A high incidence of WT1 abnormality in bilateral Wilms tumours in Japan, and the penetrance rates in children with WT1 germline mutation

Y Kaneko *,1,2, H Okita 2, M Haruta 1,2, Y Arai 3, T Oue 2, Y Tanaka 2, H Horie 2, S Hinotsu 2, T Koshinaga 2, A Yoneda 2, Y Ohtsuka 2, T Taguchi 2 and M Fukuzawa 2

1Department of Cancer Diagnosis, Research Institute for Clinical Oncology, Saitama Cancer Center, Ina, Saitama 362-0806, Japan; 2Japan Wilms Tumor Study Group (JWiTS), Itabashi-Ku, Tokyo 173-8610, Japan and 3Division of Cancer Genomics, National Cancer Center Research Institute, Chuo-Ku, Tokyo 104-0045, Japan

Background: Bilateral Wilms tumours (BWTs) occur by germline mutation of various predisposing genes; one of which is WT1 whose abnormality was reported in 17–38% of BWTs in Caucasians, whereas no such studies have been conducted in East-Asians. Carriers with WT1 mutations are increasing because of improved survival.

Methods: Statuses of WT1 and IGF2 were examined in 45 BWTs from 31 patients with WT1 sequencing and SNP array-based genomic analyses. The penetrance rates were estimated in WT1-mutant familial Wilms tumours collected from the present and previous studies.

Results: We detected WT1 abnormalities in 25 (81%) of 31 patients and two families, which were included in the penetrance rate analysis of familial Wilms tumour. Of 35 BWTs from the 25 patients, 31 had small homozygous WT1 mutations and uniparental disomy of IGF2, while 4 had large 11p13 deletions with the retention of 11p heterozygosity. The penetrance rate was 100% if children inherited small WT1 mutations from their fathers, and 67% if inherited the mutations from their mothers, or inherited or had de novo 11p13 deletions irrespective of parental origin (P = 0.057).

Conclusions: The high incidence of WT1 abnormalities in Japanese BWTs sharply contrasts with the lower incidence in Caucasian counterparts, and the penetrance rates should be clarified for genetic counselling of survivors with WT1 mutations.

Wilms tumour (WT; OMIM 194070) arises from the developmental kidney (Rivera and Haber, 2005). Wilms tumour and retinoblastoma are typical embryonal tumours. The WT1 gene was altered in <25% of sporadic WTs (Haruta et al, 2012), whereas the RB1 gene was shown to be altered in >90% of hereditary and non-hereditary retinoblastoma (Leiderman et al, 2007), indicating genetic heterogeneity and homogeneity of WT and retinoblastoma, respectively. Bilateral WT is thought to be hereditary, and the germlinal mutation of WT1 located in 11p13 and alterations of 11p15 were reported in 17–38% and 55%, respectively, of bilateral WTs in the series reported from USA, UK and Australia (Huff, 1998; Scott et al, 2012; Hu et al, 2013). Carriers with WT1 mutations are now increasing because multidisciplinary therapies have improved the survival rates of patients with bilateral WTs and those with a unilateral WT (UWT) with a WT1 germline mutation (Royer-Pokora et al, 2008; Hu et al, 2013). The penetrance rates of WT1-mutant familial WT (FWT) are needed for genetic counselling of survivors with WT1 mutations.
the incidence of WT1 and 11p15 abnormalities in bilateral WTs of East Asian children, and have not yet tried to estimate the penetrance rates of WT1-mutant FWT.

WT1 is a multifunctional protein that acts as a transcriptional activator or repressor, is predominantly expressed in the embryonic kidney, and plays a pivotal role in its development (Huff, 2011). Insulin-like growth factor II (IGF2; OMIM 147470) is an imprinted gene expressed by the paternal allele, and encodes a foetal polypeptide growth factor (Foulstone et al., 2005). In contrast, WT1 is biallelically expressed in normal foetal tissues and WTs (Little et al., 1992). The loss of heterozygosity (LOH) and loss of imprinting (LOI) of IGF2 have been reported in 30–40% and 30–70% of sporadic WTs, respectively, and these alterations cause the overexpression of IGF2, which is involved in Wilms tumorigenesis (Schroeder et al., 1987; Ravenel et al., 2001; Haruta et al., 2008).

Both WT1 and IGF2 genes are located on the short arm of chromosome 11 (11p) and uniparental disomy (UPD) on 11p, involving either the region limited to 11p15 or that including both 11p15 and 11p13, is regularly accompanied by maternal allele loss and paternal allele duplication (Schroeder et al., 1987). We previously reported that small homozygous WT1 mutations and paternal UPD (pUPD) of 11p occurred in one-third of unilateral and bilateral WTs with various WT1 abnormalities (Haruta et al., 2008). Based on these genetic findings of human WT, Hu et al. (2011) showed that the combined occurrence of the upregulation of IGF2 and ablation of WT1 resulted in WT in transgenic Wt1-Igf2 mice; however, the upregulation of IGF2 or ablation of WT1 by themselves did not lead to malignant tumours.

The inheritance of WT1 mutations have been poorly studied in FWTs, and only 13 hereditary WT families with WT1 abnormalities have been described in the literature (Yunis and Ramsay, 1980; Kousseff and Agatucci, 1981; Nakagome et al., 1980; Kousseff and Agatucci, 1981; Nakagome et al., 1980). Both WT1 and IGF2 abnormalities have been described in the literature (Yunis and Ramsay, 1980; Haruta et al., 2008). The inheritance of WT1 abnormalities from their fathers or mothers, defined more than the mean percentage + 2 s.d. of five normal kidney and two PB samples was 53.6 ± 5.6%, and we defined more than the mean percentage + 2 s.d. as the hypermethylation state. Methylation specific-MLPA analysis was used to detect the methylation status of the IC1 (H19-DMR) and IC2 (KvDMR) regions. We determined the methylation status of 11p15 region in tumour and PB samples by combined bisulphite restriction assay (COBRA; Watanabe et al., 2006) and/or methylation specific (MS)-MLPA (Salsa MS-MLPA kit, ME030BWS/SRS) assay. Combined bisulphite restriction assay of CTCF6 at H19-differentially methylated region (H19-DMR) showed that the mean methylation percentage ± 2 s.d. of five normal kidney and two PB samples was 35.6 ± 5.6%, and we defined more than the mean percentage + 2 s.d. as the hypermethylation state.

