Correlation between a specific Wilms tumour suppressor gene (WT1) mutation and the histological findings in Wilms tumour (WT)

R Shibata, A Hashiguchi, J Sakamoto, T Yamada, A Umezawa, J Hata

NATIONAL MUTATION REPORT

Wilms tumour (WT) is the most common malignant neoplasm of the kidney in childhood and accounts for approximately 8% of all childhood solid tumours. Fetal rhabdomyomatous nephroblastoma (FRN) is a histological variant of WT characterised by a predominance of rhabdomyogenic components. Clinically, WT of the FRN type presents as a huge mass in younger patients and about 30% of them have bilateral disease. The tumour rarely metastasises or shows aggressive behaviour and it has a good prognosis. The Wilms tumour suppressor gene (WT1) on chromosome 11p13 was identified in 1990 and encodes a transcriptional factor containing a domain of four zinc finger motifs. Schumacher et al reported that a germline mutation in WT1 predisposes to the development of tumours with stromal predominant histology. We analysed germline and tumour WT1 in seven cases of WT that were diagnosed as FRN histologically, or contained rhabdomyogenic components, and found the same mutation in five of them.

PATIENTS AND METHODS

Patients

Seven cases of WT who were treated surgically between 1998 and 2001 and contained abundant striated muscle as a stromal component histologically were studied. Cases 3, 4, 5, and 6 also had genitourinary tract malformations (table 1). Cases 3 and 6 were associated with cryptorchidism, case 4 with bilateral cryptorchidism and hypospadias, and case 5 with left ovarian dysgenesis. The tumour was bilateral in every case except case 2. None of the patients showed evidence of renal dysfunction or renal failure. Fresh tumour tissue and peripheral blood samples were obtained from all seven patients. Non-tumorous renal tissue was obtained from case 2. Informed consent was obtained from all patients or their parents.

Histopathological analysis

The resected tumours or biopsy specimens were fixed with 10% formalin and embedded in paraffin. Paraffin sections were stained with haematoxylin and eosin (HE), and histological subtyping of WT was performed according to the classification proposed by the Japanese Society of Pathology. We defined FRN as more than one-third of the tumour mass showing striated muscle differentiation and the nephroblastic type was defined as a tumour which showed triphasic histology containing striated muscle, but its proportion of the tumour was less than one-third.

Table 1  Clinical and histological features and the results of WT1 mutations

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/gender</th>
<th>Clinical description of anomalies</th>
<th>Laterality</th>
<th>Histological subtype</th>
<th>Germline mutations</th>
<th>Tumour mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11 mth/F</td>
<td>(-)</td>
<td>Bilateral</td>
<td>FRN</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>2</td>
<td>1y /M</td>
<td>(-)</td>
<td>Unilateral</td>
<td>FRN</td>
<td>(-)</td>
<td>1168C→T(R390X) LOH</td>
</tr>
<tr>
<td>3</td>
<td>11 mth/M</td>
<td>Cryptorchidism</td>
<td>Bilateral</td>
<td>FRN</td>
<td>1168C→C/T</td>
<td>1168C→T(R390X) Homo</td>
</tr>
<tr>
<td>4</td>
<td>9 mth/M</td>
<td>Cryptorchidism, hypospadias</td>
<td>Bilateral</td>
<td>Nephroblastic type striated muscle (+)</td>
<td>1168C→C/T</td>
<td>1168C→T(R390X) Homo</td>
</tr>
<tr>
<td>5</td>
<td>7 mth/F</td>
<td>Ovarian dysgenesis</td>
<td>Bilateral</td>
<td>FRN</td>
<td>1168C→C/T</td>
<td>1168C→T(R390X) Homo</td>
</tr>
<tr>
<td>6</td>
<td>1 y/M</td>
<td>Cryptorchidism</td>
<td>Bilateral</td>
<td>Nephroblastic type striated muscle (+)</td>
<td>1168C→C/T</td>
<td>1168C→T(R390X) LOH</td>
</tr>
<tr>
<td>7</td>
<td>6 mth/F</td>
<td>(-)</td>
<td>Bilateral</td>
<td>Nephroblastic type striated muscle (+)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

