**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cancer, only one in ten children with cancer survives in Kenya while seven in ten survive in developed countries (unpublished data from Kenyatta National Hospital by Jessie Githanga in Feb 2013).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

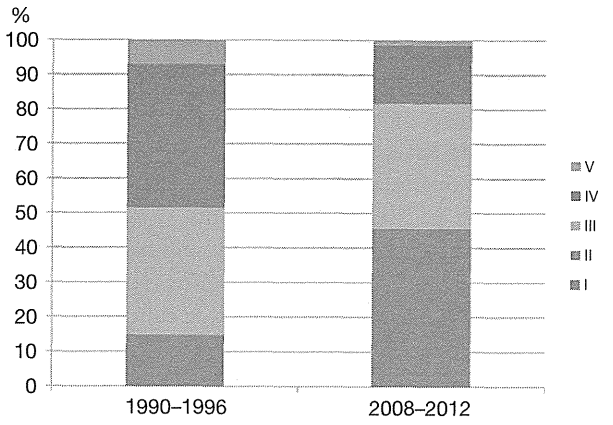


**Figure 1** Stage distribution of Wilms tumors in patients treated between 1990 and 1996 and between 2008 and 2012 in Kenya.

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.

## Acknowledgements

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【第55回日本小児血液・がん学会学術集会】プレナリーセッション

# Wilms腫瘍（腎芽腫）の発生に関わるジェネティック・エピジェネティック異常，および遺伝性・両側性Wilms腫瘍の原因遺伝子

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要　旨

近年のがんゲノム研究により，小児がんは，胎児の臓器形成にかかわる数個の遺伝子異常が月単位の短期間に生じ，発生することがわかってきた．はじめて，Wilms腫瘍モデルマウスの作製が，報告された．*Wt1*発現を消失させ，*Igf2*を高発現させたトランスジェニックに腫瘍は発生したが，それぞれの遺伝子異常のみをもつマウスには発生しなかった．さらに最近，Let-7 miRNAの発現低下による*LIN28B*遺伝子の高発現，miRNAプロセシング遺伝子である*DROSHA*や*DICER1*の変異によるmiRNAの産生低下，partial reprogrammingによるポリコーム複合体標的遺伝子の高発現など，腎前駆細胞に生じるジェネティック・エピジェネティック異常によって，マウスにWilms腫瘍を発生させることができたと報告された．Wilms腫瘍は臨床的にheterogeneousな疾患であるが，その背景にある，多様な腫瘍化の分子機構が解明されようとしている．*WT1*胚細胞変異をもつ家族性Wilms腫瘍家系の報告は少なく，浸透率は不明である．日本Wilms腫瘍研究グループ（JWiTS）では両側性・遺伝性Wilms腫瘍の遺伝子解析研究を開始した．この研究により，Wilms腫瘍の浸透率を明らかにできれば，遺伝カウンセリングの基盤となるデータを提供できるのではなかと期待している．

キーワード：Wilms腫瘍，*LIN28*，*WT1*，*IGF2*，microRNA
Key words: Wilms tumor, *LIN28*, *WT1*, *IGF2*, microRNA

## I　はじめに

小児がんと成人がんでは組織型，発生頻度，治癒率などが著しく異なる．近年のがんゲノム研究により，成人がんでは通常，いくつかの腫瘍化にかかわる多数の遺伝子変異が長期間にわたり蓄積した結果，発生すると報告された．一方，小児がんでは，胎児の臓器形成にかかわる数個の遺伝子異常が月単位の短期間に生じ，発生することがわかってきた[1]．

Wilms腫瘍の発生にかかわる遺伝子として11p13の*WT1*と11p15の*WT2*が知られている．*WT1*は，泌尿生殖器異常を伴うWillms tumor-aniridia-genitourinary malformation-mental retardation（WAGR）症候群および若年性腎障害を特徴とするDrash症候群の原因遺伝子である[2,3]．一方，*WT2*は過成長を特徴とするBeckwith-Wiedemann（B-W）症候群の原因遺伝子であると想定された[4]．その後B-W症候群は11p15に位置する複数のインプリント（刷り込み）遺伝子のどれかの異常により発生するので，*WT2*はこれらの遺伝子の総称とみなされている．11p15遺伝子群の中で胎児期の細胞増殖因子である*IGF2*の発現異常がWilms腫瘍の発生に関わることは1980年代に報告されていた．最近，胎児組織発達のタイミングを調節しているLin28遺伝子の高発現[5]，miRNAのプロセシングにかかわるRNA切断酵素（RNase III）の遺伝子変異[6]，体細胞のpartial reprogramming[7]など，新しいジェネティック・エピジェネティック異常により，Wilms腫瘍が発生するという報告が，次々になされている．

治療成績の改善によりWilms腫瘍を克服したサバイバーは増加している．Wilms腫瘍の一部は遺伝性であるので，胚細胞遺伝変異保因者の数も増加していると予想される．日本ウィルムス腫瘍研究グループ（JWiTS）では，両側性腎芽腫に対する治療研究を開始したが，その付随研究として，両側性腎芽腫の遺伝子分析研究が並行して開始された[8]．遺伝性Wilms腫瘍の遺伝子研究の現状についても，最近の知見を紹介したい．

## II　Wilms腫瘍の発生母地と*WT1*および*WNT*遺伝子群の発現

Wilms腫瘍の組織像は腎芽細胞，上皮細胞，間質細胞から構成されるが，これらの細胞は胎児期の後腎組織を構成する細胞と類似している．以上の所見により，Wilms腫瘍は腎の前駆細胞に生じた遺伝子異常により発生すると推測された．

胎児腎の発生過程において，腎前駆組織は前腎，中腎，

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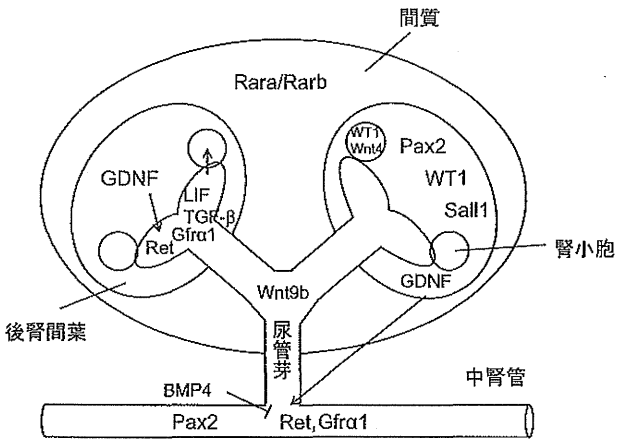


**図1**　マウスの腎発生初期の組織と発現する遺伝子群．尿管芽と後腎間葉は互いに作用する蛋白を発現し，腎を形成する[9]．

後腎の順番に形成され，後腎が最終的な腎になる．ヒトでは受精後約22日に，中間中胚葉から前腎が発生する．前腎管は胎児の尾部に延び，中腎管（Wolf管）を誘導する．胎生第5週になると総排泄腔近くの中腎管から尿管芽が発生する．尿管芽は中胚葉由来の後腎間葉（metanephric mesenchyme）と呼ばれる腎芽前駆細胞に侵入する．後腎間葉は尿管芽の分枝した先端からWnt9bシグナルを受け，腎上皮前駆細胞である造腎間葉になり，間葉-上皮転換（Mesenchymal-Epithelial Transition, MET）を経て，腎小胞renal vesicleや尿細管renal tubuleに分化する．一方，尿管芽は後腎間葉からWnt4シグナルを受け分枝し，尿管や集合管を形成する（図1）[9]．METは，成人上皮癌でみられるEMTの反対現象である．