Statistical analysis. Differences in the incidence of clinical and genetic characteristics between any two genetic subtypes of

**Materials and Methods**

Patients and samples. Forty-five tumour samples were available from 31 Japanese infants or children with bilateral WT, ranging in age between 2 and 26 months, who underwent surgery or biopsy between August 1996 and 2011 (Table 1); 11 of the 45 tumours and 7 of the 31 patients were described in a previous series of patients with WT1-mutant WT (Shibata et al., 2002; Haruta et al., 2008). In one of the seven patients, data on the 11p15 status was added and shown as Bilateral Wilms tumour 23 (BWT23) (Table 1; Shibata et al., 2002). In addition, five patients, including one with UWT of a DNWT1 mutation (UWTG1), one with familial and UWTG2, one with Wilms tumour-aniridia-genitourinary malformation-mental retardation (WAGR) syndrome-associated UWTG8 and two with sporadic and UWTS1 and 5 were incorporated into our previous study for a comparison of the data with those of WT1-mutant bilateral WTs (Table 2). Normal tissue samples were obtained from either peripheral blood (PB) or normal renal tissue adjacent to the tumour from the same patients. Tumours were staged according to the National Wilms Tumor Study Group (NWTS) staging system and most patients were treated according to the NWTS protocols (D’Angio et al., 1989; Oue et al., 2009). Malformations found in patients with bilateral WT are listed in Table 1. None of the patients in the present study showed hemihypertrophy or malformations associated with Beckwith–Wiedemann syndrome (BWS; OMIM#130650). One (BWT9) died of the disease, another (BWT27) with premature chromatid separation (PCS) syndrome died of infection (Matsuura et al., 2006) and 29 were alive at the last follow-up.

This study was approved by the Ethics Committee at Saitama Cancer Center, and written informed consent was obtained from parents for samples from the Japan Wilms Tumor Study Group (JWITS; Oue et al., 2009). Since written informed consent was not obtained in a subset of patients collected before 2001, identifying information was removed prior to their analysis, in accordance with the Ethical Guidelines for Clinical Research enacted by the Japanese Government. The Ethics Committee approved the waiver of written informed consent for the latter samples.

**Histological examination.** The diagnosis of WT was made in all 45 tumours, with routine haematoxylin and eosin-stained pathology slides by pathologists at each institution or the JWITS pathology panel according to the classification proposed by the Japanese Society of Pathology (The committee on histological classification of childhood tumors, 2008). In addition, a pathological review of 29 tissue specimens was performed by the JWITS pathology panel.

**Analysis of WT1 and allelic loss on 11p and 11q.** Copy number and LOH analysis using single-nucleotide polymorphisms (SNP) arrays, Affymetrix Mapping 50K-Xba and 250K-Nsp arrays (Affymetrix, Santa Clara, CA, USA) was conducted as described previously (Haruta et al., 2008). Copy numbers and LOH were calculated using CNAG and AsCNAR programmes with paired or anonymous references as controls (Nannya et al., 2005; Yamamoto et al., 2007). Gross WT1 deletions were analysed by Southern blotting using a WT1 CDNA probe and BCLI in chromosome band 11q13, or by SNP array or the multiplex ligation-dependent probe amplification (MLPA) method (Salsa MLPA kit, MRC-Holland, Amsterdam, the Netherlands). To detect small WT1 mutations, defined as missense, nonsense, frame-shift or splice-site mutations, all coding exons including flanking intronic sequences of WT1 were amplified from genomic DNA by PCR, and PCR products were directly sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA).
| Tumour   | Age/sex | 1p15 SNP | DMR | Status                      | Anomaly/reference/heredity | WT1 allele 1 | Other CGH changes | WT1 allele 2 | WT1 allele 3 |
|----------|---------|----------|-----|-----------------------------|-----------------------------|--------------|-------------------|--------------|--------------|
| BWT1     | 1 year/1 month | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion spanning exon 4-9 | The same as allele 1, UPD11pt-11cen | None |
| BWT2     | 1 year/1 month | 11 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 21 Mb | The same as allele 1, UPD11pt-11cen | None |
| BWT3     | 1 year/1 month | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 1.6 Mb | The same as allele 1, UPD11pt-11cen | None |
| BWT4     | 1 year/1 month | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 21.8 Mb | Deletion spanning exons 4–9 | None |
| BWT5     | 9 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 3.2 Mb | IVS6 | None |
| BWT6     | 12 months | 10 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 3.2 Mb | Deletion of 1.6 Mb | None |
| BWT7     | 7 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 21.8 Mb | Deletion spanning exons 4–9 | None |
| BWT8     | 2 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 3.2 Mb | Deletion of 1.6 Mb | None |
| BWT9     | 1 year/1 month | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 21.8 Mb | Deletion spanning exons 4–9 | None |
| BWT10    | 1 year/1 month | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 3.2 Mb | Deletion of 1.6 Mb | None |
| BWT11    | 1 year/1 month | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 21.8 Mb | Deletion spanning exons 4–9 | None |
| BWT12    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 3.2 Mb | Deletion of 1.6 Mb | None |
| BWT13    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 21.8 Mb | Deletion spanning exons 4–9 | None |
| BWT14    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 3.2 Mb | Deletion of 1.6 Mb | None |
| BWT15    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 21.8 Mb | Deletion spanning exons 4–9 | None |
| BWT16    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 3.2 Mb | Deletion of 1.6 Mb | None |
| BWT17    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 21.8 Mb | Deletion spanning exons 4–9 | None |
| BWT18    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 3.2 Mb | Deletion of 1.6 Mb | None |
| BWT19    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 21.8 Mb | Deletion spanning exons 4–9 | None |
| BWT20    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 3.2 Mb | Deletion of 1.6 Mb | None |
| BWT21    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 21.8 Mb | Deletion spanning exons 4–9 | None |
| BWT22    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 3.2 Mb | Deletion of 1.6 Mb | None |
| BWT23    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 21.8 Mb | Deletion spanning exons 4–9 | None |
| BWT24    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 3.2 Mb | Deletion of 1.6 Mb | None |
| BWT25    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 21.8 Mb | Deletion spanning exons 4–9 | None |
| BWT26    | 11 months | 12 months | ROH | Hypermethyl. (C) | No | pUPD | Deletion of 3.2 Mb | Deletion of 1.6 Mb | None |

