Abstract

To identify prognostic factors, array CGH (aCGH) patterns and mutations in WT1 and 9 other genes were analyzed in 128 unilateral Wilms tumors (WTs). Twenty patients had no aCGH aberrations, and 31 had WT1 alterations [silent and WT1 types: relapse-free survival (RFS), 95% and 83%, respectively]. Seventy-seven patients had aCGH changes without WT1 alterations (nonsilent/ non-WT1 type) and were subtyped into those with or without +12, 11q−, 16q−, or HACE1 loss. RFS was better for those with than those without +12 ($P = .010$) and worse for those with than those without 11q−, 16q−, or HACE1 loss ($P = .001, .025$, or $1.2E-04$, respectively). Silent and WT1 type and 8 subtype tumors were integrated and classified into 3 risk groups: low risk for the silent type and +12 subgroup; high risk for the no +12 plus 11q−, 16q−, or HACE1 loss subgroup; intermediate risk for the WT1 type and no +12 plus no 11q−, 16q−, or HACE1 loss subgroup. Among the 27 WTs examined, the expression of 146 genes on chromosome 12 was stronger in +12 tumors than in no +12 tumors, while that of 10 genes on 16q was weaker in 16q− tumors than in no 16q− tumors. Overexpression in 75 out of 146 upregulated genes and underexpression in 7 out of 10 downregulated genes correlated with better and worse overall survival, respectively, based on the public database. +12 was identified as a potential new marker predicting a favorable outcome, and chromosome abnormalities may be related to altered gene expression associated with these abnormalities.
Materials and Methods

Patients and Samples

One hundred and twenty-eight unilateral WT samples were obtained from 128 Japanese patients ranging in age between 2 months and 15 years who underwent surgery or biopsy between December 1987 and August 2015. Of the 128 patients, 42 registered before March 1996 were mostly treated with NWTS-3 or -4 protocol using regimen I, or EE for tumors at stage I, regimen K or K-4A for tumors at stage II, and regimen DD or DD-4A with radiotherapy for tumors at stages III/IV [14,15], and 86 registered after March 1996 were treated according to NWTS-5 protocol [5]. Outcomes of the two cohorts of patients were examined and described in the results section.

In addition, 31 WT1-mutant bilateral WTs from 23 patients, whose genetic and clinical characteristics were reported previously by our group [16], were included for the CTNNB1 analyses. Only 128 unilateral WTs were included in the study of the prognostic implications of molecular markers. Specimens were supplied by the tissue bank of the Japan Wilms Tumor Study [17] or directly sent to the Saitama Cancer Center for cytogenetic and molecular genetic analyses from several Japanese institutions. Pathologists in each institution verified that each sample for the molecular genetic analysis contained 70% or more tumor cells. Normal samples were obtained from either peripheral blood or normal renal tissues adjacent to the tumor. The study design was approved by the Ethics Committee of the Saitama Cancer Center. The clinical stage of the disease was assessed at the time of initial surgery or biopsy according to the classification of the Japanese Society of Pediatric Surgeons [18]. The therapeutic strategy was similar to that of the NWTS protocols [2,5,18,19]. As a basic principle, all patients initially underwent nephrectomy, and preoperative chemotherapy was administered after biopsy when the tumor appeared to be unresectable. Postoperative chemotherapy was performed for all but two patients who were younger than 2 years of age with stage I WT of a favorable histology weighing less than 500 g and underwent surgery in 1998.

Three patients at stage IV received open biopsy before preoperative chemotherapy, and their biopsied materials were used for the study. Nine patients at various stages received preoperative chemotherapy, and their tumor samples which showed abnormal aCGH patterns were included in the present study. In addition, 5 patients at stage III or IV who received preoperative chemotherapy and showed a normal aCGH pattern in tumors were not included in 128 patients of the study.

Histological Examination

In all cases, the diagnosis of WT was made with routine hematoxylin and eosin–stained slides by the pathology panel of Japan Wilms Tumor Study or pathologists at each institution according to the classification proposed by the Japanese Pathological Society and/or the NWTS pathology panel [20,21]. Five tumors (3.9%) with an anaplastic histology (diffuse 4, focal 1) were included among 128 tumors for the reason described in the Results section, and the other 123 tumors showed a favorable histology.

Analysis of Copy Numbers and LOH Using SNP Arrays

High-resolution SNP arrays, Affymetrix Mapping 250K-Nsp arrays (Affymetrix, Santa Clara, CA), were used to analyze the chromosomal copy numbers and LOH status of 128 unilateral and 31 bilateral tumors, as described previously [16]. Copy numbers and LOH were calculated using CNAG and AsCNAR programs with paired or anonymous references as controls [22,23].

Analysis of WT1, CTNNB1, WTX, DROSHA, DICER1, DGCR8, SIX1, SIX2, MYCN, and TP53 Abnormalities and the IGF2 Status

We examined WT1, WTX, CTNNB1, and TP53 abnormalities using MLPA (P118-C1 WT1, MRC-Holland) and/or an SNP array.
and sequencing as previously described [24,25]. Mutations in DROSHA, DICER1, DGCR8, SIX1, SIX2, and MYCN were analyzed by sequencing using the primers listed in Supplementary Table 1. The loss of imprinting (LOI), uniparental disomy (UPD), and retention of imprinting (ROI) of IGF2 were analyzed as previously described, and all UPDs of IGF2 were found to be of the paternal origin [24].

**Gene Expression Analysis**

Samples were hybridized to the Affymetrix GeneChipR Gene 1.0ST Array System for Humans, scanned, subjected to quality control standards, and normalized as previously described [26].

**Statistical Analysis**

Patients were grouped according to various biological and clinical aspects of the disease. The significance of differences in characteristics between groups was examined using the chi-squared or Fisher’s exact test, Student’s t test, and Welch’s t test. RFS was defined as the time from the date of registration to death due to any cause. OS was defined as the time from the date of registration to death from any cause. Survival functions for RFS and OS were estimated using Cox’s proportional-hazards model calculated with Stat Flex software for Windows, version 5.0 (Artec Co., Osaka, Japan).

We used the limma package to define differentially expressed genes [27]. P values were calculated by the eBayes-moderated t test and then corrected by the Benjamini-Hochberg method [28]. The criterion of differentially expressed gene was a q value <0.3.

**Results**

**Genetic and Chromosomal Abnormalities in 128 Unilateral WTs**

Mutations and deletions in 6 WT-associated genes (WT1, CTNNB1, WTX, MYCN, SIX1, and SIX2) were found at various percentages in 128 unilateral WTs (Figures 1-3, Table 1): WT1 alterations (deletion + mutation) in 31, CTNNB1 mutations in 28, WTX alterations in 34 (32 with deletions and 2 with mutations, p.Q10H/c.30G > T, or p.R353*/c.1057C > T), MYCN alterations in 11 (10 with gain and 1 with a mutation, p.P44L/c.131C > T), a SIX1 (p.Q177R/c.530A > G) mutation in 4, and SIX2 (p.Q177R/c.530A > G) mutation in 1. miRNA processing genes (miRNApgs), including DGCR8, DICER1, DIS3L2, and DROSHA, were deleted in 11 tumors, and DROSHA (p.E1147K/c.3439G > A) was mutated in 2. The miRNA genes LET7A1, LET7A2, and LET7A3 were deleted in 3, 15, and 3 tumors, respectively, and 18 tumors had 1 or 2 of these deletions.