このような形態変換過程において，Wntリガンドは標的細胞において細胞特異的転写プログラムの細胞外スイッチを入れ，Wntシグナル伝達系を活性化する．Wnt9bは尿管芽より産生されるシグナルであり，後腎間葉にWnt4を誘導する．Wnt9b欠失マウスは後腎間葉を有するが，分化障害のために造腎間葉が形成されず，低形成腎を示す．この腎では正常後腎間葉に特徴的に発現し，腎の発生に関わるPax8，Fgf8，Lh1などの転写因子が発現しない[9]．

Wnt4はWnt9bに反応して誘導され，後腎間葉とそれに続いて形成される早期の上皮に限局して発現し，腎小胞の形成にかかわる．Wnt4欠失マウスはWnt9b欠失マウスと同様に低形成腎を示し，尿細管形成が停止する．Wnt4機能消失型変異はヒトの低形成腎症候群であるSERKAL症候群の原因であることがら，Wnt4は哺乳類の腎の発生に重要であると考えられる[9]．

## III　*WT1*遺伝子の機能とWilms腫瘍の発生

*WT1*遺伝子は腎発生を司るマスター遺伝子であり，胎児の腎前駆細胞と成人腎の一部で発現する[2,3]．Wilms腫瘍の0.75%はWAGR症候群に合併して発生する．WAGR患者のリンパ球など体細胞には顕微鏡で検出可能な大きさの11p13欠失（胚細胞変異）がみられ，この欠失領域から*WT1*癌抑制遺伝子が単離された．*WT1*異常は散発性Wilms腫瘍の15～25%に報告されている．*WT1*遺伝子の大きさは約50 kbであり，10個のエキソンからなる．WT1蛋白質は主として4個のアイソフォームをもち，C端には4個のzinc fingerドメインがあり特定のDNA塩基配列と結合可能である．このドメインによりWT1蛋白質は*EGR1*，*IGF2*，*TP53*遺伝子などのプロモーター領域と結合し，その転写を制御している．

### 1．*WT1*遺伝子の発現異常とWilms腫瘍の発生

WT1は*Wnt4*の転写因子として働き，その発現を促進する．*WT1*は*Wnt4*と同様に腎の発生過程でMETを生じる組織，つまり，後腎間葉，造腎間葉，腎小胞や尿細管上皮などに高レベルで発現する[2,3]．最も強く発現するのはC字小体/S字小体で，腎の完成と共に発現は消失するが，糸球体の足細胞では成人になっても発現している．*Wt1*欠失マウスでは後腎間葉に尿管芽の侵入が起きず，後腎間葉に著しいアポトーシスを生じる．従って，腎が形成されず，胎児期に死亡するので，Wilms腫瘍の発生は観察されない．Small interfering RNA（siRNA）は標的遺伝子の発現を抑制する．マウスの胎児腎器官培養法とsiRNAを用いて後腎間葉が凝縮する時期にsiRNAを作用させて*WT1*発現を抑制すると，上皮細胞への分化が抑制され増殖が促進された．腎前駆細胞における*WT1*の発現抑制が細胞増殖を引き起こす所見は，特定の時間と組織に生じる*WT1*の機能的欠失がWilms腫瘍の発生に関わることを示唆している．

### 2．*WT1*変異型Wilms腫瘍と*CTNNB1*変異

βカテニンは，細胞表面のカドヘリンと細胞質のアクチンをつなぐ，細胞接着因子の機能と，核に移行し，転写因子と会合し，成長・増殖因子遺伝子を活性化する機能を合わせもつ．βカテニンは，細胞外分泌蛋白質であるWntがない状態の細胞では，β-カテニン分解複合体で，セリン/スレオニンキナーゼであるGSK3βによりリン酸化された後，ユビキチン化を受け，プロテアソームで分解される（図2A）．その結果，細胞質内のβカテニン蛋白質量は，低く保たれている．一方，Wntが細胞膜の受容体（Fz）とLRPで形成される共役受容体に結合すると，Wntシグナル伝達経路が活性化する（図2B）．Fzの作用を受けたDvlは

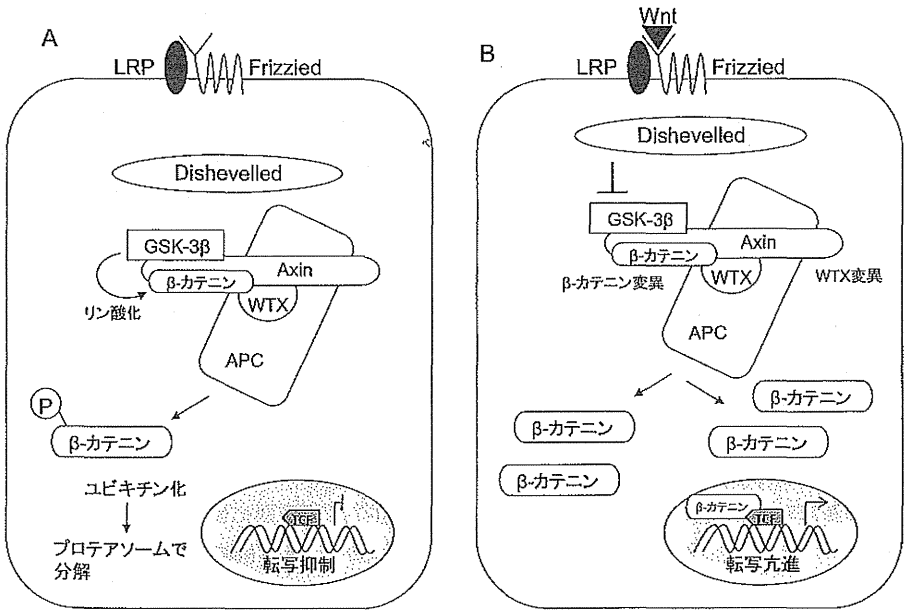


図2　正常細胞（A）と腫瘍細胞（B）のWnt/βカテニンシグナル伝達系.

GSK3β依存性のβ-カテニンのリン酸化を抑制し，低リン酸化状態になったβ-カテニンはプロテアソームによる分解をまぬがれる．その結果安定化したβ-カテニンは細胞質に蓄積し，核に移行して転写因子TCF/LEFと結合する（図2B）．β-カテニンと結合したTCF/LEFは*cycline D1*, *MYC*などの標的遺伝子の発現を促進し，様々な種類の細胞の増殖・生存にかかわる．

Wilms腫瘍の20–30%にβカテニンをコードする*CTNNB1*遺伝子の変異を認める[3,10]．この変異は，GSK3βによりリン酸化を受けるセリン，スレオニン残基に集中して発生し，機能獲得型である．変異の結果，βカテニンはリン酸化を受けなくなるので安定し，標的遺伝子の転写を促進する．後腎間葉の上皮化にはWnt/βカテニン経路からWnt/PCP経路への切り替えを必要とする．*WT1*変異型腫瘍と*WT1*野性型腫瘍の*CTNNB1*変異頻度は著しく異なり，前者で約65%であるが，後者では2–10%に過ぎない[3,10]．*CTNNB1*変異が生じているとこの切り替えが行われず，腎前駆細胞は分化できずに増殖する．*WT1*/*CTNNB1*変異型Wilms腫瘍では，間葉上皮転換（MET）がうまくいかないので，腎前駆細胞は分化の方向を誤り，筋肉細胞などへと分化すると考えられる．

ラット胎児から採取した後腎間葉細胞を増殖因子なしに培養するとアポトーシスを生じるが，機能獲得型変異をもつ*CTNNB1*遺伝子を導入すると，アポトーシスを免れ，*PAX2*を発現し，増殖する．前述のように*Wt1*欠失マウスでは後腎間葉にアポトーシスが生じる．ラット胎児の研究結果はヒト腎前駆細胞に*WT1*欠失が生じても，*CTNNB1*変異を合併するとアポトーシスを免れ，増殖する可能性を示唆する．*WT1*変異型Wilms腫瘍に*CTNNB1*変異が高頻度に合併する理由かもしれない．