Table 1. Genetic and epigenetic alterations in IGFR2 (1p 15) and WT1 (1p13) loci in 31 patients with bilateral Wilms tumours in Japan.
| Tumour   | Age/sex     | 11p15 SNP | H19-DMR Status | IGF2 Status | WT1 allele 1 | WT1 allele 2 | Other CGH changes | Anomaly/reference/heredity |
|----------|-------------|-----------|----------------|-------------|--------------|--------------|-------------------|---------------------------|
| BWT26L   | 7 months/M  | ROH       | Normal Methyl. (C) | ROI         | Wild type    | Wild type    | UPD1q            | None                      |
| BWT26R   | —           | ROH       | Normal Methyl. (C) | ROI         | Wild type    | Wild type    | UPD1q            | None                      |
| BWT27R   | 10 months/M | UPD       | Hypermethyl. (C, M) | pUPD        | Wild type    | Wild type    | +2,UPD11, +12, +17, +18 | PCS syndrome              |
| BWT28L   | 1 year 1 month/M | ROH       | Hypermethyl. (C) | LOI         | Wild type    | Wild type    | Normal            | None                      |
| BWT28R   | —           | ROH       | Hypermethyl. (C) | LOI         | Wild type    | Wild type    | +6, +12          | None                      |
| BWT29    | 9 months/F  | ROH       | Hypermethyl. (C, M) | LOI         | Wild type    | Wild type    | 1q+, +7,7q;UPD8, UPD9, +10, +12, +15q+, +20, UPD22 | None                      |
| BWT30L   | 1 year 5 months/F | UPD       | Hypermethyl. (C, M) | pUPD        | Wild type    | Wild type    | +8, +10, UPD11, +12 | Coarctation of the aorta |
| BWT30R   | ROH         | Normal Methyl. (C) | ROI         | Wild type    | Wild type    | UPD2, +5, +7, +8, +10, +12, +18 | Coarctation of the aorta |
| BWT31L   | 1 year 2 months/F | UPD       | Hypermethyl. (C) | pUPD        | Wild type    | Wild type    | 1q+, +3, +6, +8, +9, UPD11 +1cen, +12, +13, +16q | None                      |
| BWT31R   | ROH         | Normal Methyl. (C) | ROI         | Wild type    | Wild type    | Normal        | None              | None                      |

Abbreviations: BWT = bilateral Wilms tumour; C = methylation analysis of CTCF6 by COBRA; CGH = comparative genomic hybridisation; COBRA = combined bisulfite restriction assay; DMR = differentially methylated region; ex = exon; F = female; FGS = focal glomerular sclerosis; FWT1 = familial WT1; LOI = loss of imprinting; M = male; M, methylation analysis of H19-DMR by MS-MLPA; methyl = methylation; MLPA = multiplex ligation-dependent probe amplification; NE = not examined; PB = peripheral blood; PCS = premature chromatid separation; pUPD = paternal IGF2 UPD; ROH = retention of heterozygosity; ROI = retention of IGF2 imprinting; SNP = single-nucleotide polymorphism; UPD = uniparental disomy; WAGR = Wilms tumour–aniridia–genitourinary malformation–mental retardation. See Table 3 for FWT1.

* Case numbers reported by Haruta et al., 2008.
* Above pUPD, LOI or ROI indicates that normal methylation pattern at H19-DMR was identified in PB by COBRA.
* Germline origin of the mutation was confirmed.
* Case number reported by Shibata et al., 2002.
* Stage was corrected from I to V in the present Table 1.
Table 2. Genetic and epigenetic alterations in IGF2 (11p15) and WT1 (11p13) loci in 10 syndromic or familial and 20 sporadic patients with unilateral Wilms tumours

| Tumour | Age/sex | Stage | IGF2 | WT1 allele 1 | WT1 allele 2 | Malformations/heredity | References |
|--------|---------|-------|------|--------------|--------------|-------------------------|------------|
|        |         |       |      |              |              |                         |            |
| Patients with syndromic or familial unilateral Wilms tumours (n = 10) | | | | | | | |
| UWTG1  | 1 year 6 months/F | I | pUPD | ex 1, 144C > A/Y485Cl | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTG2  | 1 year 4 months/M | I | pUPD(C) | ex 9, 1168C > T/R390Xb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTG3  | 12 months/F | II | pUPD | ex 8, 1604G > A/C535Y | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTG4  | 1 year 9 months/F | III | pUPD(C) | ex 3, 666_666delinsT/S222fs299X | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTG5  | 9 months/F | II | pUPD | ex 2, 549C > T/Q184Xb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTG6  | 1 year 7 months/F | III | ROH/ROI(C) | Deletion of 2.4 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTG7  | 5 months/F | I | ROH/LROI(C) | Deletion of 6.5 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTG8  | 1 year 3 months/M | I | ROH/ROI(C) | Deletion spanning exons 1–10 | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTG9  | 1 year 3 months/M | I | ROH/ROI(C) | Deletion of 4.4 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTG10 | 1 year 3 months/M | I | ROH/ROI(C) | Deletion of 4.4 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |

Patients with sporadic and unilateral Wilms tumours (n = 20)

| Tumour | Age/sex | Stage | IGF2 | WT1 allele 1 | WT1 allele 2 | Malformations/heredity | References |
|--------|---------|-------|------|--------------|--------------|-------------------------|------------|
|        |         |       |      |              |              |                         |            |
| UWTS1  | 1 year 5 months/M | I | pUPD(C) | ex 1, 231_232insC/W816S3X | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS2  | 1 year 4 months/F | I | pUPD(C) | ex 8, 1068_1069insC/F337fs380X | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS3  | 9 months/F | I | pUPD(C) | ex 1, 204_206delinsCC/P68fs162X | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS4  | 9 months/M | I | pUPD(C) | ex 7, 1021C > T/Q241X | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS5  | 1 year 3 months/M | I | pUPD(C) | ex 8, 1084C > T/R362X | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS6  | 4 years 8 months/F | III | ROH/ROI(C) | Deletion of 3.9 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS7  | 3 years 4 months/F | II | ROH/ROI(C) | Deletion of 3.3 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS8  | 3 years 1 month/M | I | ROH/ROI(C) | Deletion of 1.3 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS9  | 1 year 11 months/M | I | ROH/ROI(C) | Deletion of 1.3 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS10 | 1 year 3 months/F | II | Monosomy 11(C) | Loss of chromosome 11 | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS11 | 1 year 11 months/M | I | ROH/ROI(C) | Deletion of 1.8 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS12 | 2 years 5 months/M | II | ROH/ROI(C) | Deletion of 3.1 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS13 | 2 years 3 months/M | I | ROH/ROI(C) | Deletion of 9.1 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS14 | 5 years 7 months/F | II | ROH/ROI(C), P | Deletion of 2.1 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS15 | 2 years 7 months/M | I | ROH/ROI(C) | Deletion of 3.5 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS16 | 1 year 9 months/M | I | ROH/ROI(C) | Deletion of 4.5 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS17 | 10 months/F | I | ROH/ROI(C) | Deletion of 18.5 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS18 | 1 year 7 months/M | I | ROH/ROI(C) | Deletion of 2.7 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS19 | 2 years 4 months/M | III | ROH/ROI(C) | Deletion of 10.8 Mb | The same as allele 1, UPD11p11-1cen | None/none | The present study |
| UWTS20 | 2 years 6 months/F | II | pUPD(C) | ex 1, 231_232insC/W816S3X | The same as allele 1, UPD11p11-1cen | None/none | The present study |

Abbreviations: C = methylation analysis of CTCF6 at H19-DMR by COBRA; COBRA = combined bisulfite restriction analysis; ex = exon; F = female; P = allelic expression analysis on the polymorphic site at exon 9 of IGF2; LOI = loss of IGF2 imprinting; M = male; pUPD = paternal IGF2 UPD; ROH = retention of heterozygosity on 11p15; ROI = retention of IGF2 imprinting; UPD = uniparental disomy; WAGR = Wilms tumour-aniridia-genitourinary malformation-mental retardation syndrome. See Table 3 for DNWT1-1 = PWT2-1.

*Above UPD indicates that the methylation status of H19DMR was not examined.

*Somatic origin of the mutation was confirmed.
tumours were examined by the χ² or Fisher’s exact test. P < 0.05 (two-sided) was considered statistically significant. The Student’s t-test or Mann–Whitney test was used to compare mean ages between patients with WT1-mutant bilateral WT and those with WT1-mutant sporadic and UWT, between patients with sporadic and UWT with a small WT1 mutation and those with sporadic and UWT with a large WT1 deletion, or between patients with paternal inheritance of a small WT1 mutation and those with maternal inheritance of a small WT1 mutation.

RESULTS

Bilateral WT1-mutant WTs with or without pUPD on 11p. Of 45 bilateral tumours from 31 patients, 35 tumours from 25 (81%) patients had WT1 abnormalities; 30 tumours from 21 patients showed small mutations in 1 WT1 allele and the same small mutation in the other WT1 allele, caused by UPD on 11p (Table 1). One tumour (BWT33R) with deletion flanking exons 1–5 also had UPD on 11p, and was added to the above 30 tumours because of the small deletion. Combined bisulphite restriction assay and/or MS-MLPA identified hypermethylation of H19-DMR, which indicated the paternal origin of IGF2 UPD in all 31 tumours except 1 whose tumour DNA was not available (BWT21R). The remaining four tumours from three patients had large deletions encompassing WT1 in one WT1 allele and a frame-shift or splice-site mutation (three tumours; BWT2R, 9L and 22R) or a small deletion spanning exons 4–9 (one tumour; BWT2L) in the other WT1 allele. All four tumours had the retention of heterozygosity (ROH) on 11p, and had normally methylated CTCF6 at H19-DMR, indicating retention of IGF2 imprinting (ROI). The methylation status of CTCF6 at H19-DMR in PB was examined in 13 of the 25 patients. All 13 patients showed the normal methylation in PB, indicating somatic origin of UPD on 11p15.

WT1-mutant UWTs with or without pUPD on 11p. To compare WT1 and IGF2 statuses between 35 WT1-mutant bilateral WTs and 10 WT1-mutant syndromic or familial UWTs or 20 WT1-mutant sporadic and UWTs, we combined our published and unpublished data on WT1-mutant WTs and presented them in Table 2. One (BWT21R) of the 35 WTs and one (UWTG1) of the 10 WTs, in which 11p UPD was identified by SNP array but methylation status of H19-DMR was not examined, were included in each group of tumours with small WT1 mutation and pUPD of IGF2 because all 30 WT1-mutant bilateral WTs with 11p UPD examined in the present study showed the hypermethylation indicating paternal 11p UPD, and the previous study indicated loss of maternal 11p allele in WTs with 11p LOH (Schroeder et al., 1987). Of the 10 patients, 5 (UWTG1-5) had small homozgyous WT1 mutations and pUPD on 11p, whereas 5 (UWTG6-10) associated with WAGR syndrome had large deletions in 1 WT1 allele and small mutations in the other WT1 allele with IGF2 ROH/ROI in the tumours (Table 2). Of the 20 patients, 5 (UWTST1-5) had small homozgyous WT1 mutations and pUPD on whole 11p in the tumours, whereas none of the remaining 15 (UWTSt6-15) had the small WT1 mutations and pUPD on 11p; 12 had IGF2 ROI, 2 had pUPD limited to the 11p15 region and 1 had monosomy 11 of paternal origin in the tumours (Table 2). Thus, a homozgyous WT1 mutation with pUPD on 11p was more frequent in 35 WT1-mutant bilateral WTs than in 10 WT1-mutant syndromic or familial UWTs (P = 0.017) or 20 WT1-mutant sporadic and UWTs (P = 3.0E–06).