Among the 128 WTs, 1q gain was found in 36 tumors, +12 in 34, +7/7q+ in 31, +13 in 20, +20/20q gain in 16, +6/6q gain in 14, 1p− in 12, 11q− in 10, 16q− in 9, 7p− in 8, 17p−/−17 in 6, and a focal deletion including HACE1 in 4 (Table 1 and Supplementary Figures 1 and 2).

**Figure 1.** Genetic and chromosomal aberrations in 31 unilateral and 31 bilateral WTs with WT1 alterations. Black squares indicate the presence of mutations or copy number gains, and gray squares indicate the presence of copy number losses. Ho in the 7p− lane indicates a focal homozygous deletion. Chr. No., chromosome number; U, uniparental disomy; UP, uniparental disomy of 11p; LOI, loss of IGF2 imprinting; ROI, retention of IGF2 imprinting; +, the patient relapsed; −, the patient did not relapse; DD, died of disease, ND, no evidence of disease.
We excluded tumors with \(-11\), UPD on whole chromosome 11 (UPD11), or UPD on 11q (UPD11q) from those with 11q\(\sim\) and tumors with \(-16\), UPD16, or UPD16q from those with 16q\(\sim\) because whole chromosome and chromosome arm deletions may be of different biological significance, and loss and UPD may also result in different biological consequences in tumors.

Five out of 128 WTs were classified as having an anaplastic histology (diffuse 4: S036, S057, S089, S122; focal 1: S125, shown in Figures 2 and 3, and Supplementary Table 2). Three tumors with diffuse anaplasia had 17p\(\sim\) or \(-17\), and a sequencing analysis of exons 2 to 10 of \(TP53\) showed a missense mutation in exon 7 (p.R248W/c.742C\(\sim\)T), another missense mutation in exon 7 (p.D281H/c.841G\(\sim\)C), or a splice site mutation in intron 8 (c. 920-2A\(\sim\)G) in one each. The other two tumors, including one with diffuse anaplasia and one with focal anaplasia, all with normal chromosome 17, showed wild-type \(TP53\).

These 5 tumors were included in the present study on 128 tumors because genetic and chromosomal changes, except for the frequent occurrence of \(TP53\) mutations, in diffuse anaplastic tumors were similar between 5 tumors with an anaplastic histology and 123 with a favorable histology. Besides, the aim of the study is to identify genetic and chromosomal markers that predict outcomes.

**RFS and OS Rates in 128 Patients Classified by Clinical, Genetic, and Chromosomal Characteristics**

No difference in RFS and OS was found between 42 and 86 patients who were registered before and after May 1, 1996 \((P = .990; P = .426)\), although if we included patients only at stages III and IV for an outcome analysis, OSs were slightly better for patients registered after May 1, 1996, than those before \((P = .092)\) (Table 1).

Patients aged 24 months or older had worse OS than those younger than 24 months \((P = .019)\) (Table 1). Patients at stage IV had worse or slightly worse RFS and OS rates than those at stages I, II, and III \((P = .006; P = .065)\). Significant differences were observed in RFS and OS between 5 patients with anaplastic tumors and 123 with favorable histology tumors \((P = .027 and P = 1.2E-05)\) (Table 1).

Patients with \(WTX\) alterations in tumors had slightly worse OS rates than those without \((P = .070)\). No significant differences in RFS and OS rates were observed between patients with \(WT1\) alterations, \(CTNNB1\) mutations, miRNAPG alterations \((DIS3L2\ deletion, DROSHA mutation/deletion, DICER1 mutation/deletion, and DGCR8 deletion), MYCN alterations \((\text{gain and mutation})\), or \(SIX1/SIX2\) mutations and those without the respective alterations.

Patients with 11q\(\sim\) or 16q\(\sim\) in tumors had worse RFS and OS rates than those without \((RFS, P = 4.9E-04 and .010; OS, P = 4.4E-06 and .006, respectively)\). Patients with \(HACE1\) loss in tumors had worse RFS rates than those without \((P = .003)\), although no significant differences were observed in the RFS rate \((P = .281)\). No significant differences were noted in RFS and OS rates between patients with and without 1q gain \((P = .515 and .456)\) (Figure 4, A and B). Patients with +12 in tumors had slightly better RFS and OS rates than those without \((P = .062 and .242)\). No
significant differences were observed in RFS and OS rates between patients with or without +7/7q gain, those with or without +6/6q gain, those with and without 1p−, and those with or without 7p−.

Three Types and Eight Subtypes of WTs Classified by Genetic and Chromosomal Findings

Among 128 unilateral WTs, 108 tumors had some aCGH abnormalities (gain, loss, and UPD), while the other 20 had no abnormalities (silent type) (Supplementary Table 2). WT1 is a master gene in kidney development and the most common WT predisposing gene [3,29]. Furthermore, 31 tumors with WT1 alterations had some aCGH aberrations, and their abnormal patterns were distinct from the other 77 tumors (Supplementary Table 3 and Figures 1-3). Thus, the 108 tumors were subclassified into 31 tumors with WT1 alterations (WT1 type) and 77 without (nonsilent/non-WT1 type).

Among various aCGH aberrations, +12, +20/20q gain, 11q−, 16q−, HACE1 loss, and 17p−/−17 were associated with better or worse RFS or OS rates (Table 1). In addition, +12, 11q−, 16q−, 17p−/−17, and HACE1 loss were only found in the 77 nonsilent/non-WT1 tumors. These 77 tumors were further classified into 4 pairs of 2 subtypes (+12 and no +12, 11q− and no 11q−, 16q− and no 16q−, or HACE1 loss and no HACE1 loss) (Supplementary Table 3). The presence or absence of 17p−/−17 was excluded from the subtype analysis because a small number of tumors with 17p−/−17 and the prognostic significance of 17p−/−17, which is causally associated with TP53 alterations, have been reported previously [30].