私たちは3組の*WT1*変異型両側性Wilms腫瘍を分析し，片側には*WT1*変異をもう片方には*WT1*変異と*CTNNB1*変異を認めた[11]．一方，Fukuzawaらは同じ腎に発生したWilms腫瘍と腫瘍前駆組織であるnephrogenic restを分析し，前者には*WT1*変異と*CTNNB1*変異を認めたが，後者には*WT1*変異のみを認めた[12]．両報告は*WT1*変異型Wilms腫瘍の発生において，*CTNNB1*変異は*WT1*変異の後に生じることを示している．

### 3. *WTX*遺伝子変異とWilms腫瘍

Wilms腫瘍の一部にXq11.2バンドを欠失する腫瘍がある．*WTX*遺伝子は，この欠失部位から単離された癌抑制遺伝子である[13]．*WTX*遺伝子は1,135アミノ酸をコードしており，*WT1*と同様に胎児期の後腎間葉や糸球体前駆組織である上皮構造に発現している．このように，両遺伝子はWilms腫瘍の前駆細胞と考えられる胎児腎組織に発現しており，胎児腎形成にかかわる．一方，*WTX*胚細胞変異は，線状広範性骨過剰症osteopathia striata congenita with cranial sclerosisの原因になるが，この先天異常患者にWilms腫瘍の合併は報告されていない[14]．胎児腎に，*WTX*変異の生じるタイミングが，腫瘍化には重要であることを示唆する．

Major等はWTX蛋白質がβカテニン，AXIN1，βTrCP2，APCなどとβカテニン崩壊複合体を形成することを示した（図2A）[15]．さらに，WTXがβカテニンのユビキチン化や分解を促進することを発見した．WTXはWntシグナ

リング系を抑制することにより，腫瘍抑制作用を示すのではないかと考えられている．*WTX*異常と*CTNNB1*変異を合併するWilms腫瘍は，まれである．この所見はWNT/βカテニン・シグナル伝達系の異常部位が，βカテニンであっても，WTXであっても，結果として同じシグナル異常が生じ，Wilms腫瘍の発生に関与していると予想される（図2B）．

WTXは細胞質と核を往復するシャトル蛋白質であり，WT1と結合し，核でWT1を介したAmphiregulin（糖蛋白であり，神経鞘腫由来の増殖因子）の転写を促進する．*WT1*と*WTX*は，腎前駆細胞で共発現している．*WTX*変異はWT1蛋白質結合部位に集中しており，変異によりWT1の正常な機能に影響与える[16]．このように，WTXの機能は多面的であり，その異常のWilms腫瘍化における役割も多様である．

## IV　*IGF2*（insulin-like growth factor 2）遺伝子の高発現とWilms腫瘍の発生

### 1. Wilms腫瘍とBeckwith-Wiedemann（B-W）症候群

Wilms腫瘍を合併する先天異常症候群としてB-W症候群が知られている．B-W症候群は臍ヘルニア，巨舌，巨躯を主症状とする先天奇形症候群である．一部の患者に11p15トリソミーや，11p15に切断点を持つ転座がみられたこと，また連鎖解析の結果から，その遺伝子座は11p15に位置すると決められた．11p15領域には2か所のインプリンティング（刷り込み）を受けるドメインがあり，それぞれ複数の遺伝子で構成される（図3）[17]．B-W症候群では，11p15のテロメア側にある*CDKN1C/KCNQ1OT1*領域か，セントロメア側にある*IGF2/H19*領域の，どちらかのインプリンティング遺伝子群に異常がみられる．*CDKN1C/KCNQ1OT1*領域では，母由来のKvDMR1（imprint center 1, IC1）の脱メチル化，*IGF2/H19*領域では母由来*H19*-DMR（imprint center 2, IC2）のメチル化，つまり，どちらのドメインにおいても，母由来アレルが父由来アレルの特徴を獲得することにより，B-W症候群は発生する．B-W症候群の12.5%にさまざまな腫瘍が発生するが，最も頻度の高いのはWilms腫瘍である．B-W症候群に合併する胎児性腫瘍のほとんどは，*IGF2-H19*遺伝子群の異常により発生したと報告されている．

### 2. Wilms腫瘍において*IGF2*の過剰発現をもたらす二つの機構

IGF2は胎児期に働く細胞増殖因子であり，胎児腎で発現するが，出生後の腎では発現消失する．前述したように，*IGF2*と*H19*の両遺伝子は11番染色体短腕p15に隣り合って位置し，インプリンティングを受ける（図4A）．すなわち正常細胞では，*IGF2*は父由来アレルからのみ，*H19*は母由来アレルからのみ発現する．*H19*遺伝子の上流にはDMR（differential methylated region）と呼ばれる領域があり，その中にinsulator proteinであるCTCFの結合部位がある．父方アレルでは*H19*-DMRのCTCF結合部位（CpG islands）がメチル化されており，CTCFが結合できないので，*H19*下流のエンハンサーが*IGF2*に発現シグナルを伝達し，*IGF2*が発現する．反対に母方アレルではCTCF結合部位は非メチル化状態にあり，CTCFが結合し，エンハンサーシグナルはCTCFで遮断されるため，*IGF2*に届かず，*H19*が発現する（図4A）．このように，*H19*-DMRのメチル化により，*IGF2*と*H19*の刷り込みは維持されている．

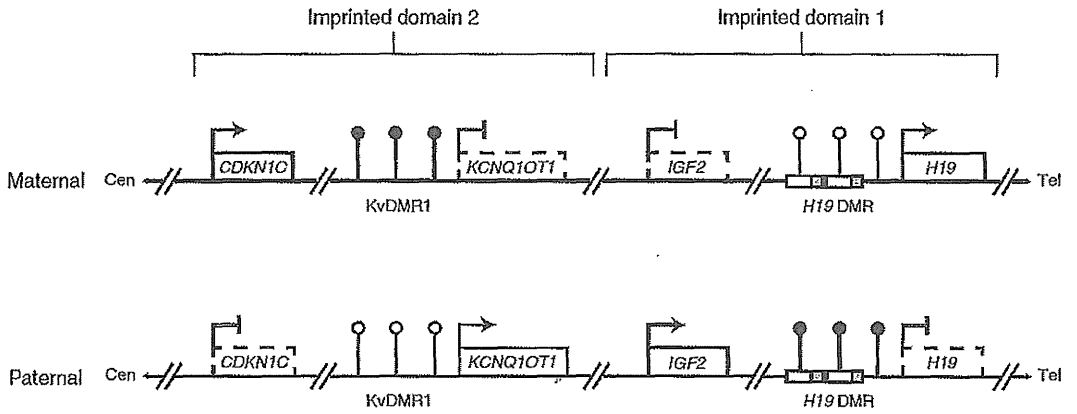


図3　11p15インプリンティング領域の模式図（文献17より引用）．11p15インプリンティング領域には2か所のドメインがあり，differentially methylated region（DMR）により4個の遺伝子発現が制御されている．父由来アレルでメチル化されている*H19*-DMR（imprinting center 1, IC1）により，*IGF2-H19*が，母由来アレルでメチル化されている*Kv*DMR1（imprinting center 2, IC2）により*CDKN1C-KCNQ1OT1*が制御されている．発現遺伝子は実線で，非発現遺伝子は破線で囲んで示す．各DMRのCpG islandメチル化は●で，同非メチル化は○で示す．