When we analysed the 3 groups of patients with WT1-mutant WTs, the mean age of 25 patients with bilateral WT was 12.4 months, that of 10 patients with syndromic or familial and UWT was 14.3 months and that of 20 patients with sporadic UWT was 25.6 months. The 25 and 10 patients were younger than the 20 patients, respectively (P = 0.001 and P = 0.006), whereas no difference in age was found between the 25 and 10 patients (P = 0.286). When we selected the 20 patients with sporadic and UWTs, the mean age of 5 patients with homozygous WT1 mutations and paternal 11p UPD was 13.2 months and that of 15 patients with large deletions encompassing WT1 with or without pUPD limited to the 11p15 region in the tumours was 29.7 months. The 5 patients with homozygous WT1 mutations and pUPD on 11p were younger than the 15 patients with large 11p3 deletions (P = 0.002).

WT1-wild-type bilateral WTs and the IGF2 status. Ten bilateral tumours from six patients had wild-type WT1; six tumours had +12, and 2 had 1q+, +6, +8. or pUPD of whole chromosome 11 and one had pUPD of 11p (Table 1). None of the six patients showed characteristics of BWS. Single-nucleotide polymorphisms analysis and COBRA of CTCF6 at H19-DMR and/or MS-MLPA revealed pUPD on 11p or whole chromosome 11 in three tumours, ROI of IGF2 in four and LOI of IGF2 in three (Table 1). Of three tumours with pUPD on 11p or whole chromosome 11, the corresponding PB showed normal methylation at H19-DMR in one (BWT30L) and was not available in two; in one of the two, the contralateral tumour had ROH/ROI (BWT31R), denying the constitutional 11p15 UPD (BWT31L). Of three tumours with LOI of IGF2, the corresponding PB showed normal methylation at H19-DMR in one (BWT29) and was not available in two bilateral WTs (BWT28L, 28R) from one patient, not denying the possibility of constitutional hypermethylation of maternal H19-DMR (Table 1).

Of 27 tumours, whose methylation status at IC2 was examined by MS-MLPA, 26 and 1 showed hypomethylation and normal methylation, respectively; the results were consistent with pUPD and LOI of 11p15, respectively.

Histology of bilateral and FWTs. Of 29 WT1-mutant bilateral WTs, which were available for pathological review, 25 and 4 tissue specimens were obtained before and after chemotherapy, respectively. Of the 25 tumours, 12 were classified as the mesenchymal type; 4 and 2 of the 12 also had intra-lobar nephrogenic rests (ILNR) and foetal rhabdomyomatous nephroblastoma, respectively, 11 were classified as the mixed type; 1 also had ILNR, 1 was classified as nephroblastoma with ILNR and 1 was classified as ILNR only. Of 4 specimens obtained after chemotherapy, 3 were classified as the mesenchymal type; one also had ILNR, and one as nephroblastoma with ILNR.

Of seven WT1-wild-type bilateral WTs from six patients, which were available for pathological review, five and two specimens were obtained before and after chemotherapy, respectively. Of the five tumours, two (BWT28, and 29) were classified as the epithelial type, two (BWT26 and 30) as the mixed type and one (BWT27) as the mesenchymal type. While the mesenchymal and mixed types were found in both WT1-mutant and WT1-wild-type bilateral WTs, the epithelial type was only found in WT1-wild-type bilateral WTs. Two specimens obtained after chemotherapy showed either the mixed or mesenchymal type. None of the tumour specimens from 31 patients exhibited features of focal or diffuse anaplasia.

The penetrance rates of WTs with inherited or DNWTT1 abnormalities, classified according to parental inheritance and WT1 abnormality types. We summarised 22 children from 14 families who inherited WT1 mutation/deletion from their mothers or fathers, and 8 children who had de novo large deletion encompassing WT1 of known parental germ cell origin from the present study and literatures listed in PubMed. The 22 individuals included 3 patients with WTs (FWT4-1, 4-3 and FWT12-1; Figure 1, Table 3); although molecular analyses have not been done in their PB and tumour samples, the pedigrees showed that the 3 patients who developed WT were thought to inherit WT1 mutations/deletions from their father or mother.
Figure 1. Pedigrees of patients with WTs with small WT1 germline mutations inherited from fathers (FWT1-6) and mothers (FWT7-10), and those of patients with large 11p13 deletions encompassing WT1 inherited from fathers (FWT11) or mothers (FWT12-14). DNWT1-8 indicates eight families having the de novo deletion of paternal or maternal germ cell origin. Open boxes and circles containing a small circle indicate males and females, respectively, with familial 11p13 deletions. Open boxes and circles indicate males and females, respectively, who developed WT. Filled boxes and circles indicate males and females, respectively, who did not develop WT. Open boxes with an inverted triangle indicate males with the deletion who did not develop WT. Oblongs and ovals indicate paternal and maternal penetrance analysis of patients with large 11p13 deletions encompassing WT1. In all families, the paternal germ cell origin of the mutation was determined by SNP markers (SNP A1946935, 2241668, 4231943 and seven others). In this study, all nine patients who inherited a small WT1 mutation from their fathers developed WT; a girl (FWT6-2) was excluded from the penetrance analysis because she died of renal failure at the age of 23 weeks before the possible development of WT (Fencl et al, 2012). Of nine WTs with the paternal inheritance of WT1 mutation, four tumours [FWT1 (BWT23L, 23R), 2-1, 2-2] showed homozygous WT1 mutations, four tumours showed homozygous WT1 mutations, and two (FWT3, 4-2) showed homozygous WT1 mutations, suggesting that the six tumours may have had the same WT1 and IGF2 abnormalities (Figure 1 and Table 3). Of 6 individuals who inherited small WT1 mutations from their mothers, 4 developed WT; in addition to the small germline mutation in 1 allele 1 had a 26 base-pair deletion that differed from the first mutation (FWT7-1), 1 had a large 11p13 deletion (FWT7-2), 1 had a wild-type WT1 (FWT10) in the other allele in their tumours and the WT1 status in the tumour was not examined in the last patient (FWT8-1; Pritchard-Jones et al, 2000; Zirn et al, 2005; Regev et al, 2008; Melchionda et al, 2013). Thus, all three
Table 3. Genetic and epigenetic alterations in IGFBP2 (11p15) and WT1 (11p13) loci in 34 patients with Wilms tumours of familial or de novo WT1 mutations