Clinical Characteristics of Three types and Eight Subtypes of WTs

The median ages of patients with silent-, WT1-, and nonsilent/non-WT1-type tumors were 7.5, 18, and 44 months, respectively, and showed a similar male to female ratios (10/10, 15/16, and 39/38, respectively). Regarding the stage distribution, silent-type tumors showed earlier stages than WT1- (P = .014) or nonsilent/non-WT1- (P = .017) type tumors, and WT1- and nonsilent/non-WT1-type tumors showed a similar stage distribution (P = .589) (Supplementary Table 2). Regarding the 11p15.5 status, i.e., the LOI, ROI including 11p15.5 loss, and UPD of IGF2, including 11p15.5 gain, ROI was more frequent in silent-type tumors than in WT1- (P = .024) or nonsilent/non-WT1- (P = .017) type tumors, and LOI was more frequent in nonsilent/non-WT1-type tumors than in silent- (P = .035) or WT1- (P = .04E-04) type tumors, and UPD was not found in silent-type tumors, and its frequency was similar between WT1- and nonsilent/non-WT1-type tumors (P = .392) (Supplementary Table 4). Thus, silent-type tumors were characterized by a younger age, earlier stages, and frequent ROI, whereas nonsilent/ non-WT1-type tumors were characterized by an older age and frequent LOI, and WT1-type tumors were characterized by an intermediate age between the other two types, a similar stage distribution to nonsilent/non-WT1-type tumors, and infrequent LOI.
Table 1. RFS and OS Rates in 128 Patients with Unilateral WTs Classified by Clinical, Genetic, and Chromosomal Characteristics

| Characteristics          | RFS                      | OS                      |
|--------------------------|--------------------------|-------------------------|
|                          | No. of Patients          | Survival Rates at the   | No. of Patients          | Survival Rates at the   |
|                          | (No. of Events)          | Last Follow-Up          | (No. of Events)          | Last Follow-Up          |
|                          |                          | 95% CI                  |                          | 95% CI                  |
|                          |                          | P Value                 |                          | P Value                 |
| All patients             | 128 (20)                 | 0.82                    | 0.75-0.90                | 128 (10)                | 0.88                    | 0.81-0.96                |
| Age                      |                          |                         |                          |                         |                         |                         |
| Low <24 months           | 59 (7)                   | 0.88                    | 0.79-0.96                | 59 (1)                  | 0.98                    | 0.94-1                   |
| High ≥24 months          | 69 (13)                  | 0.78                    | 0.66-0.88                | 69 (9)                  | 0.80                    | 0.69-0.93                |
| Stage I/II/III            | 113 (14)                 | 0.86                    | 0.79-0.93                | 113 (7)                 | 0.91                    | 0.84-0.98                |
| Stage IV                 | 15 (6)                   | 0.54                    | 0.25-0.82                | 15 (3)                  | 0.72                    | 0.44-0.99                |
| Stage I/II/III/IV         |                          |                         |                          |                         |                         |                         |
| Registration period      |                          |                         |                          |                         |                         |                         |
| 1987-Feb/1996            | 42 (7)                   | 0.83                    | 0.71-0.94                | 42 (5)                  | 0.86                    | 0.75-0.98                |
| Mar/1996-2015            | 86 (13)                  | 0.80                    | 0.69-0.92                | 86 (5)                  | 0.88                    | 0.77-0.99                |
| Stage III                | 26 (1)                   | 0.96                    | 0.8-1                    | 26 (6)                  | 1                       | 1-1                      |
| Stage III/IV             | 58 (8)                   | 0.81                    | 0.66-0.96                | 58 (3)                  | 0.90                    | 0.77-1                   |
| Histology                |                          |                         |                          |                         |                         |                         |
| Anaplastic (diffuse 4, focal 1) | 5 (2)                  | 0.95                    | 0.85-1                   | 20 (0)                  | 1                       | 1-1                      |
| Favorable                | 123 (18)                 | 0.83                    | 0.76-0.91                | 123 (8)                 | 0.90                    | 0.84-0.97                |
| WT1 alterations (mutation + deletion) | 31 (5)                | 0.83                    | 0.70-0.97                | 31 (2)                  | 0.93                    | 0.83-1                   |
| No WT1 alterations       | 97 (15)                  | 0.82                    | 0.72-0.91                | 97 (8)                  | 0.86                    | 0.76-0.96                |
| CTNNB1                   |                          |                         |                          |                         |                         |                         |
| Mutation                 | 28 (4)                   | 0.84                    | 0.70-0.99                | 28 (1)                  | 0.96                    | 0.87-1                   |
| Wild-type                | 100 (16)                 | 0.82                    | 0.73-0.90                | 100 (9)                 | 0.86                    | 0.76-0.95                |
| WTX alterations (deletion + mutation) | 34 (7)                  | 0.75                    | 0.58-0.93                | 34 (5)                  | 0.79                    | 0.59-0.98                |
| No WTX alterations       | 94 (13)                  | 0.85                    | 0.77-0.93                | 94 (5)                  | 0.91                    | 0.84-0.99                |
| miRNAPG alterations (mutation + deletion) | 12 (3)                | 0.74                    | 0.49-0.99                | 350 (12)                 | 0.91                    | 0.74-1                   |
| No miRNAPG alterations   | 116 (17)                 | 0.83                    | 0.75-0.91                | 116 (9)                 | 0.88                    | 0.79-0.96                |
| SIX1/SIX2 mutation       | 5 (1)                    | 0.80                    | 0.45-1                   | 715 (5)                 | 1                       | 1-1                      |
| No SIX1/SIX2 mutation    | 123 (19)                 | 0.82                    | 0.75-0.90                | 123 (10)                | 0.88                    | 0.80-0.95                |
| MYCN alterations (G + mutation) | 11 (3)                | 0.73                    | 0.46-0.99                | 326 (11)                 | 0.82                    | 0.59-0.91                |
| No MYCN alterations      | 117 (17)                 | 0.83                    | 0.75-0.91                | 117 (8)                 | 0.89                    | 0.80-0.97                |
| 1q gain                  | 36 (7)                   | 0.80                    | 0.66-0.93                | 515 (36)                 | 0.86                    | 0.72-0.99                |
| No 1q gain               | 92 (13)                  | 0.83                    | 0.73-0.92                | 92 (6)                  | 0.89                    | 0.79-0.98                |
| +12                      | 34 (2)                   | 0.94                    | 0.86-1                   | 34 (1)                  | 0.97                    | 0.91-1                   |
| No +12                   | 94 (18)                  | 0.78                    | 0.68-0.88                | 94 (9)                  | 0.85                    | 0.76-0.95                |
| +7/7q gain               | 31 (5)                   | 0.81                    | 0.71-0.97                | 914 (31)                 | 0.77                    | 0.52-1                   |
| No +7/7q gain            | 97 (15)                  | 0.82                    | 0.73-0.91                | 97 (6)                  | 0.91                    | 0.83-0.98                |
| +13                      | 20 (4)                   | 0.80                    | 0.62-0.97                | 547 (20)                 | 0.84                    | 0.68-1                   |
| No +13                   | 108 (16)                 | 0.83                    | 0.74-0.91                | 108 (7)                 | 0.89                    | 0.81-0.97                |
| +20/20q gain             | 16 (4)                   | 0.71                    | 0.46-0.96                | 281 (16)                 | 0.51                    | 0.13-0.90                |
| No +20/20q gain          | 112 (16)                 | 0.84                    | 0.77-0.92                | 112 (6)                 | 0.93                    | 0.87-0.99                |
| +6/6q gain               | 14 (1)                   | 0.93                    | 0.79-1                   | 381 (14)                 | 1                       | 1-1                      |
| No +6/6q gain            | 114 (19)                 | 0.81                    | 0.73-0.89                | 114 (10)                | 0.87                    | 0.79-0.95                |
| 1p−                      | 12 (2)                   | 0.77                    | 0.49-1                   | 970 (12)                 | 0.86                    | 0.60-1                   |
| No 1p−                   | 116 (18)                 | 0.84                    | 0.76-0.91                | 116 (9)                 | 0.89                    | 0.81-0.96                |
| 11q−                     | 10 (5)                   | 0.50                    | 0.19-0.81                | 4.9E-04 (10)             | 0.47                    | 0.05-0.89                |
| No 11q−                  | 118 (15)                 | 0.85                    | 0.78-0.92                | 118 (6)                 | 0.92                    | 0.85-0.99                |
| 16q−                     | 9 (4)                    | 0.44                    | 0.03-0.86                | 0.10 (9)                 | 0.53                    | 0.13-0.93                |
| No 16q−                  | 119 (16)                 | 0.85                    | 0.79-0.92                | 119 (7)                 | 0.92                    | 0.86-0.98                |
| 7p−                      | 8 (2)                    | 0.75                    | 0.45-1                   | 418 (8)                 | 1                       | 1-1                      |
| No 7p−                   | 120 (18)                 | 0.83                    | 0.75-0.90                | 120 (10)                | 0.88                    | 0.80-0.95                |
Regarding the clinical characteristics of patients with 8 subtype tumors, the median age of 34 patients with +12 subtype tumors and 43 with no +12 tumors were 44 and 41 months, respectively, and similar, whereas the 34 patients had a lower male to female ratio than the 43 patients (14/30 vs. 24/19, \(P = .024\)). The stage distribution was similar between these two subtypes (early stages I + II/advanced stages III + IV; 23/11 vs. 26/17, \(P = .341\)), and the incidence of the IGF2 LOI status was also similar between the two subtypes (Supplementary Table 4).

