of the neural crest cell lineages which differentiate into melanocytes and enteric glia. The involvement of Sox10 in the development of enteric neurones has also been reported in the Dom (Dominant megacolon) mouse model of Hirschsprung’s disease. It was shown that a single base insertion in the mouse Sox10 gene was responsible for the megacolon phenotype of the Dom mutant.17 18 Interestingly, in the Waardenburg-Shah patients as well as the Dom mutant mouse model, the intestinal aganglionosis phenotype appears to originate from heterozygous SOX10/Sox10 mutations, suggesting that the phenotype could be the result of haploinsufficiency.

In this study, we examined mutations in three WS4 patients, two of Chinese origin and one English. We identified two novel SOX10 mutations among three WS4 patients we investigated. We also reviewed all the SOX10 mutations reported so far to correlate SOX10 mutations with the aganglionosis phenotype observed in the patients.

Patients
Patient 1 is a Chinese girl, born at term, who was admitted to hospital because of failure of passage of meconium within the first 24 hours of life, followed by vomiting and a distended abdomen. She has bilateral hearing loss, light brown hair, and vivid blue irides with grey speckles. The diagnosis of Hirschsprung’s disease was made on the basis of history, physical findings, and barium enema results, and was confirmed by the pathological findings of the resected specimen. At operation, the aganglionic segment was found to involve the distal sigmoid colon and rectum. A pull through procedure was performed. There is no family history.

Patient 2 is a Chinese boy, born at term, who developed abdominal distension and vomiting on day 2 of life with delayed passage of meconium. He had dysmorphic facial features with ptosis of the right eye. He is profoundly deaf and suffers from mental and global developmental retardation. He has blue eyes on both sides and one strand of white hair over the forehead. All these are features of Waardenburg syndrome. On laparotomy, frozen section histology of intestinal biopsies showed total colonic aganglionosis and ileostomy was performed. Subsequently, total colostomy and Duhamel pull through was performed. There is no family history.

Patient 3 is an English boy, born at term, who presented with failure of passage of meconium, bile stained vomiting, and a distended abdomen on day 1. Rectal biopsy confirmed the diagnosis of Hirschsprung’s disease. He had rectosigmoid aganglionosis. Temporary colostomy was performed initially and definitive pull through operation done subsequently. The presence of a white forelock and deafness led to the diagnosis of Waardenburg syndrome. There is no family history.

Methods
DNA was extracted from peripheral blood samples collected from patients and their family members using QiaAmp Blood Kit (Qiagen)
or by phenol chloroform extraction method. Six sets of primers (sequences summarised in table 1) were used to amplify SOX10 gene fragments covering exons 2 to 5 and intron-exon junctions by PCR. The PCR products were purified and the DNA sequences determined by an automated sequencer (ABI Prism 310) after cycle sequencing reactions (dRhodamine kit). All sequences were determined from both forward and reverse orientations (table 1). Mutations were confirmed by sequencing duplicate PCR templates from separate reactions.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer sequence</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>forward 5'GCC TGG AGG CTC CAC CTT CTG C-3'</td>
<td>443</td>
</tr>
<tr>
<td></td>
<td>reverse 5'TCC TGC AGC CTG CGC CAA CGG-3'</td>
<td>689</td>
</tr>
<tr>
<td>3</td>
<td>forward 5'GCC TGT GCC CAC GTC CTG TCT C-3'</td>
<td>527</td>
</tr>
<tr>
<td></td>
<td>reverse 5'CAG TCC CGC TCT CAG GTG GAC G-3'</td>
<td>661</td>
</tr>
<tr>
<td>4</td>
<td>forward 5'AGT CCA CAA ATC ATA GGG CAC A-3'</td>
<td>696</td>
</tr>
<tr>
<td></td>
<td>reverse 5'TAG AGT CCA GGG TCT CAT TGC C-3'</td>
<td>749</td>
</tr>
<tr>
<td>5a</td>
<td>forward 5'ATG GTC AGA GTA GTC AAA CTG G-3'</td>
<td>749</td>
</tr>
<tr>
<td></td>
<td>reverse 5'AGG CAT GTG AGA GTA GTC AAA CTG G-3'</td>
<td>749</td>
</tr>
<tr>
<td>5b</td>
<td>forward 5'CAG ATC GCC TAC TCC TCT CTA A-3'</td>
<td>749</td>
</tr>
<tr>
<td></td>
<td>reverse 5'AGG CAT GTG AGA GTA GTC AAA CTG G-3'</td>
<td>749</td>
</tr>
<tr>
<td>5c</td>
<td>forward 5'ACC ACT CCT ATG ACT CCT GTT T-3'</td>
<td>749</td>
</tr>
<tr>
<td></td>
<td>reverse 5'AGA GGG GAC TAC TGA GAT AAA T-3'</td>
<td>749</td>
</tr>
</tbody>
</table>

