containing higher than 10× coverage in all comparative samples were used for the analysis.

Histological Analysis and Immunostaining

Normal and tumor tissue samples were fixed in 10% buffered formalin for 24 hr and embedded in paraffin. Sections (4 μm) were stained with hematoxylin and eosin (H&E), and serial sections were used for the immunohistochemical analyses. The primary antibodies used were anti-Oct3/4 (1:100 dilution; BD Biosciences), anti-Ki-67 (1:100 dilution; Dako), anti-insulin (1:500 dilution; Dako), anti-BrdU (1:500 dilution; Abcam), anti-2A (1:250 dilution; Millipore), anti-Lin28b (1:100 dilution; Cell Signaling Technology), and anti-GFP (1:500 dilution; Invitrogen).

ACCESSION NUMBERS

The Gene Expression Omnibus accession number for the microarray and RRBS data reported in this paper is GSE52304.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, seven figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2014.01.005.

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Social and biological factors influencing the outcomes of children with Wilms tumors in Kenya and other Sub-Saharan countries

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Abstract: Wilms tumor (WT) is a common pediatric solid tumor, and the 5-year event-free survival rate of patients with this tumor has reached 85-90% in developed countries, whereas those in developing countries were reported to be less than 50%. To overcome these disparities, physicians and investigators in developed and developing countries are currently performing research with the aim of the better management of children with WT in Kenya and other Sub-Saharan countries. Axt and colleagues published a study that increased understanding of clinicopathology of WT in Kenya on the basis of a comprehensive web-based WT registry. The study revealed that patients enrolled in the National Health Insurance Fund (NHIF) showed better completion rate of therapy and better event-free survival than those not enrolled, indicating insufficient health coverage for those not enrolled in the NHIF. Approximately 20-30% of Kenyan population is estimated to be covered by some forms of health insurance, mostly by the NHIF. This could be improved through various approaches. The report described that 2-year event-free survival rate was 52.7% for all patients, although loss to follow up was 50%; the findings indicate large problems both in the study results and also in the completion of treatment. It is crucial to determine at which point patients stopped their treatment and why. The development of standardized treatment protocol for WT is an urgent agenda. We hope that researchers in developed countries and health providers in Kenya can work together in future to conquer disparities in the outcomes of children with WT.

Keywords: Wilms tumor (WT); disparities of outcomes; Lost to Follow Up (LTFU); Kenya

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Wilms tumor (WT) is a common pediatric solid tumor, and the event-free survival rate of patients with this tumor is high in developed countries (1). WT as well as Burkitt lymphoma and Kaposi sarcoma are target cancers in Sub-Saharan Africa, in which limited resources are available (2), because the incidence of WT is high among pediatric cancers in Africa, and a high cure rate may be accomplished if standardized therapy could be more accessible. Epidemiological studies showed that the incidence of WT was high in Africa, low in Asia, and intermediate in Caucasian in North America (3). Furthermore, the incidence of WT in Asians was shown to be approximately one half to two-thirds of that in Caucasians in Hawaii and Britain (4,5), and was higher in Black American than in Caucasians in USA (3). These findings suggest that the different incidence rates among the three populations may be caused by different genetic backgrounds, and not environmental factors. The 5-year event-free survival rates of patients with WT in developed countries has reached 85-90%, whereas those in developing countries were reported to be 50% or less (1,6,7). To overcome these disparities, physicians and investigators in developed and developing countries are currently performing research with the aim of the better management of children with WT in Kenya and other Sub-Saharan countries (8).

Table 1 Increasing enrolment in the National Health Insurance Fund (NHIF) in Kenya

	June 2006 (million)	June 2010 (million)	June 2012 (million)
Number of members (total)	1.75	2.8	3.2
Formal sector		2.3	2.4
Informal sector		0.5	0.8
Number of members + dependents	4	9.6	12.3
Population in Kenya	34.7	40.0	43.0
Total contributions received (Ksh)	Ksh 3.5	Ksh 5.7	Ksh 9.4

Ksh, Kenyan shilling.

Cancer in Africa

The World Health Organization (WHO) has estimated that 70% of cancer deaths occur in low and middle income countries (9). In spite of the fact that cases of cancer are increasing in Africa due to various reasons such as changes to a less healthy lifestyle and an increase in oncogenic viral infections (10-12), the limited resources available for healthcare are used to control more rampant child killers such as diarrhea and pulmonary infections as well as world-focused infections such as HIV, tuberculosis, and malaria (8).