Key points

- We analysed WT1 in seven cases of Wilms tumour (WT). The histology of these cases mostly showed FRN or nephroblastic type tumours containing abundant mature rhabdomyomatous components with some immature metanephroblastic tissues.
- In five out of seven cases, we detected the same mutation, which was an 1168C to T substitution in zinc finger 3 and resulted in a 390Arg becoming a stop codon (R390X).
- Our results suggest that this specific WT1 mutation of R390X correlates with this particular histological type of WT showing abundant rhabdomyomatous components.
DNA preparation
Mononuclear cells were isolated from the patients’ peripheral blood. Tumour tissue and non-tumorous renal tissue were stored at −80°C until used. DNA was extracted from leucocytes and tumour tissue by the SDS-proteinase K method with slight modifications, as described previously. Amplification of exons 1 to 10 was performed by PCR. Direct sequencing of the PCR products was performed with a MegaBACE 1000 DNA Sequencing System (Amersham Biosciences, Florida, USA).

The precise methods have been described previously. The PCR and sequence primers used are shown in table 2.

Polymorphic analysis of WT1
We used a polymorphic marker, WT1-P, to investigate whether the mutation detected in WT1 in the tumour cells was homozygous or hemizygous as a result of loss of heterozygosity (LOH). This marker detects a GT polymorphism in the 3′ non-coding region of exon 10 of WT1. We used primers WT1P2-1

<table>
<thead>
<tr>
<th>Table 2</th>
<th>PCR and sequence primers</th>
</tr>
</thead>
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<tr>
<td>Exon</td>
<td>Name</td>
</tr>
<tr>
<td>1</td>
<td>WT2</td>
</tr>
<tr>
<td>2</td>
<td>CTX1R2</td>
</tr>
<tr>
<td>2B</td>
<td>TGCCGTCAGTCCTGCAAGGGA</td>
</tr>
<tr>
<td>3</td>
<td>CGTCAAGTCAGTCCTGCAAGG</td>
</tr>
<tr>
<td>4B</td>
<td>TGTCCTTGTCCTGACATCG</td>
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<tr>
<td>5B</td>
<td>TTGGCACTCAGTCCTGCAAGG</td>
</tr>
<tr>
<td>6B</td>
<td>AAAACCATCATTCCCCCCTTGTG</td>
</tr>
<tr>
<td>7F</td>
<td>GTGTCCTCTCTGCCCTGAGG</td>
</tr>
<tr>
<td>8C</td>
<td>AGATCCCTTCTTCCAGATC</td>
</tr>
<tr>
<td>9C</td>
<td>CACAAACAAGAGAGACCAATA</td>
</tr>
<tr>
<td>10C</td>
<td>CATCGGCGGCTTCTAGATGTC</td>
</tr>
</tbody>
</table>

Figure 1 Haematoxylin and eosin section. (A) Case 5. Immature striated muscle is the predominant stromal element. This is the typical histological appearance of FRN. (B) Case 6. Nodular blastemal pattern of the nephroblastic type of WT. A small number of tubular structures are present in the nodule. (C) Case 6. Striated muscle cells are seen as stromal components in case 6.
RESULTS

The clinical and pathological features and the results of the WT1 mutations are summarised in Table 1. All seven patients were under 1 year of age. Cases 3, 4, 5, and 6 were complicated by congenital genitourinary malformations. The WT was bilateral in every case except case 2. Cases 1, 2, 3, and 5 showed the typical histological features of FRN (Fig 1A). Cases 4, 6, and 7 were the nephroblastic type (Fig 1B) and contained striated muscle components (Fig 1C).

DNA sequence analyses indicated the same constitutional point mutations in exon 9 in cases 3, 4, 5, and 6. The mutation was a 1168C-T substitution in zinc finger 3 and resulted in a 390 Arg becoming a stop codon (R390X). There was a heterozygous mutation in the same codon in the germline of cases 3, 4, 5, and 6 (Fig 2). Case 2 showed mutation R390X in the tumour, but no mutations were found in the germline. No WT1 mutations were detected in cases 1 or 7.