Results

Two different novel SOX10 mutations were identified in the two Chinese WS4 patients. Patient 1 was diagnosed as having typical short segment HSCR; her intestinal aganglionosis extended to the rectosigmoid region. Sequence analysis showed that she had a heterozygous frameshift mutation in exon 3 of the SOX10 gene and a single nucleotide (G) at nucleotide position 168 from the start codon is deleted (fig 1B). This mutation would lead to a frameshift in the mutant protein producing a truncated SOX10 protein of 107 amino acids (fig 1E). This truncated mutant protein, which lacks the HMG DNA binding and the C-terminal trans-activating domains, is likely to be non-functional. No SOX10 mutation could be detected in her parents, indicating that the 168delG mutation in patient 1 was sporadic.

Patient 2 was diagnosed as having total colonic aganglionosis. Analysis of the SOX10 sequence showed that he had a heterozygous mutation at the stop codon in exon 5. A transversion of T to A (fig 1D) at the first base of the stop codon changed it to a codon for lysine. The mutant SOX10 open reading frame encodes an extra 86 amino acid long proline rich peptide at the C-terminus (fig 1E). Examination of the SOX10 sequence of his parents shows that he had a heterozygous mutation at the stop codon in exon 5. A transversion of T to A (fig 1D) at the first base of the stop codon changed it to a codon for lysine. The mutant SOX10 open reading frame encodes an extra 86 amino acid long proline rich peptide at the C-terminus (fig 1E).

Figure 1  Analysis of SOX10 mutations in WS4 patients. DNA sequence analysis of (A) normal exon 3 and (B) exon 3 of patient 1 shows that there is a deletion of a G residue at nucleotide position 168 (open arrowhead). Analysis of (C) normal exon 5 and (D) exon 5 of patient 2 shows a T→A transversion at the first base of the stop codon (filled arrowhead), changing it into a codon for lysine. (E) A diagram illustrating the sequences for the mutant SOX10 peptide encoded by the frameshift mutation 168delG from patient 1 and the proline rich C-terminal peptide encoded by the X467K mutation from patient 2. The normal functional domains of the SOX10 protein and all reported SOX10 mutations in WS4 patients are also summarised here, illustrating that mutations around the HMG domain all led to hypoganglionosis or short segment aganglionosis, and mutations in the transactivation domain all led to long segment or total aganglionosis.
confirmed that the X467K mutation in patient 2 was also sporadic.

Patient 3 has typical features of Waardenburg-Shah syndrome and rectosigmoid aganglionosis. Our mutation analysis on the SOX10 gene showed no nucleotide change in the entire coding region and in the intron-exon junctions.

**Discussion**

The mutation identified in patient 1 (168delG) would lead to the production of a truncated protein lacking the DNA binding and the putative transactivation domains (fig 1E). It is likely that the small polypeptide produced from the mutant allele is non-functional. Moreover, the mutant mRNA produced from this mutant allele might be unstable and easily degraded within the cell. Therefore, if the 168delG allele was dysfunctional, the phenotype displayed by patient 1 would result from a heterozygous null mutation. This would support the hypothesis that Waardenburg-Shah syndrome is the result of haploinsufficiency of a functional SOX10 allele.