Although pediatric tumors in Africa account for a small proportion of all cancers and receive less attention in health policies in each country, the importance of understanding their epidemiology and clinicopathology is significant for both scientists and health policy makers, considering the impact of the increasing burden of cancer in Africa as well as the importance of genetic and environmental understanding of pediatric cancers in general (13).

Cancer and the health system in Kenya

Axt and colleagues (7) published a study that increased understanding of the clinicopathology of WT in Kenya and also in low resource countries. With the increasing number of cases of cancer in Kenya, greater efforts have been made to create awareness and develop control policies towards cancer, especially in the last ten years. The Ministry of Health, Kenya established the "National Cancer Control Strategy 2011-2016" for the first time in its history to tackle issues impacting the lives of people in Kenya. Although population-based data do not exist in a country with a population of 43,000,000, the annual incidence of cancer has been estimated at approximately 28,000 cases and annual mortality as over 22,000 (National Cancer Control Strategy 2011-2016, the Ministry of Health Kenya). Regarding pediatric cancer, only one in ten children with cancer survives in Kenya while seven in ten survive in developed countries (unpublished data from Kenyatta National Hospital by Jessie Githanga in Feb 2013). Based on these findings, the establishment of the Kenya WT registry is meaningful for epidemiological analyses as well as a more common understanding of WT in Kenya. It could also assist many scientists in developing a more detailed research agenda because only a limited number of reliable scientific studies have been conducted, which has been attributed to patients not presenting to health facilities for a diagnosis and also poor record keeping.

This article revealed several issues caused by the weak health system in Kenya from the point of view of cancer management (7). Some have a negative impact on the production of scientific data. However, others may positively assist policy makers to strengthen the health system. These include poor access to health facilities due to long distances and financial reasons, lower awareness towards cancer among the general public, less specialized health providers, the limited number of health facilities and infrastructures, in which cancer treatment is offered, and the absence of standardized treatment protocols as well as poor record keeping. The National Health Insurance Fund (NHIF) has gradually increased and achieved an enrolment of 12.3 million members and dependents in 2012 (*Table 1*). Patients enrolled in the NHIF showed the better completion rate of therapy and better event-free survival than those not enrolled, indicating insufficient health coverage for those not enrolled in the NHIF. Social misconception is also a large factor that interferes with proper pediatric cancer management.

Study limitations

This study had some limitations due to the retrospective study design that inhibited obtaining exact factors that could improve the treatment outcomes of WT (7). This was also negatively boosted by several social factors.

For example, the improved treatment outcomes among the study populations who were enrolled in the NHIF may have been due to the direct benefit of the NHIF; however, the population enrolled in the NHIF may have been already biased by a baseline financial status, stronger health seeking behavior, and a more urban population who are employed. The same could be applied to the tribal proportions of WT cases and may be a genetic issue that many scientists can recognize; however, it could also be influenced by the original locality of tribes, financial status, and also other cultural factors. Therefore, some factors influencing treatment outcome and tribal bias of enrollment in the Kenyan Wilms Tumor Registry (KWTR) raised by the investigators should be analyzed in a prospective study. In addition, better writing and keeping of medical records in hospitals and clinics and improvement of the KWTR system are needed to determine the proper social and biological factors that influence outcome of Kenyan children with WT.

National Health Insurance System and outcomes of patients who received WT treatments

Even though several study limitations were observed, the results obtained indicated that various factors may have improved outcomes of patients that received WT treatments (7). The study revealed the clear benefit of the NHIF. As "Universal Health Coverage" is currently one of the top priority global health agendas since the 58th World Health Assembly of 2005 adopted the resolution on "Sustainable health financing, universal coverage, and social health insurance" (World Health Assembly Resolution 58.33, 2005), the clear benefit of the NHIF shown in this study should encourage the country policy makers to strategize improvements in the enrolment rate. Approximately 20-30% of Kenyan population is estimated to be covered by some forms of health insurance, mostly by the NHIF (the Government of Kenya/NHIF, 2012). This could be improved through different approaches such as compulsory enrolment by the law, improved payment systems, increased awareness of insurance benefits among the general public, improved accountability/integrity of the fund, and better benefit packages.