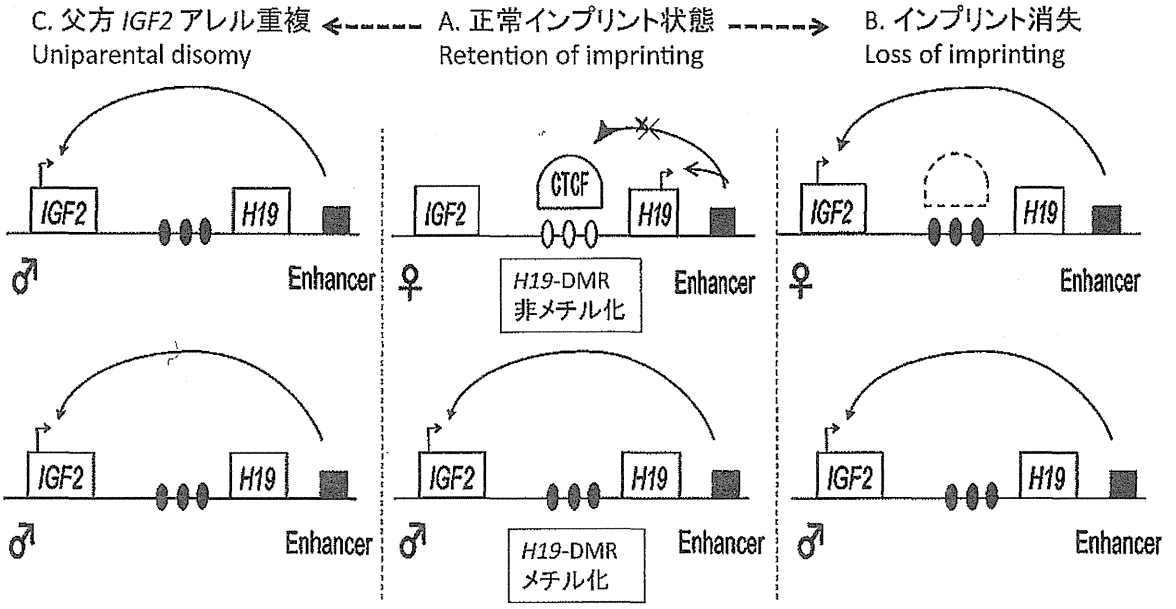


図4　*H19*遺伝子上流のDMRにあるCTCF（insulator蛋白質）結合部位のメチル化と*IGF2*遺伝子の発現．A．正常細胞の母由来アレルでは，CTCF結合部位が非メチル化状態であり，CTCFが結合する．*H19*下流からのエンハンサーシグナルはCTCFで遮断されるため，*H19*が発現する．父由来アレルではCTCF結合部位がメチル化状態であり，CTCFが結合できない．エンハンサーシグナルは*IGF2*を発現させる．B．母由来アレルのCTCF結合部位がメチル化している．これをloss of imprinting（LOI）と呼ぶ．C．母由来*IGF2*アレルが失われ，父由来*IGF2*アレルが重複している．これをpaternal uniparental disomy（UPD）と呼ぶ．LOIやUPDが生じると，*IGF2*発現アレルは倍加するので，IGF2蛋白質が過剰に産生される．

散発性Wilms腫瘍を調べると，30–70%では，母由来アレルのCTCF結合部位がメチル化している．これをloss of imprinting（LOI）と呼ぶ（図4B）．また，Wilms腫瘍の30–40%では，母由来*IGF2*アレルが失われ，父由来*IGF2*アレルが重複している．これをpaternal uniparental disomy（UPD）と呼ぶ（図4C）．LOIやUPDが生じると，*IGF2*発現アレルは倍加するので，IGF2蛋白質が過剰に産生される．そのために，Wilms腫瘍前駆細胞の増殖が促進される．これが腫瘍化のワンステップになっていると考えられる[2]．*IGF2*のLOIやUPDは肝芽腫や胎児性横紋筋肉腫など他の胎児性腫瘍においても生じており，胎児性腫瘍に共通な腫瘍増殖機構であると考えられている．

### 3．散発性Wilms腫瘍患者の末梢血に発見された11p15異常

Scott等はイギリスにおいて先天奇形のない散発性Wilms腫瘍437例の末梢血を分析し13例（3%）に11p15異常を発見した[17]．異常の内容は母由来IC1の高メチル化，父由来11p15のUPD，IC1の塩基挿入や欠失である（図3）．母由来IC1の高メチル化は一般的にモザイク状態でみられたので，受精後早期に生じたと考えられた．13例中4例は両側性腫瘍であり，その12%を占めた．4例中3例は父由来*IGF2-H19*のUPD，1例は母由来*H19*-DMRのLOIによるものであり，両親の血液には異常はみられないので，新生突然変異により生じたと考えられた．このように，臨床的に正常な表現型を示す患者の中に，生まれつきの11p15異常を示す患者がいる．

一方，日本のB-W症候群47例の11p15領域の解析結果が報告されている[9]．母由来IC1の高メチル化はアメリカやヨーロッパの患者の頻度に比して低く，染色体異常の頻度は日本で高かった．この47例中3例に肝芽腫が1例に横紋筋肉腫を合併していたが，Wilms腫瘍の合併例はなかった．我が国において散発性Wilms腫瘍患者の末梢血のIC1，IC2を分析した報告はない．

## V　Wilms腫瘍発生モデルマウスの作製

### 1．*Wt1*消失と*Igf2*高発現マウスにおけるWilms腫瘍の発生

*Wt1*欠失マウスでは後腎間葉に尿管芽の侵入が起きず，後腎間葉に著しいアポトーシスを生じる（図1）．従って，腎が形成されず，胎児期に死亡するので，Wilms腫瘍の発生は観察されない．一方，母由来*H19*-DMRを欠失し，*IGF2*を過剰発現すマウスでは，過成長がみられるが，やはりWilms腫瘍の発生は観察されない．*WT1*と*IGF2*の両遺伝子は11番染色体短腕に位置している．Haruta等は*WT1*異常のあるWilms腫瘍36例を分析し，1/3の腫瘍に*WT1*変異と父由来*IGF2*がUPDにより重複していることを報告した[11]．この所見をヒントに，Hu等は*Igf2*を過剰発現し，*Wt1*発現消失した*Wt1-Igf2*トランスジェニックマウスを作

製し，Wilms腫瘍を発生させることに成功した[18]．*Wt1*欠失は，腎前駆細胞の分化を阻害し，*Igf2*の高発現は，その増殖を促進する．両者の異常が腫瘍化には必要であることを示している．IGF2はチロシンキナーゼであるIGF1受容体のリガンドである．IGF1Rの下流にERKシグナル伝達経路があるが，このトランスジェニックマウスでは，リン酸化したERK1/2が高発現していた．

## 2. micro-RNA（miRNA）プロセシングに働くDROSHAおよびDICER1と，Wilms腫瘍におけるその遺伝子変異

miRNAは，細胞内に存在する長さ20から25塩基のRNAであり，他の遺伝子の発現を調節する．miRNAは，蛋白質には翻訳されないnon-coding RNAの一種である．核内で内在性二重鎖RNA（primary miRNA）は，RNA切断酵素（RNase III）Droshaによりヘアピン様二重鎖構造をもつ未熟miRNA（pre-miRNA）として切り出される．さらに，この前駆物質は細胞質に移行し，同じくRNA切断酵素であるDicerにより成熟二重鎖RNA（miRNA）として切り出される．細胞質内のRNA-induced silencing complex（RISC）においてmiRNAがmRNAに結合することにより，mRNAからポリペプチドへの翻訳が抑制される（図5）．