The other novel mutation we identified (X467K), which affected the stop codon of SOX10, would produce a larger protein with an extra C-terminal proline rich domain of 86 amino acids long (fig 1E). Interestingly, a mutation which would produce a similar but shorter mutant protein has been identified in a patient with complex syndromes in addition to WS4; in that case a 12 bp deletion at the stop codon led to the production of an extra 82 amino acid domain. It has been postulated that the C-terminal proline rich domain of this mutant SOX10 protein has a dominant negative effect. In terms of phenotype of the colon, both patients who have similar mutations (patient 2 in this study and that described by Inoue) have more severe intestinal aganglionosis (total colonic aganglionosis and long segment HSCR). However, patient 2 in this study does not have the dysmyelinating segment aganglionosis). However, patient 2 in this study does not have the dysmyelinating segment aganglionosis. (fig 1E).

**Acknowledgements**

This work was supported by the Swiss National Science Foundation (grant 310000-136500), the University Foundation, and the American Disabilities Association. We thank Dr M J et al for providing us with patient 1.

The reported mutations can be grouped into four categories. (1) Mutations that lead to a truncation of SOX10 protein before the HMG domain (Y83X, 168delG), or that affect the HMG domain directly (482ins6), are functionally equivalent to null mutations, as the mutant proteins would not have any DNA binding activity. These mutations tend to be correlated with a milder phenotype, that is, hypoganglionosis or short segment HSCR. (2) Mutations that lead to a truncation of SOX10 protein shortly after the HMG domain (E189X, Y207X) would produce a mutant protein that potentially would have DNA binding activity but would lack the other functional domains. These mutations have also been shown to cause mild short segment HSCR. (3) Mutations which affect the C-terminal putative transactivation domain (Q377X, 1400del12, X467K) tend to have a stronger effect, leading to long segment or total aganglionosis. (4) The other mutations which cause truncation of the SOX10 protein in between the HMG domain and the C-terminal domain (S251X, Y313X) have more variable phenotypic consequences. In particular, the Y313X mutation detected in two different families actually led to a different intestinal phenotype.

**Table 2** Summary of SOX10 mutations identified and phenotypes of affected patients

<table>
<thead>
<tr>
<th>Sex</th>
<th>SOX10 mutation</th>
<th>Effect on protein sequence</th>
<th>Affected colon phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>nt1076delGA</td>
<td>Frameshift→37aa added at 359</td>
<td>NA</td>
<td>14 (family 2)</td>
</tr>
<tr>
<td>M</td>
<td>X467K</td>
<td>86aa added at stop codon</td>
<td>Total colonic aganglionosis</td>
<td>This study (patient 2)</td>
</tr>
<tr>
<td>M</td>
<td>Y313X (C→A at nt565)</td>
<td>Truncation after HMG domain</td>
<td>Short segment HSCR</td>
<td>14 (family 2)</td>
</tr>
<tr>
<td>M</td>
<td>Y207X</td>
<td>Truncation after HMG domain</td>
<td>Short segment HSCR</td>
<td>14 (family 3)</td>
</tr>
<tr>
<td>M</td>
<td>nt482ins6</td>
<td>Duplication of LR in HMG domain</td>
<td>Short segment HSCR</td>
<td>14 (family 4)</td>
</tr>
<tr>
<td>M</td>
<td>Y168X (C→T at nt249)</td>
<td>Truncation after HMG domain</td>
<td>Short segment HSCR</td>
<td>14 (family 5)</td>
</tr>
<tr>
<td>M</td>
<td>Y168X (C→T at nt939)</td>
<td>Truncation after HMG domain</td>
<td>Short segment HSCR</td>
<td>14 (family 6)</td>
</tr>
<tr>
<td>M</td>
<td>S251X (C→A at nt752)</td>
<td>Truncation after HMG domain</td>
<td>Short segment HSCR</td>
<td>14 (family 7)</td>
</tr>
<tr>
<td>M</td>
<td>Y313X (C→A at nt939)</td>
<td>Truncation after HMG domain</td>
<td>Short segment HSCR</td>
<td>14 (family 8)</td>
</tr>
<tr>
<td>M</td>
<td>nt1076delGA</td>
<td>Frameshift→37aa added at 359</td>
<td>NA</td>
<td>14 (family 9)</td>
</tr>
<tr>
<td>M</td>
<td>Q377X</td>
<td>Truncation of C-terminal domain</td>
<td>Variable diagnosis, ranging from</td>
<td>13 (family 10)</td>
</tr>
<tr>
<td>F</td>
<td>nt1400del12 (X467C)</td>
<td>82aa added at stop codon</td>
<td>Long segment HSCR</td>
<td>15</td>
</tr>
<tr>
<td>M</td>
<td>X467K</td>
<td>86aa added at stop codon</td>
<td>Total colonic aganglionosis</td>
<td>This study (patient 2)</td>
</tr>
</tbody>
</table>