Strategy to reduce Lost to Follow Up

It is also crucial to determine at which point patients stopped their treatment and why (7). The study proved that the completion of treatment led to the significantly better outcomes of patients with WT. However, it is not easy to specify the timing and reasons for Lost to Follow Up (LTFU) from the findings of the study; the finding that fifty percent of study patients were LTFU indicate large problems both in the study results and also in the completion of treatment. The large number of LTFU may have been due to financial constraints at the individual level, distance to the treatment facility, and cultural beliefs/superstitions including witchcraft and/or misconceptions towards the WT management. In African culture, especially in rural areas, people tend to link medical conditions with religious and cultural beliefs. Therefore, when sick children are not immediately responding to "Western medicine", the guardians often try to bring them to religious leaders or traditional healers or any other forms of traditional treatment methods, which waste a lot of time and money, and increases the number of LTFU.

This finding could also be attributed to factors on the side of the health services such as inability to obtain central venous access for chemotherapy, as was described in the Discussion section, discouragement due to drugs being out of stock, and other forms of poor services. Strategies to increase the treatment completion rate are essential to improve the outcomes of the treatment for WT. As dropouts were reported during pre- and post-operative chemotherapy, it is also important to consider quality communication and sufficient explanations of the treatment to the families before and after the treatment starts. Irrespective of developed or developing countries, the success of cancer treatment often depends on the relationship between the patient/their family and health care providers and how their social and psychological issues are followed-up by a multi-disciplinary team. Therefore, comprehensive care for cancer should also be included in the strategies to increase treatment completion rates.

Treatment protocol and the outcomes of patients with WT

As was described in the study (7), the development of a standardized treatment protocol for WT is also an urgent agenda. Children in Kenya with WT are mainly treated with one of two protocols established by the Children's Oncology Group (COG) or Société Internationale d'Oncologie Pédiatrique (SIOP). The findings of this study showed that many patients dropped out during chemotherapy; therefore, using the COG protocols, in which up-front resection is performed prior to chemotherapy appears to be more appropriate so that all

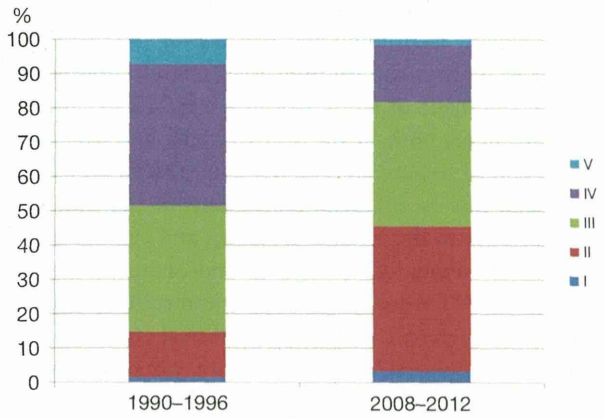

Figure 1 Stage distribution of Wilms tumors in patients treated between 1990 and 1996 and between 2008 and 2012 in Kenya.

patients could benefit from resection, which is often very essential for the management of solid tumors. However, it is also important to consider that the majority of WT cases in Kenya already presented in advanced stages at the first visit. The findings of this study showed that over 50% of WT patients in Kenya were diagnosed at stage III and IV (*Figure 1*), which is markedly different from data obtained in developed countries, in which the majority of patients were diagnosed at stage I or at most II. This could make resection without preoperative chemotherapy less successful. However, the stage distribution of WT in Kenya has been changing over the last few decades. Abdallah *et al.* reported in 2001 that 78% of WT patients in Kenya were stage III and IV (6) as opposed to the 52.8% reported in Axt and colleagues' study (7) (*Figure 1*). This indicates that the clinical features of WT have markedly changed. Therefore, continuous observations and studies are required to determine a standardized treatment protocol that provides a better outcome for Kenyan children with WT.