The other 6 subtypes were summarized as 11q−, 16q−, and/or HACE1 loss group tumors (18 patients) and no 11q−, 16q−, and HACE1 loss subgroup tumors (59 patients) because the number of each tumor subtype (11q−, 16q−, or HACE1 loss) was small, and 11q−, 16q−, and HACE1 loss overlapped in 4 tumors (Supplementary Fig. 2). The 2 groups of patients had the same median age of 44 months, a similar male to female ratio (8/10 vs. 30/29, \(P = .282\)), a similar stage distribution (early I + II, advanced III + IV; 13/5 vs. 36/23, \(P = .282\)), and a similar incidence of IGF2 LOI (Supplementary Table 4).

\textit{RFS and OS Rates in Patients with Three Types or Eight Subtypes of Tumors and Those in Three Risk Groups of Patients}

All patients with silent-type tumors were alive at the last follow-up without disease, although one patient had relapsed (RFS 95% and OS 100%), those with WTI-type tumors had RFS and OS rates of 83 and 93%, respectively, and those with nonsilent/non−WTI-type tumors had lower RFS and OS rates of 79 and 83% than the other two types without significance (Table 2). Patients with +12 subtype tumors had better or slightly better RFS and OS rates than those without \(P = .010\) and \(P = .075\) (Figure 5, A and B). Three of 43 patients with no +12 subtype tumors died around 10 years after the diagnosis, two died of WT after late relapse, and one died of secondary leukemia, which may be caused by intensive therapy consisting of CBDCa, etoposide, and doxorubicin and radiotherapy given for the relapsed tumor.

Patients with 11q− subtype tumors had worse RFS and OS rates than those without \(P = .001\) and 9.3E-05 (Supplementary Figures 3, A and B). Patients with 16q− subtype tumors had worse RFS and OS rates than those without \(P = .025\) and .031 (Supplementary Figures 3, C and D). Patients with HACE1 loss subtype tumors had worse RFS than those without, although no significant difference was observed in OS rates between patients with or without HACE1 loss subtype tumors \(P = 1.2E-04\) and .570 (Supplementary Figures 3, E and F). Thus, +12 is an exceptional factor, and patients with +12 in tumors had favorable outcomes, whereas those with three other subtypes with chromosomal loss had unfavorable ones.
Table 2. RFS and OS Rates in 128 Patients with Unilateral WTs Classified by 3 Biological Types or 3 Risk Groups

| Biological classification | RFS | | | OS | | |
|---------------------------|-----|---------------|---|---------------|---|
| No. of Patients (No. of Events) | Survival Rates at the Last Follow-Up | 95% CI | P Value | No. of Patients (No. of Events) | Survival Rates at the Last Follow-Up | 95% CI | P Value |
| Three types | | | | | | | |
| A) WT1 alterations | 31 (5) | 0.83 | 0.70-0.97 | .409 | 31 (2) | 0.93 | 0.83-1 | .323 |
| B) Silent (no genetic or chromosomal abnormalities) | 20 (1) | 0.95 | 0.85-1 | | 20 (0) | 1 | 1-1 | |
| C) Non-WT1/nonilent | 77 (14) | 0.79 | 0.68-0.89 | | 77 (8) | 0.83 | 0.72-0.95 | |
| The non-WT1/silent type (C) was classified into 8 subtypes (D, E, F, G, H, I, J, and K) | | | | | | | |
| D) +12 | 34 (2) | 0.94 | 0.86-1 | .010† | 34 (1) | 0.97 | 0.91-1 | .075† |
| E) No +12 | 43 (12) | 0.66 | 0.49-0.83 | .024† | 43 (7) | 0.75 | 0.57-0.92 | |
| 2 types and 2 subtypes (A, B, D, and E) | | | | | | | |
| F) 11q− | 10 (5) | 0.5 | 0.19-0.81 | .001 | 10 (4) | 0.47 | 0.05-0.89 | 9.3E-05 |
| G) No 11q− | 67 (9) | 0.83 | 0.73-0.94 | | 67 (4) | 0.89 | 0.78-0.99 | 2.3E-04 |
| 2 types and 2 subtypes (A, B, F, and G) | | | | | | | |
| H) 16q− | 9 (4) | 0.44 | 0.03-0.86 | .025 | 9 (3) | 0.53 | 0.13-0.93 | .031 |
| I) No 16q− | 68 (10) | 0.84 | 0.75-0.93 | | 68 (5) | 0.89 | 0.79-0.99 | |
| 2 types and 2 subtypes (A, B, H, and I) | | | | | | | |
| J) HACE1 loss | 4 (3) | 0.25 | 0.40-0.67 | 1.2E-04 | 4 (1) | 0.75 | 0.32-1 | .470 |
| K) No HACE1 loss | 73 (11) | 0.82 | 0.71-0.92 | | 73 (7) | 0.84 | 0.72-0.96 | |
| 2 types and 2 subtypes (A, B, J, and K) | | | | | | | |
| Risk classification | | | | | | | |
| 3 risk groups | | | | | | | |
| L) Low risk (silent type and +12 subgroup) | 54 (3) | 0.94 | 0.88-1 | 54 (1) | 0.98 | 0.94-1 | 9.1E-06 |
| M) Intermediate risk (WT1 type and no +12 plus no 11q−, 16q−, or HACE1 loss subgroup) | 64 (11) | 0.81 | 0.70-0.91 | 64 (4) | 0.91 | 0.82-1 | 2.5E-06 |
| N) High risk (no +12 plus 11q−, 16q−, or HACE1 loss subgroup) | 10 (6) | 0.33 | 0.40-0.67 | 10 (5) | 0.42 | 0.07-0.77 | |

