Published in final edited form as:

World J Pediatr. 2018 December ; 14(6): 585–593. doi:10.1007/s12519-018-0181-3.

Molecular and epidemiologic characterization of Wilms tumor from Baghdad, Iraq

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Abstract

Background: Wilms tumor (WT) is the most common childhood kidney cancer worldwide, yet its incidence and clinical behavior vary according to race and access to adequate healthcare resources. In order to guide and streamline therapy in the war-torn and resource-constrained city of

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Author Contributions: HMP wrote the manuscript, interpreted the data, and provided administrative and technical support. MFA designed the study, acquired data, provided administrative and technical support, and supervised the study. NMC wrote the manuscript, interpreted the data, and provided administrative and technical support. JMP developed the study methodology, acquired data, analyzed and interpreted the data, and provided administrative and technical support. BL analyzed and interpreted the data. QW analyzed and interpreted the data. RRF acquired data. HC acquired data. SU acquired data and provided administrative and technical support. HF designed the study. ARA acquired data and provided administrative and technical support. SAFA acquired data and provided administrative and technical support. AFA acquired data and provided administrative and technical support. RMA acquired data and provided administrative and technical support. SAA acquired data and provided administrative and technical support. HNL designed the study, developed the methodology, analyzed and interpreted the data, wrote the manuscript, and supervised the study. All authors approved the final version of the manuscript.

Conflict of Interest: The authors have no conflict of interests to disclose. No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this article.

Ethical Approval: Approval to conduct these studies was provided through the Vanderbilt Institutional Review Board (#100734 and #020888).

Baghdad, Iraq, we conducted a first-ever molecular analysis of 20 WT specimens to characterize the biological features of this lethal disease within this challenged population.

Methods: Next Generation Sequencing of 10 target genes associated with WT development and treatment resistance (*WT1*, *CTNNB1*, *WTX*, *IGF2*, *CITED1*, *SIX2*, *p53*, *NMYC*, *CRABP2*, and *TOP2A*) was completed. Immunohistochemistry was performed for 6 marker proteins of WT (WT1, CTNNB1, NCAM, CITED1, SIX2, and p53). Patient outcomes were compiled.

Results: Mutations were detected in previously described WT “hot spots” (e.g., WT1 and CTNNB1) as well as novel loci that may be unique to the Iraqi population. Immunohistochemistry showed expression domains most typical of blastemal-predominant WT. Remarkably, despite the challenges facing families and care providers, only one child, with combined *WT1* and *CTNNB1* mutations, was confirmed dead from disease. Median clinical follow-up was 40.5 months (range, 6 to 78 months).

Conclusions: These data suggest that WT biology within a population of Iraqi children manifests features both similar to and unique from disease variants in other regions of the world. These observations will help to risk stratify WT patients living in this difficult environment to more or less intensive therapies and to focus treatment on cell-specific targets.

Keywords

Iraq; low- and middle-income countries; next generation sequencing; pediatric cancer; Wilms tumor

Introduction

Wilms tumor (WT) is the most common childhood kidney cancer worldwide, and its incidence varies with race (1, 2). Globally, black children of sub-Saharan African ancestry consistently show the highest incidence of WT regardless of nationality, whereas children of Asian descent typically show the lowest rate. However, little has been reported on the incidence, biology or outcome from WT in the formerly suppressed and currently war-torn Middle Eastern population of Iraqi children. The only report to date describes in limited detail a 51% survival from WT for Iraqi children treated during the height of the second Persian Gulf War (3). Moreover, no report characterizing WT biology in this population has been published. Given the challenges of treating children for any cancer and of studying population-specific outcomes and disease characteristics in the midst of tyranny, war, and terrorism, this limited availability of data documenting WT behavior and biology in Iraq is understandable.