Blood transfusion and WT treatment outcomes

Blood transfusion received outside of the operating theatre was associated with a poor outcome (7). Almost all patients received a blood transfusion during their time in an operating theatre, and approximately 20% received on outside the theatre, both of which were markedly different from developed countries in which blood transfusions are not always common practice both inside and outside of the operating theatre during the management of WT. Although the reasons for the blood transfusions were not stated or analyzed, it may be attributed to extra complications during the operation because of the advanced stage of tumors, infections due to poor hygiene, the overuse of blood transfusions due to poor risk management, and preconditions such as HIV infections, malnutrition, and sickle cell diseases. Further research is needed to identify the reasons for these transfusions in order to improve the outcomes of patients with WT.

Biological differences in WT among African, Caucasian, and Asian children

Murphy and colleagues studied the molecular characteristics of 15 Kenyan WTs, age-matched North American WT controls, and found an increased mortality, higher incidence of nuclear unrest, and increased proportion of epithelial nuclear β-catenin in Kenyan WTs than in the North American counterparts (14). Anaplastic histology with intense p53 immunostaining was detected in two (13%) of the 15 Kenyan WTs, which was consistent with the incidence of NWTS (10.8%) and appears to be higher than that of anaplastic histology in Japanese WTs (3.5%) (15,16). They demonstrated that the African WT specimens expressed markers of adverse clinical behavior and treatment resistance and may require more intensive treatment protocols.

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 (17). We reported that if only sporadic tumors were included, the frequencies of WT with *WT1* abnormalities (22.8%) would be similar between Japanese and Caucasian populations; however, an exact comparison is difficult because of the absence of data on the population-based incidence of *WT1* alterations in WT (18). The study on 15 Kenyan WTs only detected one tumor with a *WT1* mutation (6.7%) (14), which indicated that the higher incidence of African WT may be caused by the increased incidence of *WT1*-wild-type WTs.

IGF2, insulin-like growth factor II, is an imprinted gene expressed from the paternal allele, and encodes a fetal polypeptide growth factor (19). We and other studies previously reported that loss of *IGF2* imprinting was markedly lower in Japanese children than in their Caucasian counterparts, and showed that the lower incidence of WT with the loss of *IGF2* imprinting may be implicated in the lower incidence of WT in Japan (18,20). Unfortunately, no studies have examined the *IGF2* status in African WTs. Thus, studies of the molecular characteristics of African WTs have just begun, and future studies will clarify whether genetic and epigenetic differences correlate with the different incidence

rates of WT among different ethnic populations.

Conquering the disparities in the outcomes of children with WT between developed and developing countries

As described earlier, limited resources are used for common diseases such as diarrhea, pulmonary infection, HIV, tuberculosis, and malaria. However, disparities in the outcomes of children with pediatric cancer such as WT between developed and developing countries cannot be ignored. Axt and colleagues described the present medical situation for treating WT in Kenya, and made recommendations to accomplish better treatment outcomes (7). Researchers in developed countries examine the biology of WT, and believe that this research will improve the outcomes of subgroups of patients with WT who fail to respond to the present standardized regimens. Physicians and other health providers in Kenya take care of children with WT as well as common, but possibly life-threatening diseases. We hope that both these groups can work together in future to conquer disparities in the outcomes of children with WT between developed and developing countries. Further updated studies from both groups are essential for obtaining this goal.

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

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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 germinal 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 WT survivors. However, investigators have never examined

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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*, 1984; Lavedan *et al*, 1989; Pelletier *et al*, 1991; Kaplinsky *et al*, 1996; Jeanpierre *et al*, 1998; Pritchard-Jones *et al*, 2000; Shibata *et al*, 2002; Zirn *et al*, 2005; Regev *et al*, 2008; Fencl *et al*, 2012; Melchionda *et al*, 2013). In addition, the parental origins of *de novo* small *WT1* mutations and large 11p13 deletions encompassing *WT1* were reported previously in two and eight individuals, respectively (Huff *et al*, 1990; Nordenskjold *et al*, 1994). The aim of the present study was to determine the incidence rates of *WT1* and *IGF2* abnormalities in bilateral WTs in Japanese children, and was to compare the results with those reported in bilateral WTs of Caucasian children. In addition, we summarised the present and previous findings on the penetrance rate for children who inherited various types of *WT1* abnormalities from their fathers or mothers, or had *de novo WT1 (DNWT1)* abnormalities that occurred in the paternal or maternal germ cell, and tried to clarify whether parental inheritance and *WT1* abnormality types may affect the penetrance rate of hereditary WT.