The results of the polymorphic analysis of 11p13 are also summarised in Table 1. The germline in case 5 contained 124 bp and 132 bp bands at WT1-P. These bands were also detected in the bilateral tumours (Fig 3A), and we concluded that the WT1 mutation in the tumours were heterozygous. Cases 3 and 4 also showed heterozygous mutations in tumour tissue (data not shown). DNA isolated from mononuclear cells and the non-tumorous renal tissue of case 2 contained 124 bp and 130 bp bands at WT1-P. The tumour showed only a 124 bp band and it was concluded to show LOH (Fig 3B). Case 6 also showed LOH in tumour tissue at WT1-P (data not shown).

DISCUSSION

We found the same mutation of WT1 in five of the seven cases of WT that were classified as FRN histologically or contained abundant rhabdomyogenic components. Our findings suggested that this specific WT1 mutation, R390X, is correlated with the histological features of WT.
Cases 3, 4, 5, and 6 were found to have the same constitutional point mutations, a change from 1168C to T in zinc finger 3, resulting in "Arg becoming a stop codon. Case 2 had the R390X mutation in the tumour. During translation, this nonsense mutation leads to protein truncation, and the result is that the last zinc finger necessary for DNA binding of WT1 is missing. Interrupted DNA binding to the target genes of WT1 with loss of the last zinc finger has previously been shown by electrophoretic mobility shift assays. Loss of WT1 function has been reported to be the underlying cause of tumour development. Miyagawa et al reported that loss of WT1 function leads to ectopic myogenesis in WT and suggested that normal expression of WT1 might prevent the metanephris-mesenchymal stem cells of the kidney from differentiating into skeletal muscle. Three of the five cases that had the WT1 mutations, cases 2, 3, and 5, were classified as FRN. Cases 4 and 6 were the nephroblastic type and contained striated muscle as stromal components. It could not be concluded that all WTs with the R390X mutation are FRN histologically, but the results at least suggested a correlation between this mutation and rhabdomyomatous histology of WT.

In earlier studies, mutation R390X had been found in patients with sporadic unilateral WT, in a patient with bilateral WT, and in a patient with WT associated with urogenital malformation. This mutation has also been detected in a patient with acute promyelocytic leukaemia and a patient with an isolated genital malformation without WT. However, the histological findings in the WTs with the R390X mutation have not been published previously. Histological information in these cases would show whether our hypothesis is more likely or not.

Schumacher et al reported that a germline mutation in WT1 predisposes to the development of tumours with predominantly stromal histology. Case 2 had no germline mutation; however, since Nakadate et al reported that not only germinal WT1 mutation/deletion but somatic WT1 deletion/mutation correlated with the predominantly stromal histology; the somatic mutation appears to be associated with the histology in case 2.

Some authors have reported WT1 mutations in WT patients with congenital genitourinary malformations in the absence of renal disorders. Huf et al hypothesised that the truncated protein resulting from WT1 mutation is non-functional and causes genitourinary malformation by a dose effect of decreasing normal WT1 protein. Cases 3, 4, 5, and 6, which had WT1 mutations, exhibited congenital genitourinary malformations, suggesting that the loss of WT1 function may cause genitourinary malformation.

Bilateral WT is explained by the two hit inactivation mechanism of a tumour suppressor gene, as proposed by Knudson and Strong. Cases 3, 4, 5, and 6 presented with bilateral disease, and a hereditary mutation was detected in their germline and either LOH (case 6) or a homozygous mutation (cases 3, 4, and 5) was found in their tumour specimens. These findings support the two hit inactivation model in bilateral WT. Based on the results of this study, the loss of WT1 expression caused by the R390X mutation appears to be related to the histological findings, genitourinary malformations, and tumorigenesis, at least in bilateral WT. The tumours in cases 1 and 7 contained rhabdomyomatous components and had bilateral disease, but no WT1 mutation was detected in them. They had no evidence of congenital genitourinary malformations. Another mechanism may be related to the tumorigenesis of bilateral WT without congenital genitourinary malformations.

CONCLUSION

We found that a specific WT1 mutation of R390X correlates with the histology of WT. WTs with this mutation tend to be FRN or to contain rhabdomyogenic components: histologically, this mutation appears to be strongly correlated with not only the histological features but with tumorigenesis in bilateral WTs with congenital genitourinary malformations. However, this report is on only seven cases and we need to investigate more cases of unilateral FRN or cases with FRN but no congenital abnormalities to see if they show WT1 mutation or not.

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