| Tumour | Age/sex | Children inherited small WT1 mutations from their fathers (n = 6) | WT1 allele 1 | WT1 allele 2 |
|--------|---------|---------------------------------------------------------------|--------------|--------------|
| FWT1   | 7 months | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | OD, FGS/BWT |
| FWT2   | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | GU/UWT |
| FWT3   | 3 years   | F father had a WT1 mutation and no history of WT              | H291fs306X   | LOH at WT1 mutation and a history of WT | Pelletier et al., 1991 |
| FWT4   | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | GU/UWT |
| FWT5   | 1 year    | F father had a WT1 mutation and no history of WT              | S223N        | LOH at WT1 mutation and a history of WT | Jeanpierre et al., 1991 |
| FWT6   | 1 year    | F father had a WT1 mutation and no history of WT              | R366P        | CNS/UWT      | Fencl et al., 2012 |
| FWT7   | 2 years   | F father had a WT1 mutation and no history of WT              | H319fs377X   | LOH at WT1 mutation and a history of WT | Lavedan et al., 1989 |
| FWT8   | 9 years   | F father had a WT1 mutation and no history of WT              | X450W        | Renal failure/UWT | Zirn et al., 2000 |
| FWT9   | 6 years   | F father had a WT1 mutation and a history of WT               | Y109X        | GU/WT        | Regev et al., 2008 |
| FWT10  | 1 year    | F father had a WT1 mutation and no history of WT              | P328fs380X   | UWT          | Kousseff and Agatucci, 1981 |
| FWT11  | 2 years   | none                                                          | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT12  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT13  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT14  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT15  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT16  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT17  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT18  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT19  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT20  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT21  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT22  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT23  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT24  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT25  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT26  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT27  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT28  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT29  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT30  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT31  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT32  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT33  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |
| FWT34  | 1 year    | F father had a WT1 mutation and no history of WT              | T/R390X      | The same as allele 1, UPD11q13q14 | AN, GU UWT |

Abbreviations: AN = aniridia; BWT = bilateral WT; COBRA = combined bisulfite restriction assay; CNS = congenital nephrotic syndrome; GU = genitourinary malformation; LOH = loss of heterozygosity; M = male; MR = renal malformation; NR = not relevant; OD = oligodactyly; OD, FGS/BWT = oligodactyly, focal glomerular sclerosis, bilateral WT; OD, GU, MR = oligodactyly, genitourinary malformation, renal malformation; OD, GU, MR/UWT = oligodactyly, genitourinary malformation, renal malformation, unilateral WT; OD, GU/WT = oligodactyly, genitourinary malformation, unilateral WT; OD/WT = oligodactyly, unilateral WT; UWT = unilateral WT; UWTG1 = unilateral WT, familial WT1 mutation; UWTG2 = unilateral WT, de novo WT1 mutation; WT = Wilms tumour.

Genetic and epigenetic alterations in IGFBP2 (11p15) and WT1 (11p13) loci in 34 patients with Wilms tumours of familial or de novo WT1 mutations.
patients who inherited the WT1 mutation from their mothers and whose WT1 status in tumours were examined were not likely to have UPD on 11p in their tumours (Figure 1 and Table 3). The mean ages at diagnosis were 14.2 months for the nine patients with the paternal inheritance and 55.3 months for the four patients with the maternal inheritance; the mean age of the nine patients who inherited the mutations from their fathers was younger than that of the four patients who inherited the mutations from their mothers (P = 0.011 by Mann–Whitney test).

Fifteen individuals from 12 families were shown to have inherited (FWT11-14) or de novo large 11p13 deletions (DNWT1-8) of paternal or maternal origin (Figure 1 and Table 3; Yunis and Ramsay, 1980; Koussev and Agatucci, 1981; Nakagome et al., 1984; Lavedan et al., 1989; Huff et al., 1990; Nordenskjold et al., 1994). All four parents who transmitted large 11p13 deletions had balanced chromosomal involvements involving the 11p13 band. Ten patients developed WTs, on which sequencing analysis of WT1 was not conducted to identify the status of the other WT1 allele in the tumours. Thus, the penetrance rate was 100% (9/9) for individuals who inherited small WT1 mutations from their fathers, and was 67% (14/21) for individuals who inherited small WT1 mutations from their mothers or large 11p13 deletions or had de novo large 11p13 deletions irrespective of the parental origin. Thus, the 9 individuals were more likely to develop WT than the 21 individuals (P = 0.057).

**DISCUSSION**

The incidence of WT1 abnormalities in the bilateral WTs of Japanese children was 81%, and this incidence was markedly higher than those reported in American, UK and Australian children (the mean percentage of three series, 32%; P = 9.4E − 05; Table 4; Huff, 1998; Scott et al., 2012; Hu et al., 2013). The present series included 31 patients and 45 bilateral WTs, and the UK series included 11 patients and 11 bilateral WTs; the statuses of WT1 and 11p13 were precisely examined in 44 of the 45 and all 11 tumours (Table 4; Scott et al., 2012). The incidence of WT1 abnormality is more frequent in Japanese tumours than in British tumours; however, if we consider that the incidence of WT among Japanese is half of that in Caucasians, the population-based rate of bilateral WT with WT1 abnormality may be similar between the two populations (Figure 2). Diller et al. (1998) found constitutional WT1 abnormalities in 8 of 157 (5%) American children with a history of WT. Likewise, Little et al. (2004) found constitutional WT1 abnormalities in 6 (2%) of 282 British children with non-syndromic WT. Unfortunately, no studies on constitutional WT1 abnormalities have been performed in Japanese children with WT that precluded the comparison of the incidence of constitutional WT1 abnormalities between the two ethnic populations. In contrast to the equivocal findings in the incidence of WT1 abnormality, that of IGF2 LOI was clearly higher in British children with WT1-wild-type or WT1-wild-type plus WT1-mutant bilateral WT than Japanese counterparts (Table 4 and Figure 2; Scott et al., 2012). Thus, it is clear that the incidence of IGF2 LOI in Caucasian bilateral WTs is higher than that in Japanese counterparts, and that the incidence of WT1 wild-type was low in Japanese bilateral WTs.

