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Identification of a constitutional mutation in the WT1 gene in Taiwanese patients with Wilms tumor

Meng-Yao Lu[1], Wen-Chung Wang[2], Chiao-Wen Lin[3], Alice Chang[3], Yen-Chein Lai[3*]

[1]Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan
[2]Department of Obstetrics and Gynecology, Jen-Ai Hospital, Taichung, Taiwan
[3]School of Medical Laboratory and Biotechnology, Chung Shan Medical University, Taichung, Taiwan
Email: lmy1079@gmail.com, leopard.wang@gmail.com, a1993129@yahoo.com.tw, *yenchein@csmu.edu.tw

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ABSTRACT

The overall frequency of WT1 gene alterations in Wilms tumor is still unclear in Taiwan. Here we conducted molecular genetic analysis of the WT1 gene in Taiwanese patients with Wilms tumor. Polymerase chain reaction and direct sequencing were performed on DNA samples from blood and paraffin-embedded tumor specimens. A constitutional mutation in the WT1 gene was found in one DNA sample from peripheral blood lymphocytes. The remaining DNA samples from peripheral blood lymphocytes and paraffin-embedded tumor specimens were tested negative for both constitutional mutations and somatic mutations. Thus, mutations at other Wilms tumor loci may play an important role in Wilms tumor development.

KEYWORDS

Wilms Tumor; WT1; Tumor Suppressor Gene; Nephroblastoma; Denys-Drash Syndrome

1. INTRODUCTION

Wilms tumor, or nephroblastoma, is the most common pediatric renal malignancy [1,2]. Several genetic loci have been shown to be associated with tumor formation, including WT1 on chromosome 11p13, WT2 on chromosome 11p15, FWT1 on chromosome 17q12-q21, and FWT2 on 19q [3,4]. WT1 is the first and most important gene to be isolated [5]. The WT1 gene contains ten exons of approximately 50 kb [6,7] and encodes a zinc finger protein that binds DNA and regulates transcription of target genes [8]. Many mutations in the WT1 gene have been described (The Human Gene Mutations Database, HGMD) and WT1 gene mutations have been observed in approximately 20% of patients with Wilms tumor [9,10]. Genotype-phenotype associations at WT1 have also been demonstrated [11,12]. To date, molecular genetic studies have mainly been conducted in Western countries. The risk of Wilms tumor conferred by mutations in these genes is poorly characterized in Taiwan. In this study, we performed genetic analysis to clarify the role of the WT1 gene in the renal tumorigenesis of Wilms tumor in Tai-wan.

2. MATERIALS AND METHODS

2.1. Study Subjects

Four blood (W1 to W4) and eight paraffin-embedded tumor tissue (W5 to W12) samples from Wilms tumor patients were provided by the Department of Pediatrics of National Taiwan University Hospital. W3 and W12 were isolated from the same individual. DNA sample collection for the study protocol was approved by the Institutional Review Board of Chung Shan Medical University Hospital and informed consent was obtained from each subject.

2.2. Isolation of DNA from Blood

Genomic DNA was isolated from peripheral blood lymphocytes using the QIAamp DNA blood kit (QIAGEN GmbH., Hilden, Germany). DNA was also prepared from sections of paraffin embedded tissue using the QIAamp tissue kit (QIAGEN GmbH., Hilden, Germany). The DNA was finally dissolved in 100 µl of TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA). Concentrations of the DNA stocks were estimated by spectrophotometer. Each genomic DNA sample was adjusted to 100 ng/µl to serve as a template for subsequent analyses.

*Corresponding author.

2.3. Polymerase Chain Reaction

The ten exons of the WT1 gene were amplified in 10 fragments using the published primers [10]. A slight modification was made in PCR primers and experimental conditions as shown in **Table 1**. The PCR fragments were amplified in a Perkin-Elmer 2400 DNA thermal cycler in a final volume of 30 µl that contained one-fold Qiagen PCR buffer [Tris-HCl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$; pH 8.7 at 20˚C], one-fold Q-solution, 0.015 units/µl Taq DNA polymerase supplied from Taq DNA polymerase kit (Qiagen), 500 nM for each primer, 200 µM dGTP, dATP, dCTP and dTTP (Promega) and 3 ng/µl template. The PCR conditions were initial denaturation at 95˚C for 5 min, followed by 35 cycles of 1 min at 95˚C, 1 min at annealing temperature, and 2 min at 72˚C, with final extension at 72˚C for 10 min.