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 *BCL1* in chromosome band 11q13, or by SNP arrays 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).

COBRA of the CTCF6 site at *H19*-DMR or MS-MLPA of the IC1 (*H19*-DMR) and IC2 (KvDMR) regions. We determined the methylation status of 11p15 region in tumour and PB samples by combined bisulfite restriction assay (COBRA; Watanabe *et al*, 2006) and/or methylation specific (MS)-MLPA (Salsa MS-MLPA kit, ME030BWS/SRS) assay. Combined bisulfite 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 53.6 ± 5.6%, and we defined more than the mean percentage + 2 s.d. as the hypermethylated state. Methylation specific-MLPA analysis was used to detect the methylation status of the IC1 (*H19*-DMR) and IC2 (KvDMR) regions. The methylation statuses were defined according to the manufacturer's instructions.

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

Table 1. Genetic and epigenetic alterations in *IGF2* (11p15) and *WT1* (11p13) loci in 31 patients with bilateral Wilms tumours

Tumour	Age/sex	11p15 SNP	*H19*-DMR	*IGF2* Status	*WT1* allele 1	*WT1* allele 2	Other CGH changes	Anomaly/reference/heredity
BWT1R	1 year 1 month/F	UPD	Hypermethyl. (C)	pUPD	ex 1, 370C>T/G124X	The same as allele 1, UPD11pt-11cen	del(7)(q11.1q21.11)	None
BWT2L	1 year 2 months/F	ROH	Normal Methyl. (C)	ROI	Deletion of 21.8 Mb	Deletion spanning exons 4–9	None	WAGR syndrome/No. 1[a]
BWT2R	—	ROH	Normal methyl. (C)	ROI	Deletion of 21.8 Mb	ex 7, 927-956del30ins5/T309fs379X	None	WAGR syndrome/No. 16[a]
BWT3R	1 year 2 months/M	UPD	Hypermethyl. (C, M)	pUPD	Deletion in ex 1~5	The same as allele 1, UPD11pt-11cen	UPD3pter-3p14.2,7p-q+	None/No. 2[a]
BWT4L	12 months/M	UPD	Hypermethyl. (C)	pUPD[b]	ex 9, 1168C>T/R390X	The same as allele 1, UPD (MLPA)	NE	None
BWT4R	—	UPD	Hypermethyl. (C, M)	pUPD[b]	ex 9, 1168C>T/R390X	The same as allele 1, UPD, 11pt-p13	7p-q+	None
BWT5L	9 months/M	UPD	Hypermethyl. (C, M)	pUPD	ex 9, 1168C>T/R390X	The same as allele 1, UPD 11pter-p12	None	Hypospadia/cryptorchidism/No. 26[a]
BWT6R	12 months/M	UPD	Hypermethyl. (C, M)	pUPD	IVS1+1G>A	The same as allele 1, UPD (MLPA)	NE	None
BWT7R	7 months/F	UPD	Hypermethyl. (C, M)	pUPD[b]	ex 1, 268delA/S90Afs128X	The same as allele 1, UPD11pt-11cen	None	None
BWT8R	2 months/M	UPD	Hypermethyl. (C, M)	pUPD[b]	ex 3, 646delC/L216Cfs2X	The same as allele 1, UPD11pt-11cen	1q+	None
BWT9L	1 year 1 month/M	ROH	Normal Methyl. (C)	ROI	ex 1, 172delC/P58Rfs32X	Deletion of 1.6 Mb	None	Urogenital anomaly/No. 19[a]
BWT9R	—	UPD	Hypermethyl. (C, M)	pUPD	ex 1, 172delC/P58Rfs32X	The same as allele 1, UPD11pt-11cen	UPD17q21.33-qter	Urogenital anomaly/No. 31[a]