NA: data not available from the reference.

*Hypoganglionosis was based on description cited in the reference.

**References**


2. Waardenburg-Shah syndrome is a rare malformation, yet mutations in the three known causative genes (EDN3, EDNRB, SOX10) have so far only explained some of the patients. On the other hand, SOX10 mutation per se might not be sufficient to cause WS4. A SOX10 missense mutation (S135T) has been reported in a case of Yemenite deaf-blind hypopigmentation syndrome.**
HMG domain and the transactivation domain of the SOX10 protein is highly conserved between the group E SOX proteins (96% homology)22 and it could be involved in protein-protein interaction,23 such that when its structure is affected by mutation it could lead to more severe biochemical consequences.

Among the 12 cases reported, half of the mutations are nonsense mutations resulting in a truncation of the SOX10 polypeptide along its length (table 2). The truncated mutant mRNA produced by the mutant alleles could be subjected to rapid decay because of the mRNA surveillance mechanisms within the cells,24 rendering a haploinsufficient condition. In the case of the Y313X mutation, variant mutant mRNA stability in the two patients might account for the different intestinal phenotype observed.16 In view of the structure of the SOX10 protein and its organization of functional domains, we postulate that the WS4 features could be the result of a dominant negative effect of the mutant protein, but not necessarily of haploinsufficiency of normal SOX10. Particularly in those cases where mutations of SOX10 occurred in the C-terminal transactivation domain, the mutant SOX10 protein could gain novel functions and contribute to a more severe phenotype.

As also observed in other studies, there remain a large number of Waardenburg-Shah patients who do not have any SOX10 mutations within the coding region and splice junctions. Therefore, other mutations within the SOX10 gene locus or other genes may be responsible for their hypopigmentation and intestinal aganglionosis phenotype. We also found silent and polymorphic DNA changes in the SOX10 gene of Hirschsprung's disease patients without Waardenburg syndrome (Shah KN et al, unpublished data). Although these nucleotide changes do not affect the amino acid sequence of SOX10 protein, given the multigenic nature of Hirschsprung's disease, the importance of these silent and polymorphic changes in the aetiology of Hirschsprung's disease has yet to be evaluated.

We are grateful to Dr Peter Gornall, Consultant Paediatric Surgeon, Birmingham for allowing us to include his patient (patient 3) for study. This study was supported by the Research Grant Council, Hong Kong (Project No 7300/99M).

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**Electronically**

- Two novel SOX10 mutations were identified in two of the three WS4 patients we examined. One of the mutations was a single nucleotide deletion in exon 3 (168delG), which led to a frameshift mutation producing a truncated SOX10 protein. The other mutation was at the stop codon in exon 5, which led to a mutant protein with an extra 86 amino acid long, proline rich peptide at the C-terminus. Both of these mutations were sporadic.

- We analysed all the reported SOX10 mutations in WS4 patients and found that mutations around the HMG domain would lead to hypoganglionosis or short segment HSCR; mutations that affect the C-terminal transactivation domain would lead to long segment or total aganglionosis.

**SOX10 mutations affecting the C-terminal transactivation domain might produce mutant proteins that have a dominant negative effect, which account for a more severe intestinal aganglionosis phenotype in the patients.**

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7 Puffenberger E, Housa K, Washington S, Nakao K, deWitt D, Yagunawa M, Chakravarti A. A missense mutation of the endothelin-B receptor gene in multigenic Hirsch-


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