2.4. Direct Sequencing

PCR products were purified using QIAquick PCR Purification kits (Qiagen GmbH., Hilden, Germany). The purified PCR products were sequenced using the cycle-sequencing method with fluorescently labelled dideoxy chain terminators from ABI Prism kit (Applied Biosystems, Taipei, Taiwan) in an ABI Model 377 automated DNA sequencer, according to the distributor's protocol. The sequencing primers were the same as those for the preceding PCRs. When a mutation was detected, the nucleotide sequence was confirmed on both strands.

3. RESULTS

We found a constitutional mutation in the WT1 gene in one DNA sample from peripheral blood lymphocytes. Wilms tumor W2 showed a deletion of one base pair at codon 115 CCT in exon 1 (**Figure 1(A)**), designated 359delC according to the Reference Sequence (RefSeq) of GenBank (accession number M80217). This mutation causes a reading frame shift after codon 115 and premature chain termination at codon 217.

W2 also showed a homozygous point mutation at codon 323 in exon 7 (**Figure 2(B)**), 71A > G (GenBank M80229). This is a silent mutation, without a change in the amino acid Arg. The remaining samples also showed this silent mutation, 71A > G in exon 7 (**Figure 2(A)**), however in heterozygous form.

Except for W2, the DNA samples from peripheral blood lymphocytes and paraffin-embedded tumor specimens tested negative for both constitutional mutations and somatic mutations in the exon regions. There were nucleotide alterations in the intron regions in almost all samples (**Table 2**). However, splicing status was not af-

Table 1. Primers for the amplification of WT1 exons.

Exon	Name	Primer sequence	Size (bp)	Temperature
1	1A	5' CGAGGAGCAGTGCCTGAGCG	248	68˚C
	1R	5' GCGGAGAGTCCCTGGCGC		
2	2A	5' CGAGAGCACCGCTGACACTG	189	66˚C
	2R	5'GAGAAGGACTCCACTTGGTTCCG		
3	3A	5' CCAGGCTCAGGATCTCGTGT	238	61˚C
	3R	5' AAGGACCCAGACGCAGAGC		
4	4A	5' TGCTTTTGAAGAAACAGTTGTG	169	58˚C
	4R	5' GGAAAGGCAATGGAATAGAGA		
5	5A	5' GGGCTTTTCACTGGATTCTG	147	60˚C
	5R	5' CCATTTGCTTTGCCATCTCC		
6	6A	5' GTGAGCCACACTGAGCCTTT	198	61˚C
	6R	5' GGCCGGTAAGTAGGAAGAGG		
7	7A	5' GGCTTAAAGCCTCCCTTCCT	253	60˚C
	7R	5' TGAGAGCCTGGAAAAGGAGC		
8	8A	5' GAGATCCCCTTTTCCAGTATCA	175	60˚C
	8R	5' ACAGCTGCCAGCAATGAGAA		
9	9A	5' CATTGTTAGGGCCGAGGCTA	218	60˚C
	9R	5' CTTTTCCAATCCCTCTCATCA		
10	10A	5' TGTGCCTGTCTCTTTGTTGC	224	60˚C
	10R	5' GTTCACACACTGTGCTGCCT		

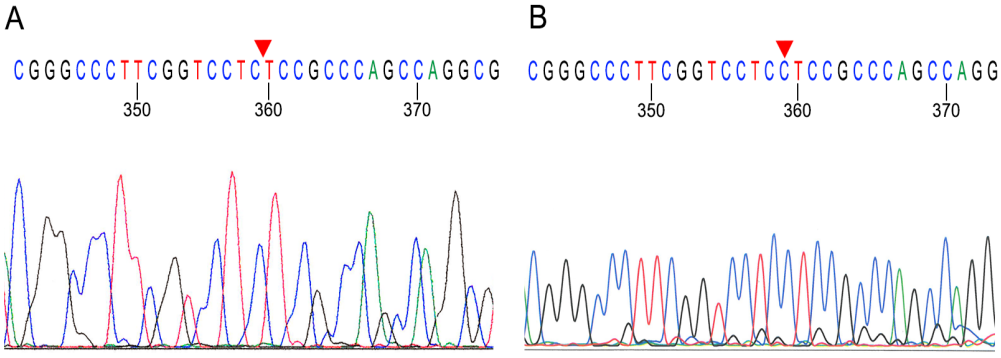

Figure 1. Partial sequencing chromatograms of Wilms tumor W2 show a base pair deletion 359delC in exon 1 (A). W7 does not show this deletion (B). Red triangles indicate the positions of mutations.