BWT10L	1 year 2 months/F	UPD	Hypermethyl. (C)	pUPD[b]	ex 9, 1186G>A/D396N	The same as allele 1, UPD11pt-11p12	None	Drash syndrome/No. 32[a]
BWT10R	—	UPD	Hypermethyl. (C)	pUPD[b]	ex 9, 1186G>A/D396N	The same as allele 1, UPD11pt-11cen	UPD3pter-p21.33	Drash syndrome/No. 33[a]
BWT11L	12 months/F	UPD	Hypermethyl. (C, M)	pUPD[b]	ex 7, 938C>A/S313X	The same as allele 1, UPD11pt-11cen	None	None
BWT11R	—	UPD	Hypermethyl. (C, M)	pUPD[b]	ex 7, 938C>A/S313X	The same as allele 1, UPD (MLPA)	NE	None
BWT12R	11 months/F	UPD	Hypermethyl. (M)	pUPD	ex 6, 818C>G/Y271X	The same as allele 1, UPD11pt-11cen	None	None
BWT13L	12 months/M	UPD	Hypermethyl. (M)	pUPD	IVS2-1G>A	The same as allele 1, UPD11pt-11cen	UPD3pt-3p21	None
BWT13R	—	UPD	Hypermethyl. (M)	pUPD	IVS2-1G>A	The same as allele 1, UPD11pt-11p13	None	None
BWT14L	11 months/F	UPD	Hypermethyl. (M)	pUPD[b]	ex 9, 1180C>T/R394W	The same as allele 1, UPD11pt-11cen	None	Drash syndrome
BWT15L	9 months/M	UPD	Hypermethyl. (C, M)	pUPD	ex 8, deletion	The same as allele 1, UPD11pt-11cen	None	None
BWT15R	—	UPD	Hypermethyl. (C, M)	pUPD	ex 8, deletion	The same as allele 1 (UPD)/11pt-p13	None	None
BWT16L	12 months/M	UPD	Hypermethyl. (C)	pUPD	ex 8, 1084C>T/R362X	The same as allele 1, UPD11pt-11cen	None	None
BWT17	12 months/M	UPD	Hypermethyl. (C)	pUPD	ex 1, 97_98ins5/Q32Rfs59X	The same as allele 1, UPD11pt-11cen	7p-q+	None
BWT18L	11 months/F	UPD	Hypermethyl. (C, M)	pUPD[b]	ex 4, 714G>A/W238X	The same as allele 1, UPD11pt-11cen	None	None
BWT18R	—	UPD	Hypermethyl. (C, M)	pUPD[b]	ex 4, 714G>A/W238X	The same as allele 1, UPD11pt-11p12	UPD3pt-3p21	None
BWT19R	1 year 2 months/F	UPD	Hypermethyl. (C, M)	pUPD[b]	ex 4, 682delC/Q228Kfs2X	The same as allele 1, UPD11pt-11cen	None	None
BWT20L	1 year 1 month/F	UPD	Hypermethyl. (C, M)	pUPD	ex 9, 1168C>T/R390X	The same as allele 1, UPD11pt-11p13	None	None
BWT20R	—	UPD	Hypermethyl. (C, M)	pUPD	ex 9, 1168C>T/R390X	The same as allele 1, UPD11pt-11cen	None	None
BWT21R	10 months/F	UPD	N. D.	UPD	ex 9, 1180C>T/R394W	The same as allele 1, UPD (MLPA)	NE	Drash syndrome
BWT22R	2 years 2 months/M	ROH	Normal Methyl. (C)	ROI[b]	Deletion of 3.2 Mb	IVS6+1-IVS6+3del3	3p-,4q-,7p-	Hypospadia/cryptorchidism
BWT23L	7 months/F	UPD	Hypermethyl. (C, M)	pUPD	ex 9, 1168C>T/R390X[c]	The same as allele 1, UPD11pt-11p12	None	OD, FGS/No. 5[d]//FWT1
BWT23R	—	UPD	Hypermethyl. (C, M)	pUPD	ex 9, 1168C>T/R390X[c]	The same as allele 1, UPD11pt-11cen	None	OD, FGS/No. 5[d]/FWT1
BWT24R	1 year 8 months/F	UPD	Hypermethyl. (C, M)	pUPD[b]	ex 1, 144C>A/Y48X[c]	The same as allele 1, UPD11pt-11cen	7p-,7p-q+, 9q-	*de novo* mutation/No. 36[a,e]/DNWT9-2
BMT25L	1 year 7 months/F	UPD	Hypermethyl. (C, M)	pUPD[b]	ex 9, 1168C>T/R390X[c]	The same as allele 1, UPD11pt-11cen	1q+	None/FWT2-2