RFS: A vs. B, P = .245; A vs. C, P = .784; A vs. D, P = .181; A vs. E, P = .218; B vs. C, P = .191; B vs. D, P = .933; B vs. E, P = .056; I vs. M, P = .00; I vs. N, P = 8.4E-07; M vs. N, P = .001. OS: A vs. B, P = .286; A vs. C, P = .425; A vs. D, P = .512; A vs. E, P = .192; B vs. C, P = .466; B vs. E, P = .109; I vs. M, P = .281; I vs. N, P = 5.9E-04; M vs. N, P = 1.1E-04.

† P value evaluated from two subtypes of patients;

* P value evaluated from two types and two subtypes of patients. Please also see Figure 5 and Supplementary Figure 3.

Figure 5. Relapse-free and overall survival curves for 3 or 4 groups of patients. Patients were classified by no CGH aberrations (silent type), WT1 alterations (WT1 type), and the presence or absence of +12 (+12 and no +12 subtypes) (A, B) in tumors or by three risk groups (low risk, silent type and +12 subgroup; intermediate risk, WT1 type and no +12 plus no 11q−, 16q−, or HACE1 loss subgroup; high risk, no +12 plus 11q−, 16q−, or HACE1 loss subgroup) (C, D).
As mentioned in the previous section, 18 tumors had 11q−, 16q−, and/or HACE1 loss. Eight of the 18 tumors also had +12 (Supplementary Figure 2). For the risk classification, the 8 tumors were included in +12 subgroup and the remaining 10 tumors were classified as no +12 plus 11q−, 16q−, or HACE1 loss subgroup. We integrated two types and eight subtypes of tumors, classified them into three risk groups, and examined RFS and OS rates in three risk group of patients. Fifty-four patients with silent type and +12 subgroup of tumors were classified as low risk; 10 with no +12 plus 11q−, 16q−, or HACE1 loss as high risk; and 64 with WT1-type and no +12 plus no 11q−, 16q−, or HACE1 loss subgroup tumors as intermediate risk (Figure 6). Low-risk patients had better RFS and OS rates than high-risk patients (P = 8.4E-07 and 5.9E-07) and had better RFS rate than intermediate-risk patients (P = .049), whereas low-risk and intermediate-risk patients had comparative OS rates (P = .281). Intermediate-risk patients had better RFS and OS rates than high-risk patients (P = .013 and 1.1E-04) (Table 2 and Figure 5, C and D).

Multivariate Outcome Analysis of 7 Clinical, Genetic, and Chromosomal Factors in 128 Patients with WT

A multivariate Cox proportional-hazard regression analysis confirmed the relationship between 16q− and a poor outcome after adjustments for age and stage [RFS: hazard ratio (HR) 5.21, P = .007; OS: 5.66, P = .025] (Table 3). The relationship between 11q− or HACE1 loss and a poor outcome was not evaluated due to collinearity. The relationship between +20/20q gain and a poor outcome was not confirmed after adjustments for age and stage (RFS, HR 1.37, P = .599; OS, HR 2.91, P = .111). The relationship between +12 and a favorable outcome was confirmed or suggested after adjustments for age and stage [RFS: HR 0.23, P = .050; OS: HR 0.19, P = .112], and was confirmed or suggested after adjustments for 11q−, 16q−, or HACE1 loss, or +20/20q gain in addition to age and stage (RFS: HR 0.096, P = .004; 0.24, P = .057; not evaluable due to collinearity; 0.19, P = .034, respectively; OS: HR not evaluable due to collinearity, 0.19, P = .122; 0.11, P = .075; 0.10, P = .047, respectively).

Ten WT1s with 11q−, 9 with 16q−, and 16 with +20/20q gain each were classified into those with or without +12. RFS and OS rates were better in WT1s with 11q−, 16q−, or +20/20q gain each plus +12 than in those with 11q−, 16q−, or +20/20q gain only, with or without significance (Supplementary Table 5), and these effects of +12 on favorable outcomes may have contributed to significant P values in the multivariate analyses when each abnormality was added to the three factors (age, stage, and +12) (Table 3). Thirty-six WT1s with 1q gain were classified into those with or without +12. RFS and OS rates were better in 17 patients with 1q gain plus +12 than in 19 patients with 1q gain only in tumors with or without significance (P = .045 and P = .358) (Figure 4, C and D). Therefore, the effect of +12 on a favorable outcome was also identified in WT1s with 1q gain.

Differential Gene Expression Profiles Between WT1s With or Without +12, and Those With or Without 16q−

We examined the gene expression profiles of 27 WT1s and 2 normal kidney tissues; 20 out of 27 tumors were included in the present study on 128 WT1s. Of the 27 tumors, 6 had WT1 alterations, 7 had no aCGH aberrations (silent type), and 14 had the non-WT1/nonsilent type; 4 had +12 only, 1 had 16q− only, 3 had both +12 and 16q−, and 6 had neither +12 nor 16q−. The expression of 324 genes was stronger in 7 tumors with than in those without +12; 146 and 178 of the 324 genes were located on chromosome 12 and other chromosomes, respectively (Supplementary Fig. 4). Comparisons of 1198 probes on chromosome 12 and 22,357 probes on other chromosomes revealed that upregulated genes were more likely to be located on chromosome 12 (P < 10−16, Fisher’s exact test). The expression of 23 genes was weaker in 4 tumors with than in those without 16q−; 10 and 13 genes were located on chromosome arm 16q and other chromosome arms, respectively (Supplementary Fig. 5), indicating that downregulated genes were more likely to be located on 16q (P < 5.439 *10−12, Fisher’s exact test).

Relationship between the Overexpression of Each Upregulated Gene in WT1s with +12 and Better OS Rates and Between Downregulated Genes on Chromosome Arm 16q or Upregulated Genes on Other Chromosome Arms in WT1s with 16q− and Worse OS Rates Based on the TARGET OCG Dataset 148 or 125

Two datasets are available in a public database (R2) (http://hgserv1.amc.nl/cgi-bin/r2/main.cgi) to investigate the relationship between the over- or underexpression of upregulated or downregulated genes in WT1s with +12 or 16q− and the better or worse OS rates of patients with WT1s, and we firstly used dataset 148 (Tumor Wilms (TARGET) – OCG – 148 – MAS5.0 – u133pa) rather than dataset 125 (Tumor Wilms (TARGET) – OCG – 125 – MAS5.0 – u133p2) because the former
dataset and present study had more similar patient characteristics, including stage distribution and mortality rates, than the latter.