Torrezan等は1家系のエクソーム解析を実施し，発端者のWilms細胞に*DROSHA*体細胞変異を発見した[6]．次いでWilms腫瘍222例において，10個のmiRNAプロセシングにかかわる遺伝子の塩基配列解析を実施し，12%（26例）に*DROSHA*変異を発見した．その81%は同一の変異E1147Kであった．*DROSHA*変異のない腫瘍においては，*DGCR8*，*DICER1*，*XPO5*，*TARBP2*などのやはりmiRNAプロセシングにかかわる遺伝子に変異が検出された．さらに*DROSHA*-E1147K変異を有する腫瘍や細胞株を検討すると，変異のほとんどは片アレルにのみ生じており，dominant negative効果により．野生型*DROSHA*の発現を抑制していると考えられた．また，*DROSHA*変異腫瘍では，特定のmiRNA群に発現抑制が生じていた．次に行われたWilms腫瘍66例の解析では，miRNAプロセシング遺伝子の変異が33%にみられたのに対し，既にWilms腫瘍に報告されている*WT1*，*CTNNB1*，*WTX*，*TP53*，*DIS3L2*，*FBXW7*などの遺伝子変異は23%と低頻度であった．興味深いことに*DROSHA*変異を示す10腫瘍中4腫瘍に*WT1*欠失の合併がみられた．*DROSHA*変異は体細胞変異であり，胚細胞変異を伴う家族性腫瘍は報告されていない．

*DICER1*は細胞質でmiRNAプロセシングにかかわる遺伝子であるが，その変異はこれまでに，胸膜肺芽腫，多結節性甲状腺腫，嚢胞性腎腫瘍，卵巣性索間質腫瘍，子宮頚部胎児性横紋筋腫や，まれではあるがWilms腫瘍に報告されてきた[19]．報告の多くは，家族性腫瘍にみられる胚細胞変異である．

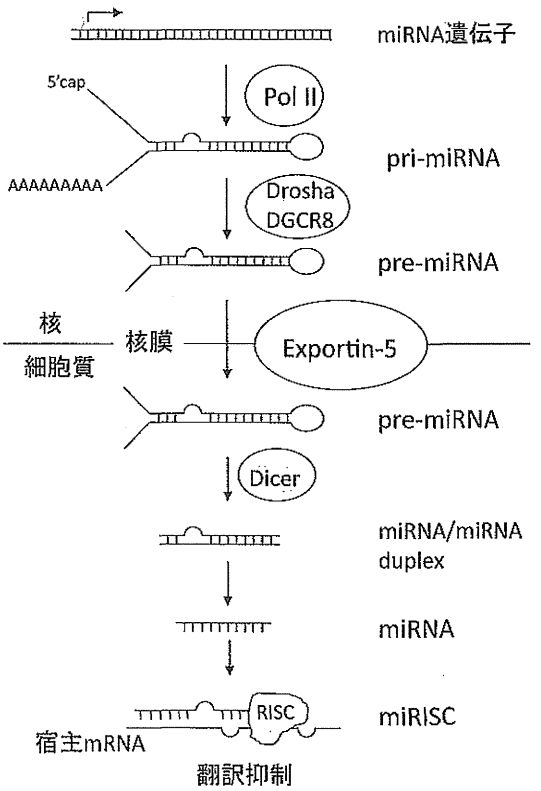


図5　RNA干渉の模式図．miRNA遺伝子からRNA Polymerase IIにより転写された長い前駆miRNAはpri-miRNAと呼ばれる．核においてpri-miRNAは，RNA切断酵素（RNase III）であるDroshaにより切断され，約70塩基の断片であるpre-miRNAが産生される．pre-miRNAはexportin 5により核から細胞質に運び出された後，別のRNase IIIであるDicerにより切断され，成熟miRNAが産生される．RNA-induced silencing complex（RISC）に取り込まれたmiRNAは，自分自身の塩基配列と対合できる標的mRNAと結合した後に，mRNA崩壊や，蛋白質の翻訳抑制などの作用を示す．

## 3. *Lin28*は腎前駆細胞の増殖を維持することにより，Wilms腫瘍を発生させる

*Lin28A*とそのパラログである*Lin28B*はRNA結合蛋白をコードしており，多能性幹細胞に高発現している．iPS細胞を誘導する山中4因子は*OCT3/4*，*KIF4*，*SOX2*，*MYC*であるが，Yu等は*KIF4*，*MYC*の代わりに*LIN28*，*NANOG*を導入することにより，ヒトiPS細胞を誘導できることを示した[20]．一方，*LIN28*はさまざまな腫瘍で高発現していることが知られている．Urbach等は*LIN28B*を発達過程や成長したマウスの腎に高発現させるために，*LIN28B*と*Wt1*を高発現するトランスジェニックマウスを作製した[5]．*Wt1*は後腎のもとになる中間間葉に発現している．胎児期に両遺伝子を高発現させると，生後2週間以内にすべてのマウスに腎腫瘍が発生した．*Lin28a*は，胎生13.5週までの正常マウス腎に発現しているが，その後，衰退する．一方，*Lin28b*は胎児マウス腎ではまったく発現していない．これらの所見から，*Lin28*が発現している間は，後腎間葉

の最終的な上皮細胞への分化は起こらず，腎前駆細胞は活発に増殖する．そのため，Lin28の発現消退が生じないと，腎前駆細胞に分化が起きず，最終的にWilms腫瘍が発生すると考えられる．*Let-7*はmiRNAであり，*Lin28*発現を抑制する．*Lin28b*を高発現しているマウス腎腫瘍では，*Let-7*発現が低下していた．*Lin28*と*Let-7*は相互に抑制する作用がある．イギリスのヒトWilms腫瘍77例の検討によると，*LIN28B*高発現は30%の腫瘍にみられ，腫瘍組織内の発現部位は腎芽細胞に限局し，再発，死亡と強い関係がみられた．Urbach等はLIN28/LET-7パスウェイが新しいWilms腫瘍の治療標的になるのではないかと提唱している[5]．

### 4. 体細胞のpartial reprogrammingにより発生するマウスWilms腫瘍

体細胞のreprogramming（初期化）によりiPS細胞は誘導される．Reprogrammingを中断させるpartial reprogrammingにより，Wilms腫瘍を発生させるマウスモデルが，最近，我が国より報告された．Ohnishi等は正常2倍体胚胞にreprogramming遺伝子（山中4因子）を発現するES細胞を注入し，キメラマウスを作製した[7]．このマウスでは，ドキシサイクリン投与により山中4因子の発現を調節可能である．生後4週のキメラマウスにドキシサイクリンを4週間投与し，山中4因子を継続して発現させると，さまざまな器官に奇形腫が発生した．しかし，4因子を7日間発現させた後，ドキシサイクリンを中止し，4因子の発現を止めたマウスには（partial reprogramming），さまざまな上皮組織に異形成（dysplasia）が生じた．異形成細胞は周囲の組織に浸潤し，腫瘍の特徴を示した．ドキシ休薬により腎に発生した腫瘍は，腎前駆細胞と同様に*Six2*，*Eya1*，*Lgr5*などの遺伝子が発現していた．これらの遺伝子は，分化に伴いES細胞においてポリコーム複合体により発現が抑制されることが知られている．ドキシ休薬誘導腎腫瘍では，体細胞のreprogrammingが中断されるため，ポリコーム複合体による遺伝子発現の抑制がかからず，その標的遺伝子の発現が継続したと考えられた．また，これらの腫瘍では，グローバルなDNAメチル化パターンに変化が生じていたが，遺伝子変異は認められなかった．これらの所見から，エピジェネティック異常によりWilms腫瘍を発生可能とするマウスモデルが提唱された．

## VI　家族性Wilms腫瘍

### 1. Wilms腫瘍の地理的疫学

Wilms腫瘍は，我が国では年間80～100例発生するが，その発生頻度は欧米の1/2～1/3であり，アジア全体の発生頻度も低い．アメリカのWilms腫瘍の平均発生年齢は42～47カ月となっているが，私たちの158例の解析結果では31カ月であり，日本では低いようだ．私たちとFukuzawa等の解析により，*WT1*，*CTNNB1*，*WTX*遺伝子異常は日欧間に差がないが，*IGF2*-インプリント消失（LOI）の頻度が我が国で低く，Wilms腫瘍全体の頻度が低い一因であると考えられた[10,21]．