The incidence rates of WT are known to vary, being markedly lower in East Asian children than in their Caucasian counterparts (Parkin et al., 1988). We previously reported that if only sporadic WTs were included, the frequencies of WT with WT1 abnormality were similar between East-Asian and Caucasian populations (Haruta et al., 2012). Furthermore, we reported a lower incidence of WT with IGF2 LOI in Japanese children than in American children (P = 0.041), and we and others proposed that the lower incidence of IGF2 LOI may be one of the reasons for the lower incidence of WT in Japan (Fukuzawa et al., 2004; Haruta et al., 2012). Contrary to the equivocal findings in the incidences of WT1 abnormality in sporadic or bilateral WTs between the two populations, the difference in the incidence of IGF2 LOI in bilateral WTs is clear (P = 0.036); the relationship is comparable to that of IGF2 LOI in sporadic WTs between the two populations. Beckwith–Wiedemann syndrome is an imprinting-related growth disorder. Five to 10% of patients with BWS have methylation of H19-DMR on both parental chromosomes, resulting in IGF2 LOI (Cerrato et al., 2008). Interestingly, Japanese patients with BWS were shown to have a significantly lower frequency of H19-DMR hypermethylation than North American and European patients, whereas the incidences of pUPD on 11p15 were comparable, suggesting that susceptibility to epigenetic alterations differs between the two populations (Sasaki et al., 2007). The constitutional 11p15 abnormalities in patients with WT were reported from UK and Netherlands. The UK series included 437 patients with non-syndromic WT and found 11p15 abnormalities in 13 (3%) patients; of the 13 patients 4 had bilateral WT and 6 had hypermethylation of H19-DMR (Scott et al., 2008). The Netherlands series included 109 patients with syndromic or non-syndromic WTs and found 8 (7.3%) children with 11p15 abnormalities; of the 8 patients 3 had bilateral WT, 4 had BWS and 3 had hypermethylation of H19-DMR (Segers et al., 2012). Of 13 patients whose methylation status of H19-DMR in PB was examined in the present series, all including 1 (BWT29) with IGF2 LOI in the tumour showed the normal methylation pattern. Thus, the involvement of the constitutional IGF2 LOI in Japanese bilateral WTs could not be identified. The same decreased susceptibility to the epigenetic change reported in BWS may have also caused the decreased incidence of bilateral WTs with IGF2 LOI (Sasaki et al., 2007). The study for constitutional 11p15 abnormalities in Japanese WTs is needed to prove the hypothesis.

**Table 4. Incidence rates of WT1 and IGF2 abnormalities in Japanese and British, American or Australian series of bilateral Wilms tumour**

|        | WT1 abn + wt | pUPD | ROI | LOI | WT1 abn | pUPD | ROI | LOI | WT1 wt | pUPD | ROI | LOI |
|--------|--------------|------|-----|-----|---------|------|-----|-----|-------|------|-----|-----|
| A. Japan (the present study) | 45* (31) | 34* | 8   | 3   | 35* (25) | 31* | 4   | 0   | 10 (6) | 3    | 4   | 3   |
| B. UK (Scott et al., 2012) | 11 | 5 | 0 | 6 | 4 | 4 | 0 | 7 | 1 | 0 | 6 |
| Constitutional defect-associated | 5 | 5 | 0 | 0 | 4 | 4 | 0 | 1 | 1 | 0 | 0 |
| Sporadic | 6 | 0 | 0 | 6 | 0 | 0 | 0 | 6 | 0 | 0 | 6 |
| C. USA (Huff, 1998) | 15 | NE | NE | NE | 4 | NE | NE | NE | 11 | NE | NE | NE |
| D. Australia (Hu et al., 2013) | 8 | NE | NE | NE | 3 | NE | NE | NE | 5 | NE | NE | NE |

Abbreviations: abn = abnormality; LOI = loss of IGF2 imprinting; NE = not examined; pUPD = paternal uniparental disomy; ROI = retention of IGF2 imprinting; SNP = single-nucleotide polymorphisms; wt = wild type. Numbers indicate numbers of tumours, and those in parentheses indicate numbers of patients in the Japanese series. Numbers in other series indicate both patient and tumour numbers. WT1 abnormality and WT1 wild type (patients): A versus B P = 0.011; A versus B + C + D P = 9.4E − 05. WT1 abnormality and WT1 wild type (tumours): A versus B P = 0.012; A versus B + C + D P = 5.1E − 05. LOI and pUPD + ROI (tumours with WT1 abnormality and those with WT1 wild type): A versus B P = 0.001. LOI and pUPD + ROI (tumours with WT1 wild type): A versus B P = 0.036.

*Include one tumour (BWT21R) in which pUPD was detected by SNP array analysis but methylation status at H19-DMR was not examined.*
The present study included six patients having bilateral WTs with wild-type WT1, and one of them had PCS syndrome, which was caused by a BUB1B mutation and known to be associated with a predisposition to WT (Matsuura et al., 2006). The FWT genes, FWT1 and FWT2, were located at 17q21 and 19q13, respectively, and the lack of a linkage to these loci in some WT families was reported previously and indicated the existence of additional FWT genes (Ruteshouser and Huff, 2004). Very recently, Hanks et al. (2014) identified germline mutation of the CTR9 gene in 3 of 35 WT families using an exome and sequencing analysis and proposed it as a new WT predisposing gene. These genes may be candidates for germline mutations in the other five patients with WT1-wild-type bilateral WTs in the present series.