Among the 146 upregulated genes on chromosome 12 in WT's with +12, the higher expression levels of 75 genes were associated with better OS rates based on dataset 148. Furthermore, among the 178 upregulated genes on other chromosomes in WT's with +12, the higher expression levels of 75 genes were associated with better OS rates (Table 4, Supplementary Table 6, and Supplementary Figure 4). Thus, upregulated genes on chromosome 12 were more frequently associated with favorable outcomes than those on the other chromosomes (P = .001). CDK4 on chromosome 12 was upregulated in WT's with +12; however, no significant P values were obtained based on dataset 148. Because a CDK4 inhibitor is clinically available, we also used dataset 125 and found that higher expression levels of CDK4 were associated with better OS rates (Table 4).

Some of the upregulated genes in WT's with +12, which were associated with better outcomes when overexpressed in WT according to dataset 148 of a public database (R2), were categorized into 7 groups based on the DAVID analysis: ubiquitination-related, 9 genes; chromatin-related, 12; TP53 pathway-related 11; DNA damage and response, 4; mRNA processing, 11; mitosis and cell division, 5. In addition, four genes were categorized as immune response (Table 5).

As described in the previous paragraph, we initially used dataset 148. Among the 10 downregulated genes on chromosome arm 16q in WT's with 16q−, the lower expression levels of only two genes (GABARAPL2 and ATMIN) were associated with worse OS rates, those of three genes (FTO, CYB5B, and AP1G1) with better OS rates, and those of three genes (TERF2IP, MON1B, and MAP1LC3B) with no significant difference in OS rates. No data existed for the other two genes (CENPBD1 and ZFP90). When we analyzed the three genes with no significant differences in OS rates and two genes with no data using dataset 148, the lower expression levels of these five genes were associated with worse outcomes when we used dataset 125 (Table 6 and Supplementary Figure 5).

In contrast, while no genes on 16q were upregulated in tumors with 16q−, 16 genes on non-16q chromosome arms were upregulated, and the higher expression levels of three genes (LGALS14, INT51, and MMP9) were associated with worse OS rates. No data existed for the other two genes (CENPBD1 and ZFP90). When we analyzed the three genes with no significant differences in OS rates and two genes with no data using dataset 148, the lower expression levels of these five genes were associated with worse outcomes when we used dataset 125 (Table 6).
mutation (c.del133_135TCT, p.del45S), and 7 out of the 10 accompanied UPD3p, including 3p22.1, at which mutation (c.del133_135TCT, p.del45S), and 7 out of the 10 accompanied UPD3p. These results indicate that the CTNNB1 mutation is homozygous in some WTs caused by UPD3p and is strongly associated with the specific mutation del133_135TCT, p.del45S.

**Discussion**

We investigated chromatosomal, genetic, and epigenetic alterations in 128 unilaterial WTs and proposed a biological classification consisting of 3 types: silent, WT1, and non-silent/non-WT1 types, and 4 sets of 2 subtypes: +12 or no +12, 11q− or no 11q−, 16q− or no 16q−, and HACE1 loss or no HACE1 loss (Figure 6). The prognostic implications of silent, 11q−, and 16q− have been previously reported by other groups; however, the favorable outcomes of patients with +12 tumors and unfavorable outcomes of those with +12, and the downregulated genes may have led to the unfavorable outcomes of patients having tumors with 16q−.

The public database provides Kaplan-Meier survival curves for patients with WT classified by the expression levels of various genes, and we used it to investigate the relationship between each upregulated gene in tumors with +12 and better OS rates, and that between each downregulated gene on chromosome 16q or each upregulated gene on the non-16q chromosome arms in tumors with 16q−, and worse OS.

Davoli and colleagues reported that the distribution and potency of TSGs, oncogenes, and essential genes critical for survival on chromosomes may explain copy number alterations in whole chromosomes and chromosome arms during cancer evolution through a process of cumulative haploinsufficiency and triplosensitivity. The present results that showed significantly higher numbers of upregulated genes on chromosome 12 in WTs with +12 than in those without, and significantly higher numbers of downregulated genes on 16q in tumors with 16q− than in those without, concur with our statement. We speculated that the upregulated genes may have resulted in the favorable outcomes of patients having tumors with +12, and the downregulated genes may have led to the unfavorable outcomes of patients having tumors with 16q−.

Table 4. Upregulated Genes on Chromosome 12 or Other Chromosomes That Are Associated with Better OS When Overexpressed in Patients with +12 in WT

| Gene Symbol | FDR | P Value | Bonferroni | Probe Set |
|-------------|-----|---------|------------|-----------|
| 1 KRAS      | 0.23| 8.7E-12 | 1.2E-09    | 214352_s_at |
| 2 PRDM4     | 0.04| 1.3E-08 | 1.7E-06    | 218329_at  |
| 3 WPPI      | 0.28| 6.1E-07 | 8.1E-05    | 217822_at  |
| 4 YAF2      | 0.10| 1.4E-06 | 1.9E-04    | 206238_s_at |
| 5 KANSL2    | 0.22| 1.9E-06 | 2.5E-04    | 221821_at  |
| 6 LRPS      | 0.20| 7.2E-06 | 9.6E-04    | 205606_at  |
| 7 KDM5A     | 0.28| 1.2E-05 | 1.6E-03    | 202040_s_at |
| 8 COL2A1    | 0.29| 1.9E-05 | 2.5E-03    | 217404_s_at |
| 9 CDC2D2    | 0.22| 2.0E-05 | 2.7E-03    | 218175_at  |
| 10 NOP2     | 0.20| 2.1E-05 | 2.8E-03    | 214227_at  |
| 11 BRAP      | 0.24| 2.5E-05 | 2.7E-03    | 213473_at  |
| 12 LEMD3    | 0.03| 3.0E-05 | 4.0E-03    | 206967_at  |
| 13 ZCCHPC    | 0.18| 3.4E-05 | 4.6E-03    | 218478_s_at |
| 14 CNP2     | 0.29| 4.3E-05 | 5.8E-03    | 209979_at  |
| 15 TDG       | 0.22| 7.8E-05 | 1.0E-03    | 202040_s_at |
| 16 CAND1     | 0.13| 1.2E-04 | 1.6E-02    | 208839_s_at |
| 17 DDX23     | 0.20| 1.3E-04 | 1.7E-02    | 40465_at   |