### 2. *WT*変異型両側性Wilms腫瘍の遺伝子研究

日本Wilms腫瘍研究グループ（JWiTS）では「本邦における両側性腎腫瘍に対する統一プロトコール腎機能温存率と治療の完遂率の評価：両側性腎芽腫の温存」に関する治療研究を2014年6月より開始した．同時に付随研究として「両側性腎芽腫の遺伝子分析と遺伝相談への応用」を開始した[8]．これまでの研究で，日本人に発生した30例の両側性Wilms腫瘍の80%に*WT1*胚細胞変異が見られた．親の同意を得て*WT1*胚細胞変異解析を3家系において実施した．一家系では，父親にWilms腫瘍の既往があり，*WT1*変異を父親から受け継いだ子供に腫瘍が発生した．別の一家系では，Wilms腫瘍の既往のない父親が*WT1*変異の保因者であり，*WT1*変異を受け継いだ子供に腫瘍が発生した．また，もう一家系では，両親のリンパ球の*WT1*遺伝子は正常であり，両親と患者の血液と腫瘍のSNP（single-nucleotide polymorphisms）解析より，父親の胚細胞に生じた新生突然変異により腫瘍が発生したと考えられた．このように，*WT1*変異のある両側性Wilms腫瘍であっても，患者と親の末梢血の*WT1*塩基配列，SNPやDNAコピー数を分析することにより，*WT1*異常が親から伝承されたのか，新生突然変異であるのかがわかる．また，*WT1*異常を保有していてもWilms腫瘍を発病しない保因者の存在がわかる．*WT1*異常をもつ家族性Wilms腫瘍家系は世界で13家系しか報告されておらず，浸透率（*WT1*胚細胞変異の保因者の中でWilms腫瘍を発生する患者の頻度）が不明である．この研究により，Wilms腫瘍の浸透率を明らかにできれば，遺伝カウンセリングの基盤となるデータを提供できるのではなかと期待している．

### 3. *WT1*変異のない両側性Wilms腫瘍の遺伝子研究

JWiTSの両側性Wilms腫瘍の遺伝子研究で収集した80%の腫瘍に*WT1*変異があることを既に述べたが，残り20%は正常の*WT1*を示した．そのうちの1例はpremature chromatid separation（PCS）症候群であった．*BUB1B*遺伝子は，細胞分裂中期において，染色体の正常な分配を制御するチェックポイント遺伝子のひとつであるが，その変異がPCS症候群の原因である[22,23]．

PCS症候群のリンパ球を培養後，観察すると分裂中期細胞の50%以上に未熟な染色分体chromatidの分離と異数性細胞モザイクが認められる．臨床症状としては発育障害，小頭症，白内障，けいれん，多嚢胞性腎，乳幼児期肥満で

あり，Wilms腫瘍や横紋筋肉腫を好発する．胎児性腫瘍に対する化学療法にはvincristineを併用することが多いが，その標的は分裂期細胞の紡錘糸であり，細胞分裂機構が二重に障害されるため，重度の副作用が出やすく，その投与には注意が必要である．

家族性Wilms腫瘍は全体の2%程度であり，その頻度は低い[24]．前述のように，*WT1*変異による家系が一部にみられる．欧米の連鎖解析の結果より，17q21と19q13のDNAマーカーにそれぞれ連鎖するWilms腫瘍家系が報告され，遺伝子座位は*FWT1*および*FWT2*と命名された．しかしながら，両遺伝子は現在までに単離されていない．11p13（*WT1*），17q21，19q13に連鎖を示さないWilms腫瘍家系が報告されており，ほかの家族性Wilms腫瘍遺伝子の存在も示唆される．

## VII　おわりに

小児固形腫瘍は，胎児期に器官形成にかかわる遺伝子のジェネティック・エピジェネティック異常により発生することが実証されつつあることを述べた．実際，*WT1*と*WTX*は胎児の腎発生に関わる遺伝子であることが証明され，ジェネティック異常により発生する腫瘍であることが明らかにされた．一方，1980年代よりWilms腫瘍の30%において，母由来*H19*-DMRに高メチル化（Loss of imprinting, LOI）が生じており，その結果*IGF2*が過剰発現していることが報告された．最近では，miRNAである*Let-7*の発現低下により高発現する*LIN28B*遺伝子，miRNAプロセシング遺伝子である*DROSHA*や*DICER1*の変異によるmiRNAの産生低下，partial reprogrammingなど，腎前駆細胞に生じるエピジェネティック異常が腫瘍化にかかわることがわかってきた．Wilms腫瘍は臨床的にheterogeneousな疾患であるが，その背景にある，多様な腫瘍化の分子機構が解明されようとしている．

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# Problems during the Long-Term Follow-Up after Surgery for Pediatric Solid Malignancies

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## Abstract

**Introduction** With the recent improvements in the prognosis of pediatric malignancies, the number of patients surviving long-term after surgery has been increasing. Therefore, the late effects of cancer treatments are important issues. In this study, we analyzed the problems associated with the treatment of pediatric patients during the long-term follow-up after surgery.

**Patients and Methods** A total of 64 patients with pediatric malignancies who underwent surgical treatment and were followed up for more than 5 years and who were older than 13 years of age were included in this study. The average age was 20.8 (13–33) years, and the follow-up ranged from 5 to 31 years (mean, 17.7 years). Twenty-one patients (32.3%) received high-dose chemotherapy (HDC) and nine (14.1%) received radiotherapy.

**Results** In this study, 46 patients (71.9%) developed at least one problem during the follow-up period. With regard to the surgical problems, 14 patients underwent nephrectomy, and 1 of them developed renal failure. One patient received cystectomy with urinary tract reconstruction. One patient received a partial vaginectomy. Two cases with ovarian tumors received oophorectomy, one of whom also received partial hysterectomy. Other complications such as ileus, scoliosis, and leg length discrepancies were seen in some patients. In terms of the medical problems, 15 patients showed growth retardation and 2 were treated with growth hormone therapy. Gonadal dysfunction was observed in 23 patients, and 8 of them were treated with hormone replacement therapy. Six patients developed hypothyroidism, two of whom were treated with thyroid hormone replacement therapy. Other medial issues, such as hearing impairment, low bone mineral density, and hepatitis, were seen in some patients. The rate of growth retardation, gonadal dysfunction, and hypothyroidism were significantly higher in the patients who received HDC ($p < 0.05$). There was one case of second malignancy of the parotid gland.

**Conclusion** Various treatment-related complications may occur even many years after treatment, especially in patients who receive HDC. Medical problems, especially endocrine disorders, appear to be more serious than surgery-related problems. Lifetime medical surveillance and continuous follow-up by not only pediatric surgeons but also by various specialists, such as pediatric oncologists, pediatric endocrinologists, urologists, and gynecologists, are necessary.

**Keywords**
- childhood cancer survivor
- late effect
- chemotherapy
- surgery
- radiation therapy

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## Introduction

With the recent improvements in the diagnosis and treatments of pediatric malignancies, the number of patients surviving long-term after surgery has been rapidly increasing. Approximately 80% of children with cancer now survive longer than 5 years. Patients who have survived more than 5 years after the treatments are generally identified as childhood cancer survivors (CCSs). Large cohort studies of CCS have reported that these patients are at high risk for developing late treatment-related complications, such as organ dysfunction, second neoplasms, and psychosocial problems.[1–4] The importance of the quality of life of these patients is now being recognized, and the late effects of cancer treatments are essential and important issues.

However, these have not been well recognized by pediatric surgeons, especially before 2000. In 2005, pediatric oncologists and endocrinologists started the "long-term follow-up clinic" for CCS in our hospital, and started to determine the late effects in the patients who received multimodal anticancer therapy and survived more than 5 years or become adolescents and young adults.[5] Therefore, the recent patients with pediatric solid tumors treated by surgery are also followed by the long-term follow-up clinic. The aim of the present retrospective study was to assess the surgical and medical disorders of CCSs who were surgically treated and followed for at least 5 years in our pediatric surgical clinic to clarify their problems and to establish a suitable follow-up system for these patients.