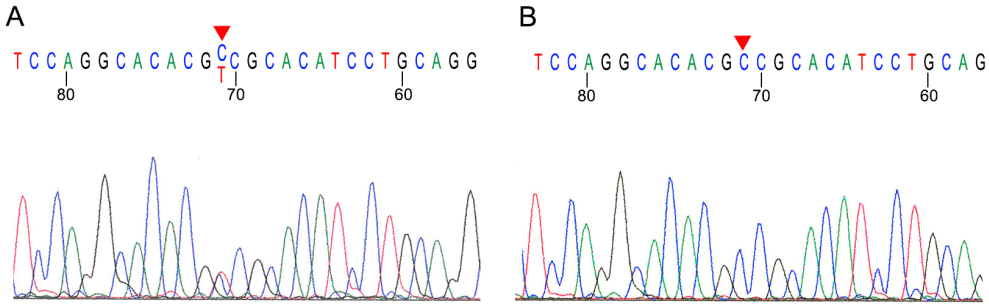

Figure 2. Partial sequencing chromatograms of Wilms tumor W3 show a heterozygous 71A > G point mutation in exon 7 (A). W2 shows a homozygous 71A > G point mutation (B) in reverse direction. Red triangles indicate the positions of mutations.

Table 2. Nucleotide alterations in the intron regions of WT1 gene.

Fragment	Nucleotide alteration	Intron	GenBank
1	45insC	1	M80218
2	44C > G, 67delA	2	M80219
3	54insT; 71insT	3	M80220
4	14insC	4	M80221
5	156insG, 164insC	6	M80228
6	25delT, 220delC, 225insC, 265insT	6, 7	M80229
7	43insA[a], 57delC[b]	7	M80230
8	149delG	8	M80231

Most alterations were observed in all samples. [a]43insA was observed only in W9 and W11. [b]57delC was observed only in W1 and W3.

fected. These alterations may be attributed to ethnic effects as they were also observed in the DNA from a healthy individual (data not shown).

4. DISCUSSION

According to statistics compiled by the Childhood Cancer Foundation, about 10 new cases of Wilms tumor are reported in Taiwan every year. This accounts for 3.4% of total childhood malignancies (www.ccfroc.org.tw). Breslow *et al*. have shown that 1% - 3% of cases of Wilms tumor are familial and that a predisposition to Wilms tumor is most likely caused by rare germline mutations acting in a dominant fashion [13]. Constitutional mutations in the WT1 gene on chromosome 11p13 predispose an individual to Wilms tumor and are associated with genitourinary abnormalities [4]. Several genetic syndromes (e.g., Denys-Drash syndrome) have been correlated with intragenic WT1 mutations [12]. In contrast to other WT1 mutations, these mutations are dominant and mainly found in the zinc finger region encoded by exon 7 through exon 10 of WT1 [5].

None of the patients in this study were diagnosed with Denys-Drash syndrome or Beckwith-Wiedemann syndrome. In addition, none presented with familial Wilms tumor. Evidence of WT1 inactivating mutations was apparent in only one of the 11 cases on mutational analysis, suggesting that direct alterations of the WT1 gene are present in only a very small fraction of Wilms tumor patients in Taiwan. This result is consistent with the findings of a previous study [14]. Less than 10 percent of sporadic Wilms tumors are associated with WT1 mutations. Thus, mutations at other Wilms tumor loci may play an important role in Wilms tumor development.

The WT1 gene is located at chromosome 11p13 [6,7]. It was discovered as a part of the WAGR syndrome (Wilms tumor, Aniridia, Genitourinary anomaly, and mental Retardation). Those who present with this syn-

drome have germline deletion on chromosome 11p13 [5]. This gene has 10 exons and encodes a protein called WT1 which has tumor suppression function [15]. High expression of this protein on developing kidney results in suppression of growth-related gene [16]. Tumor suppression gene is usually involved in a two-hit mechanism [17]. In our study, there was only one case with one mutant allele from blood sample. This may be explained by WT1 product acting as dimeric or multimeric protein [18]. When one allele produces an abnormal product, it presents as negative dominant event with loss of function. When there is mutation on only one allele, there are normal polypeptides with preserved function. The phenotype of Denys-Drash syndrome involves mutations in the zinc finger domain [19], so does that of Frasier syndrome [20], neither of which was observed in this study.

In conclusion, our data showed a low frequency of constitutional and somatic WT1 mutations in children with nonsyndromatic Wilms tumor. This is the first study to detect WT1 mutation in children with Wilms tumor in Taiwan.

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