Table 5. Groups of Upregulated Genes on Chromosome 12 or Other Chromosomes That Are Associated with Better OS When Overexpressed in Patients with +12 in WT

| Biological Function (Gene Nos.) | Upregulated Genes on Chromosome 12 | Upregulated Genes on Other Chromosomes |
|---------------------------------|------------------------------------|---------------------------------------|
| Ubiquitination-related (n = 9)   | BRAP, CAN1, KRAS, FBXW14, MDM2, MED21, RNF34, UBE2B | KBTBD2, BRCA2, H2AFV, PAM, TAF5, TRP |
| Chromatin-related (n = 12)       | BAZ2A, KANSL2, KDM5A, TDG, ARID2, SMARCC2, TIMELESS | BAZ2A, H2AFV, PAM, TAF5, TRP |
| TP53-related (n = 11)            | TDP, MAPKAPK2, MDM2, POLE, REC8, RNF34, TIMELESS, TRAP1 | TDP, PAXIP1, TAF5 |
| DNA damage response (n = 4)      | TDP, POLE, TIMELESS | TDP, PAXIP1 |
| mRNA processing (n = 11)         | CNOT2, DDX3, SFSWAP, WPB11, ZCCHPC, CPSF6, EIF4B, PAN2, SART3 | CNOT2, DDX16, SNRPE |
| Mitosis and cell division (n = 5) | ASUN, CCNT1, KNTC1, TIMELESS | CLTA |
| Immune response (n = 4)          | SART3, TBK1 | ICOSLG, LGALS3BP |
may be upregulated by the transcriptional activation of the upregulated genes on chromosome 12, were associated with better OS rates (Table 4, Supplementary Table 6, and Supplementary Figure 4). Some of these genes were grouped as ubiquitination-related (CAND1), chromatin-related (KDM5A), TP53-related (TGD), DNA damage response (TGD), mRNA processing (CNOT2), mitosis and cell division (ASUN), and immune response (SART3) (Table 5). The mechanisms by which these groups of upregulated genes contribute to the favorable outcomes of patients have not yet been elucidated.

**CDK4** is 1 of the 146 upregulated genes on chromosome 12 in tumors with +12, and an oncogene whose product forms a complex that plays an important role in cell cycle G1/S phase progression [35]. The present study showed that the higher expression level of CDK4 was associated with better OS rates based on dataset 125 (Table 4). The markedly stronger expression of CDK4 than CDK6 was previously reported in WTs [36]; however, the chromosomal status of the tumors was not examined in that study. We speculated that the overexpression of CDK4 and some other oncogenes promotes the proliferation of WT cells, and these cells are very susceptible to cytotoxic drugs, resulting in a favorable response in and outcome for patients with WTs with +12. The favorable effects of CDK4/6 inhibitors were reported in clinical trials for breast cancer [37]. The substitution of cytotoxic drugs for CDK4/6 inhibitors may be an important subject for the circumvention of adverse effects caused by cytotoxic chemotherapy in the treatment of WT.

The lower expression levels of 7 out of the 10 downregulated genes on 16q and the higher expression levels of 5 out of the 16 upregulated genes on the non-16q chromosome arms were associated with worse OS rates (Table 6 and Supplementary Figure 5). Downregulated genes included ATMIN (DNA damage response gene), GABARRP2L2 (autophagy-related), CEND1 (control of chromosomal segregation), and ZFP90 (a negative regulator of NRSF/REST) [38,39]. Upregulated genes, which may be derepressed by the deletion of repressor genes on 16q, included LGALS14 (a strong inducer of T-cell apoptosis), INTS1 (RNA polymerase II-associated complex), MMP8 (matrix metalloproteinase family), ZBED6CL (repression of IGF2 expression), and SLC9C2 (putative Na(+)/H(+) exchangers) [40]. Matrix metalloproteinases play a pivotal role in tumor growth and the multistep processes of invasion and metastasis [40], and the upregulation of MMP8 may be causally related to the unfavorable outcomes of patients having WTs with 16q-. The mechanisms by which these downregulated genes on 16q contribute to the unfavorable outcomes of patients having WTs with 16q- need to be clarified.

Whole chromosomal aneuploidy results from errors in the chromosomal segregation of duplicated chromosomes. Our previous study on 10 bilateral WTs with no WT1 alterations included one tumor with +12 and UPD11, which developed in an infant with premature chromosome separation syndrome [16]. Premature chromosome separation syndrome is caused by biallelic mutations in BUB1B, biallelic single nucleotide substitutions in the upstream region of BUB1B, or compound monoallelic BUB1B mutations and monoallelic single nucleotide substitutions in the BUB1B upstream region [41–43]. BUB1B is a spindle assembly checkpoint gene, and RASSF1A plays some roles at a mitotic checkpoint [44]. We previously reported that BUB1B was not mutated in 25 WTs, including 6 with +12, and the expression levels of BubR1, a protein product of BUB1B, decreased and RASSF1A promoter regions were methylated in hyperdiploid and pseudodiploid WTs but not in diploid WTs [45]. Yost and colleagues recently reported that all six children with biallelic mutations in TRIP13, another spindle assembly checkpoint gene, developed WT [46]. These findings suggest that the downregulation of mitotic checkpoint genes may cause hyperdiploid WTs with +12.

Gadd and colleagues recently examined the genetic landscape of 117 WTs and found that genetic alterations preserved the progenitor state and abnormal induction of embryonal kidney cells [47]. They also stated that decreased LET7A expression, caused by an LET7A deletion or LIN28B upregulation and miRNAPG mutations, appears to perpetuate the progenitor state and prevent progenitor cell maturation. LIN28B is located at q16, and +6/6q gain was almost exclusively found in +12 subtype tumors but rare in no +12 subtype tumors, whereas deletions in LET7A and miRNAPGs were frequent in no +12 subtype tumors but rare in +12 subtype tumors (Figures 2 and 3). The upregulation of LIN28B by +6q gain, LET7A deletion, and miRNAPG deletion may result in reduced expression levels of LET7A [47]. The present results suggested that +12 and no +12 subtype tumors both preserve the progenitor states through the
We found focal deletions including WT5 or WT6, indicating a low incidence of these genes as predisposing genes. In a child with bilateral, young-onset WT disrupted 1q gain, further studies are needed to clarify the role of this gene.

The 6q21 breakpoint of the congenital t(5;6)(q21;q21) translocation has been reported by other groups [5,31,32]. Ten genes on 16q were downregulated, and the lower expression levels of seven of them were associated with poor outcomes, indicating the enhanced proliferation or resistance to chemotherapy of tumor cells caused by a haploinsufficiency of possible TSGs on 16q. Unfortunately, there was only one tumor with 11q− out of the 27 tumors on which the expression array analysis was performed, and it was not possible to examine the relationship between the lower expression levels of downregulated genes on 11q and poor outcomes in the present study.