## Patients and Methods

From 1984 to 2008, 132 patients with malignant solid tumors underwent surgical treatment at Osaka University Hospital and lived to be older than 13 years of age, which is the starting age for the adolescent. Among them, 26 patients died of disease, 42 patients were lost to follow-up, and the remaining 64 patients were continuously followed up in the pediatric surgical clinic for more than 5 years. These patients, adolescent–young adult CCSs, were included in this study. They comprised 32 males and 32 females. The tumors included 22 neuroblastomas, 12 renal tumors, 11 rhabdomyosarcomas, 10 hepatoblastomas, 7 malignant germ cell tumors, 1 primitive neuroectodermal tumor, and 1 granulosa cell tumor of the ovary. The average age of the patients was 20.8 (range, 13–33) years, and the follow-up duration ranged from 5 to 31 years (mean, 17.7 years). The mean age at the diagnosis of the underlying tumor was 3.3 (range, 0–15) years.

The treatment regimens (chemotherapy, radiation therapy, surgery, and high-dose chemotherapy [HDC] with hematopoietic stem cell transplantation) and various late effects, problems, and complications were retrospectively assessed from the medical records. The chi-square test and Fisher exact test were used to assess the difference between the groups. A $p$ value of less than 0.05 was considered to indicate statistical significance.

## Results

### Treatments

The treatment protocols for cancer have changed over time. All of the 64 patients received surgical treatments. Fifty-five patients (85.9%) received chemotherapy, 21 (32.3%) received HDC, 12 (18.5%) received radiation therapy, and 9 patients (14.1%) received surgery alone. Forty-six patients (71.9% of followed cases; 43.4% of surviving cases) developed at least one problem during the follow-up period.

### Surgical Problems

The most important problem related to surgery was resection of the affected organs. Fourteen patients (3 with neuroblastoma and 11 with renal tumors) underwent nephrectomy, and 1 of them developed renal failure and required renal transplantation. One patient with rhabdomyosarcoma received cystectomy with urinary tract reconstruction. One patient with a germ cell tumor received partial vaginectomy. Two cases with ovarian tumors received oophorectomy, one of whom also received partial hysterectomy.

Various other surgery-related problems, orthopedic disorders (such as scoliosis and leg length discrepancies), Horner syndrome, adhesive ileus which required laparotomy, portal vein obstruction, and gallstones, were seen in the CCSs (►Table 1).

### Medical Problems

Growth retardation was defined as a height shorter than − 2.0 standard deviation (SD) of the normal population. Fifteen patients showed growth retardation. Among them, two were diagnosed to have growth hormone (GH) deficiency and were treated with GH therapy.

Gonadal dysfunction was evaluated by the serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), and estradiol (females) or testosterone (males). Patients showing high levels of LH or FSH were considered to have primary hypogonadism. Gonadal dysfunction was observed in 23 patients (10 boys and 13 girls), and 8 patients were treated with hormone replacement therapy. ►Fig. 1 shows a typical case of growth retardation with gonadal dysfunction. A 2-year-old female with stage IV neuroblastoma was treated with surgery, chemotherapy, and HDC with hematopoietic stem cell rescue (HSCR). Her height was under − 2SD of the normal range, and puberty was delayed, when she was 10 years old. GH therapy was started when she was 12 years old, and gonadal hormone therapy replacement therapy was started when she was 15 years old, both of which were effective, and her height caught up to the normal range and puberty was observed.

The thyroid status of patients was evaluated by the serum thyroid stimulating hormone (TSH) and free T4 levels. Six patients showed high levels of TSH and were diagnosed with primary hypothyroidism, and two of them were treated with thyroid hormone replacement therapy. ►Table 1 shows the effects of HDC on the growth and endocrinological disorders. The rates of growth retardation, gonadal dysfunction, and hypothyroidism were significantly higher in the patients who

Table 1 Problems seen in the 64 CCSs followed in the pediatric surgical clinic

| | |
|---|---|
| **Surgical problems** | **27/64 (42.2%)** |
| Organ resection | 18 |
| Nephrectomy | 14 |
| Oophorectomy | 2[a] |
| Cystectomy | 1 |
| Partial vaginectomy | 1 |
| Partial hysterectomy | 1[a] |
| Leg length discrepancies | 3 |
| Horner syndrome | 2 |
| Adhesive ileus | 1 |
| Scoliosis | 1 |
| Portal vein obstruction | 1 |
| Gallstone | 1 |
| **Medical problems** | **35/64 (54.7%)[b]** |
| Growth retardation | 15 |
| Gonadal dysfunction | 23 |
| Low bone mineral density | 10 |
| Hypothyroidism | 6 |
| Hearing impairment | 6 |
| Hepatitis | 2 |
| Renal dysfunction | 1 |
| Diabetes | 1 |
| Anemia | 1 |
| **Second malignancy** | **1/64 (1.6%)** |
| **Total** | **46/64 (71.9%)[c]** |

[a]One patient received both oophorectomy and partial hysterectomy
[b]Some patients had multiple medical problems
[c]16 patients has both surgical and medical problems
Abbreviation: CCSs, childhood cancer survivors.

were treated with HDC than in those who did not receive this more aggressive treatment ($p < 0.05$) (►Table 2).

The bone mineral density of the lumbar spine was measured by dual energy X-ray absorptiometry in 18 patients. The bone mineral density in childhood was expressed as SD from the mean for age- and sex-matched controls according to the report by Tanaka.[6] Ten patients showed a bone mineral density lower than normal range and were diagnosed to have a low bone mineral density. Other medical issues, such as hearing impairment, hepatitis, renal dysfunction, diabetes mellitus, and anemia were also seen in the CCSs (►Table 1). There was one case of second malignancy, where a parotid gland tumor developed in a patient after the treatment of rhabdomyosarcoma in the cheek.

## Discussion

In the present study, among the 64 patients followed in our pediatric surgical clinic for more than 5 years and who became older than 13 years old, 46 patients developed at least one surgical or medical problem during the follow-up period. During the same period, we treated 132 patients, and 26 patients died of disease. Therefore, 106 patients had become CCRs. These results indicate that 72% of the followed patients and 43% of the total CCRs developed problems during the long-term follow-up. These percentages are compatible with those of the previous reports. For example, Oeffinger et al calculated the frequencies of chronic conditions in 10,397 survivors and 3,034 siblings and reported that 62.3% had at least one chronic condition; 27.5% had a severe or life-threatening condition (grade 3 or 4).[2] Geenen et al performed a retrospective cohort study of 1,362 5-year survivors of childhood cancer treated at a single institution in the Netherlands, and reported that almost 75% of survivors had one or more adverse events, and 24.6% had five or more adverse events.[3]

The most important surgical problem is the resection of affected organs. The kidney was the most frequently resected organ, 14 of 64 cases received nephrectomy. Nephrotoxicity is also a known acute side effect of several treatments, including cisplatin, carboplatin, ifosfamide, and radiotherapy, and can cause impaired glomerular filtration, proteinuria, and tubulopathy.[7] Elli et al investigated the blood pressure profile in 25 children with unilateral Wilms tumor and reported that the daytime and nighttime systolic blood pressure and nighttime diastolic blood pressure measurements were significantly increased in the patient group compared with healthy children.[8] Survivors with impaired renal function due to childhood cancer treatment are usually symptom free. To reduce the risk of long-term nephrotoxic events in CCS, the renal function and blood pressure should be frequently checked for a long time, especially in the patients who received nephrectomy.