A previous study showed that the WT1 mutations observed in bilateral WT were of germline origin (Huff, 1998). Another study reviewed WT1 germline mutations in 117 patients with WTs (Royer-Pokora et al., 2004). Of the 117 patients, 44 had bilateral WT, indicating that a large proportion of germline WT1 mutations are associated with bilateral WT, although the inheritance is not known in all patients. The present study included 25 patients with WT1-mutant bilateral WTs, and the status of WT1 in PB was only examined in 3 patients and 6 parents from 3 families; 2 were shown to have inherited the mutation from their father with or without a past history of WT and 1 was identified to have a DNWT1 mutation of paternal germ cell origin. Thus, mutation analyses of WTs were performed.

Figure 2. Abnormalities of the WT1 and IGF2-LOI in bilateral WTs in Japanese (the present series) and British children (Scott et al., 2012). The bar length for Japanese is half of that for Caucasians because the incidence rate for Japanese is half of that for Caucasians.

Figure 3. Parental inheritance of small WT1 mutations and large 11p13 deletion and a model of Wilms tumorigenesis. Black and white circles in terminal 11p represent methylated and unmethylated statuses, respectively, at CTCF6 in the IGF2-H19 region. Blue arrows indicate the expression of IGF2. × and star in the 11p13 region indicate the first and second WT1 mutations, respectively. Solid and broken arrows in the 11p13 region indicate normal and abnormal WT1 expression, respectively. Parents had balanced insertions involving the 11p13 band (H). A gap in the 11p chromatid (I and L) indicates a large deletion encompassing WT1, and × in the 11p13 region indicates the second WT1 mutations (K and N). Explanation for panels A–G, J, and M is described in the discussion.
WT1 in the PB of patients and their parents are needed to determine whether the mutation is inherited from the parents or occurred de novo.

A previous study examined the parental origin of de novo RB1 mutations, and found that the paternal origin of the mutation in all patients (Dryja et al., 1989). Regarding DNWT1 alterations, we and other researchers found that the paternal germ cell origin of the small mutation in four patients and that of the large deletion in seven patients and the maternal germ cell origin of the large deletion in one patient (Huff et al., 1990; Nordenskiodt et al., 1994). No studies have reported the maternal germ cell origin of de novo small WT1 mutations. In a review on human germinal mutations, Crow (2000) described that one marked difference between the human male and female was that there are many more germine cell divisions in the life history of a sperm than that of an egg. In WT1s with homozygous WT1 mutations and paternal IGF2 UPD, the IGF2 alteration is thought to be the second genetic event subsequent to the WT1 alteration that has been shown to occur in the paternal WT1 allele. The result that pUPD on 11p was found in the great majority of bilateral WT1s in the present study further supports the paternal germ cell origin of de novo small WT1 mutations.

We summarised the data of all 30 children from 22 families with hereditary WT, whose inheritance of the WT1 alteration was described in the present and previous studies (Table 3; Yunis and Ramsay, 1980; Koussef and Agatucci, 1981; Nakagome et al., 1984; Lavedan et al., 1989; Pelletier et al., 1991; Kaplinsky et al., 1996; Jeangierre et al., 1998; Pritchard-Jones et al., 2000; Shibata et al., 2002; Zirm et al., 2005; Regev et al., 2008; Fencl et al., 2012; Melchionda et al., 2013). We classified 30 children into 3 groups based on parental inheritance of the germline mutation and types of WT1 abnormality, and found that children who inherited small WT1 mutations from their father were more likely to have the higher penetrance rate than those who inherited small WT1 mutations from their mothers or inherited the large deletions or had the de novo large deletions irrespective of parental origin (P = 0.057; Figure 1). Why do parental inheritance and WT1 abnormality types affect the penetrance rate? Most parents had DNWT1 mutations of paternal germ cell origin, as shown in (Figure 3A). Children who had a small WT1 mutation of paternal germ cell origin easily developed WT because pUPD on 11p resulted in homozygous WT1 mutations and simultaneous overexpression of IGF2 (Figure 3B and C). Children less frequently developed WT by the second mutation in the maternally derived WT1 allele because this tumorigenic pathway needs additional genetic and/or epigenetic events (Figure 3D). In contrast, WT1s developed in children who inherited the small mutation from their mothers could not take advantage of simultaneous alterations in WT1 and IGF2 because maternally derived IGF2 is imprinted and repressed (Figure 3E and F). Children who inherited the small mutations from their mothers could develop WT if an independent WT1 mutation occurred in the paternally derived WT1 allele, which resides on the same 11p expressing IGF2/H19 locus in Beckwith-Wiedemann syndrome and Wilms’ tumour. Haem Mol Genet 17(10): 1427–1435.

Crow JF (2000) The origins, patterns and implications of human embryonic tumour. Nat Rev Genet 1(1): 40–47.

D’Angio GJ, Breslow N, Beckwith JB, Evans A, Baum H, deLorimier A, Fernbach D, Hrabovský E, Jones B, Kelalis P, Othersen HB, Tefft M, Thomas PRM (1989) Treatment of Wilms’ tumour. Haem Mol Genet 17(10): 1427–1435.

Diller L, Ghahremani M, Morgan J, Grundy P, Reeves C, Breslow N, Green D, Neuberg D, Pelletier J, Li FP (1998) Constitutional WT1 mutations in Wilms’ tumour patients. J Clin Oncol 16(11): 3634–3640.

Dryja TP, Mukai S, Petersen R, Rapaport JM, Walton D, Yandell DW (1989) Parental origin of mutations of the retinoblastoma gene. Nature 339(6225): 556–558.

Fend F, Malina M, Star A, Vieg J, Mixova D, Seeman T, Blahova K (2012) Discordant expression of a new WT1 gene mutation in a family with monozygotic twins presenting with congenital nephrotic syndrome. Eur J Pediatr 171(1): 121–124.
This work is published under the standard license to publish agreement. After 12 months the work will become freely available and the license terms will switch to a Creative Commons Attribution-NonCommercial-Share Alike 4.0 Unported License.