We also demonstrated that HACE1 loss is a marker predicting a poor outcome. This gene is a TSG involved in various cancers [48]. The 6q21 breakpoint of the congenital t(5;6)(q21;q21) translocation in a child with bilateral, young-onset WT disrupted HACE1 [49], indicating that this gene is one of the WT predisposing genes. Subsequent sequencing revealed HACE1 mutations in 1 of the 450 WTs, indicating a low incidence of HACE1 mutations in sporadic WTs. We found focal deletions including HACE1 in 4 (3.1%) out of 128 WTs. The hypermethylation of CpG islands upstream of HACE1 and its low expression level were reported in sporadic WTs. The deletion regions of four tumors also included LIN28B, and further studies are needed to clarify the role of HACE1 and LIN28B losses in Wilms tumorigenesis.

We found 1q gain in 36 (28.1%) out of 128 WTs, and a similar incidence of 1q gain was reported in COG (28.5%) and SIOP (28.5%) [6,7]. Although EFS and OS rates in patients with or without 1q gain appear to be similar among the three series of WTs, significant differences were observed in EFS and OS rates in the previous two studies but not in the present study (Table 7). We and other investigators reported that the incidence of IGF2 LOI in WTs was lower in Japanese than that of IGF2 LOI reported in Caucasians [24,50]. Someone may wonder if the contradictory results in the present study are related to biologic differences between Japanese and Caucasian WTs. Because the percentages of 1q gain or +12 in WTs were similar between Japanese and Caucasians [34], these contradictory results may be caused by the smaller number of patients in the present study than in the other two studies and/or a favorable effect of +12 on tumors with 1q gain in the present study (Figure 4, A-D).

While the present study examined genetic aberrations in a single tumor from each patient with WT, Cresswell and others examined intratumor genetic heterogeneity in 70 tumor samples from 20 patients with WT [9]. Their data showed 1q gain in 21 tumor samples from 8 patients and +12 in 27 tumor samples from 11 patients, indicating more frequent occurrence of +12 than 1q gain in their WTs. Furthermore, their results indicated that simultaneous occurrence of 1q gain and +12 was found in 14 tumor samples from 6 patients and was the most frequent combination of chromosomal aberrations. Thus, SIOP and COG should examine the favorable effect of +12 on outcomes of patients with WTs with 1q gain.

β-Catenin encoded by CTNNB1 is a key protein involved in the Wnt signaling pathway that is critical for mesenchymal-epithelial transition [51]. CTNNB1 mutations in WT are reported to be heterozygous and considered to enhance WT cell proliferation [52]. We showed that 8 out of 47 WTs with CTNNB1 mutations had homozygous CTNNB1 mutations due to partial UPD3p covering the CTNNB1 locus at 3p22.1; 7 out of the 8 WTs had the same CTNNB1 mutation (Ser45del). The reason why the mutation (Ser45del) was frequently homozygous currently remains unknown. Since CTNNB1 mutations have a gain of function property, the homozygous mutation may confer a greater proliferative capacity on tumor progenitor cells. Similar findings were reported for the CBL gene with gain-of-function mutations, which were duplicated by UPD11q, in myeloid neoplasms [53].

An aCGH analysis revealed no copy number aberrations and no allelic imbalances in 20 (15.6%) out of 128 WTs, although 7 out of the 20 had WTX alterations, CTNNB1 mutations, or LOI of IGF2, and these 20 tumors were classified as the silent type. Patients were characterized by a young age, early stage of the disease, frequent epithelial predominant histology, and favorable outcomes. Previous

| Table 7. EFS and OS Stratified by 1q Gain in WTs Reported from COG [6], SIOP [7], and the Present Study |
|---------------------------------|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| COG No. of Patients | 8-Year EFS | 95% CI | P Value | 8-Year OS | 95% CI | P Value | 8-Year EFS | 95% CI | P Value |
| 1q gain 317 (28.5%) | 77.0% | 72.0%-81.0% | <.001 | 88.0% | 83.0%-91.0% | <.001 | |
| No 1q gain 797 | 90.0% | 88.0%-92.0% | | 96.0% | 94.0%-97.0% | | 94.0% | 92.0%-95.0% | |
| The present study | No. of Patients | 8-Year EFS | 95% CI | P Value | 8-Year OS | 95% CI | P Value | 8-Year EFS | 95% CI | P Value |
| 1q gain 36 (28.1%) | 80.5% | 62.3%-89.9% | .396 | 91.6% | 73.6%-96.9% | .338 | |
| No 1q gain 92 | 86.9% | 75.8%-91.8% | | 95.6% | 87.3%-98.1% | | 94.5% | 87.6%-97.0% | |
| SIOP | No. of Patients | 5-Year EFS | 95% CI | P Value | 5-Year OS | 95% CI | P Value | 8-Year EFS | 95% CI | P Value |
| 1q gain 167 (28.5%) | 79.0% | 68.5%-82.0% | <.001 | 88.4% | 83.5%-93.6% | .01 | |
| No 1q gain 586 | 88.2% | 85.0%-91.4% | | 94.4% | 92.1%-96.7% | | 94.5% | 87.6%-97.0% | |
| The present study | No. of Patients | 5-Year EFS | 95% CI | P Value | 5-Year OS | 95% CI | P Value | 5-Year EFS | 95% CI | P Value |
| 1q gain 586 | 80.5% | 62.3%-89.9% | .297 | 91.6% | 73.6%-96.9% | .338 | |
| No 1q gain 36 (28.1%) | 88.0% | 79.0%-93.1% | | 95.6% | 87.3%-98.1% | | 94.5% | 87.6%-97.0% | |
aCGH studies also reported no chromosomal aberrations in some WTs [11–13]. Subset 1 proposed by Gadd et al. consisted of 11 tumors with an epithelial histology, patient age ranging between 6 and 91 months, and stages I and II, and showed no alterations in WTI, CTNNB1, and WTX or the LOH of 1p and 16q; 1 tumor with LOI of IGFB2 was included in this subset [33]. The favorable outcomes of epithelial predominant WTs were reported by SIOP [54]. Seven out of 20 patients with tumors classified as an epithelial predominant histology in the present study are alive with no relapse. Patients with early-stage WT with an epithelial predominant histology and no aCGH aberration (silent type) may avoid chemotherapy that may cause adverse effects without the risk of relapse.

Conclusions

We newly identified chromosome 12 gain (+12) as a potential marker without the risk of relapse. (silent type) may avoid chemotherapy that may cause adverse effects with an epithelial predominant histology and no aCGH aberration (silent type) may avoid chemotherapy that may cause adverse effects without the risk of relapse.

CRediT authorship contribution statement

Masayuki Haruta: Conceptualization, Methodology, Writing - original draft. Yasuhito Arai: Methodology & Investigation. Yukiichi Tanaka: Methodology & Investigation. Tetsuya Takimoto: Formal analysis. Ryuichi P. Sugino: Formal analysis. Yasuhiro Yamada: Methodology & Investigation. Takehiko Kamijo: Writing - review & editing, Takaharu Oue: Writing - review & editing, Takaharu Oue: Writing - review & editing. Masahiro Fukuzawa: Writing - review & editing. Tsugumichi Koshinaga: Writing - review & editing. Yasuhiro Kaneko: Conceptualization, Methodology, Writing - original draft.

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neo.2018.10.007.

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