Another important surgery-related problem is the resection of genitourinary tract tissues. In our series, one patient with rhabdomyosarcoma underwent cystectomy with urinary tract reconstruction. Such patients should be followed by urologists for the rest of their lives. One patient with a germ cell tumor underwent a partial vaginectomy, and another case with an ovarian tumor underwent oophorectomy and partial hysterectomy. These patients are not yet married, but they will have troubles in terms of sexual contact, pregnancy and delivery when they do. They will need to be carefully followed up in collaboration with gynecologists.

Growth retardation is one of the most common complications that emerge during cancer treatment, and also during the follow-up period in CCSs.[9–12] In this study, 15 of 64 CCSs (23.4%) exhibited growth retardation. The cause of growth disturbance is considered to be multifactorial, and to include nutritional insufficiency, GH deficiency, the exposure of the spine or legs to radiation, hypothyroidism and corticosteroid therapy. In our series, the patients who were treated with HDC had a significantly higher risk of growth retardation. Hypogonadism may cause insufficient pubertal height gain. In the case of hormonal insufficiency, hormone replacement therapy is reported to be effective for growth catch up.[5] The growth retardation was improved by GH therapy and gonadal hormone therapy in our patients with hormone insufficiency.

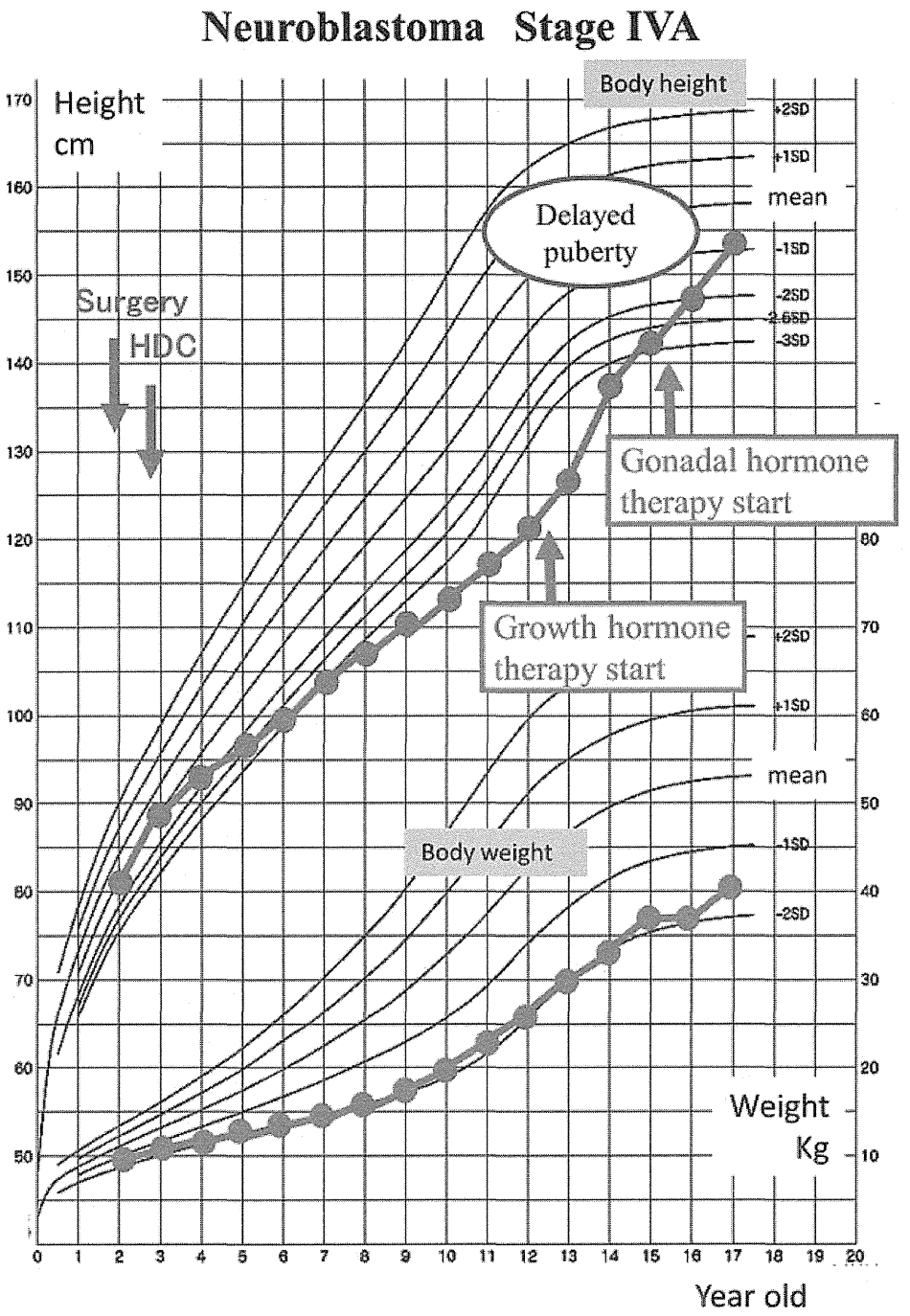


Fig. 1 The growth of a patient treated for stage IV neuroblastoma. A 2-year-old female with stage IV neuroblastoma was treated with surgery, chemotherapy, and HDC with HSCR. Her height was under − 2 SD of the normal range and puberty was delayed at 10 years old. GH therapy was started when she was 12 years old, and gonadal hormone replacement therapy was started when she was 15 years old, which were both effective, and her height caught up to the normal range and puberty was observed. GH, growth hormone; HDC, high-dose chemotherapy; HSCR, hematopoietic stem cell rescue; SD, standard deviation.

Abnormal gonadal function is also a common problem. In male patients, it is sometimes difficult to recognize gonadal dysfunction; however, high levels of FSH and decreased testicular volume indicate primary hypogonadism. These patients have the potential for experiencing male infertility and should therefore consult urologists. An important late effect among female survivors of childhood cancer is treatment-related ovarian damage and impaired fertility. Chemotherapy and radiation therapy are reported to be associated with impaired fertility.[13] Pubertal females with primary ovarian dysfunction showed high levels of FSH, LH and a low level of estrogen and amenorrhea. Recently, anti-Müllerian hormone (AMH) was shown to be a sensitive marker of the ovarian reserve. Miyoshi et al evaluated the ovarian function

Table 2 Effects of HDC on the patient growth and endocrinological disorders

| | HDC(+) ($n = 21$) | HDC(−) ($n = 43$) |
|---|---|---|
| Growth retardation | 12[a](57%) | 3 (7%) |
| Gonadal dysfunction | 17[a] (81%) | 5 (12%) |
| Hypothyroidism | 6[a] (29%) | 0 (0%) |

Abbreviation: HDC, high-dose chemotherapy.
[a]Significantly higher in the HDC (+) group than in the HDC (−) group; χ squared, $p < 0.05$.

of 53 Japanese female CCSs by measuring the serum levels of AMH and gonadotropin. Among them, 28 (53%) had a decreased AMH level, whereas only 16 (30%) had an increased FSH level.[14] The ovaries are sensitive to both chemotherapy and radiation. Therefore, the serum LH, FSH, and AMH levels should be regularly checked, and if abnormal ovarian function is suspected, the patients should be referred to gynecologists for gonadal hormone replacement therapy.

Endocrinological abnormalities are common problems, often requiring early interventions.[5,9,10] In our study, a deficiency of GH, gonadal hormones and thyroid hormone were frequently observed among the CCSs, especially treated with HDC. These patients should be followed by pediatric endocrinologists for early detection of problems and appropriate treatments.

## Conclusion

Various surgical and medical complications may occur many years after cancer treatments, especially in patients who received HDC. Among the surgical problems, resection of the affected organ was the most serious problem. Medical problems such as endocrine disorders seem to be more serious. Lifetime medical surveillance and continuous follow-up by not only pediatric surgeons but also by various specialists, such as pediatric oncologists, pediatric endocrinologists, gynecologists, urologists, and orthopedists, are necessary.

Conflict of Interest
None.

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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