WT is a genetically heterogeneous disease that arises in the setting of specific and consistent “hot spot” alterations in predisposing genetic loci, which theoretically could vary with race and therefore drive the observed disparate incidences. Specifically, mutations in *WT1*, *CTNNB1*, *WTX* (i.e., *AMER1*), and *IGF2* have been shown to occur relatively commonly in WT and have been implicated as initial hits promoting the WT sequence (4–6). Perturbations in expression of specific gene products, such as CITED1, SIX2, and NCAM, have been reported to label self-renewing blastema, the putative WT cancer stem cell and malignant analogue of the multipotent nephron progenitor (7–11). Additionally, TOP2A and CRABP2

were detected abundantly in our recent proteomic analysis of 98 WT specimens from North America and Kenya, and each is a targetable moiety specific to WT biology (e.g., etoposide and cis-all trans retinoic acid, respectively) (12). Finally, two other genes, *p53* and *MYCN*, have been shown to mutate or gain copy number respectively in treatment-resistant anaplastic WT (13–15). These genes and protein products form the basis of this targeted molecular analysis to assess similarities and differences in biological characteristics between Iraqi WT and specimens of the Western world.

While developed countries have shifted therapeutic protocols to stratify patients according to biologic risk groups, little data have been published on WT outcomes or biology from the once suppressed and now war-ravaged Iraqi population. We designed these studies to characterize the biology and clinical behavior of WT arising in Iraqi children as an initial means to streamline delivery of care under currently volatile conditions.

Methods

To help steer risk assignment in future therapeutic algorithms in this politically unstable region of Baghdad, Iraq, we examined the biologic composition of WT treated there. We performed a Next Generation Sequencing (NGS) analysis targeting 10 genes associated with Wilms tumorigenesis and disease progression along with an immunohistochemical (IHC) evaluation of 6 proteins also relevant to WT biology, as introduced above. Approval to conduct these studies was provided through the Vanderbilt University Institutional Review Board (#100734 and #020888).

WT cohort from Baghdad, Iraq

All kidney tumor patients ($n=154$) who were treated at the Children's Welfare Teaching Hospital (CWTH) in Baghdad, Iraq between June 2008 and March 2014 were selected for potential molecular evaluation. Multiple formalin-fixed paraffin embedded (FFPE) tissue blocks archived from 35 patients could be located, and all were shipped from CWTH to the Vanderbilt Children's Hospital (VCH) in Nashville, TN for analysis. The remaining bulk of patient specimens either had been retained by the families or had been shipped previously to other international collaborators for protective storage given governmental instability. No other storage conditions, such as liquid nitrogen, are available currently to archive fresh tissue specimens in this low-income setting.

After sectioning and hematoxylin and eosin (H&E) staining, a thorough pathologic review was completed at VCH to verify histologic diagnosis and to assure quality of tissue preservation. Detailed review of sections from all shipped tissue blocks ($n=35$ patients) yielded the final 20 WT specimens and 4 adjacent kidneys from which a WT arose and that were of sufficient quality for molecular analysis. Excluded blocks from the remaining 15 patients were determined either to carry an alternative diagnosis (e.g., papillary renal cell carcinoma or congenital mesoblastic nephroma) or the tissue was completely necrotic. Demographic variables (e.g., age and stage at diagnosis, gender, time to last follow up, relapse, and vital status) of these 20 patients were compiled.

Immunohistochemical analysis

To evaluate expression of proteins that are common to WT and appear fundamental to its biology, the 20 Iraqi WT specimens were examined using immunohistochemistry (IHC) for WT1, CTNNB1, NCAM, CITED1, SIX2, and p53. Briefly, and in accordance with our previously published IHC protocol, 5 µm tissue sections were subjected to antigen retrieval and then probed for each protein at the following dilutions: WT1 – 1:1000 (Santa Cruz Biotechnology, Dallas, TX), CTNNB1 – 1:100 (Cell Signaling, Danvers, MA), NCAM – 1:100 (Cell Signaling), CITED1 – 1:50 (Lab Vision, Fremont, CA), SIX2 – 1:20 (US Biological, Salem, MA), and p53 – 1:100 (Cell Signaling). Proteins were visualized using the Dako EnVision HRP+ System (Agilent Technologies, Carpinteria, CA) (7, 9, 16). A pediatric-trained pathologist (RRF) reviewed in a blinded manner all 120 immunostained WT slides to evaluate the expression domain between cellular elements (e.g., blastema and epithelia) and subcellular compartments (e.g., cytosol and nucleus). The presence or absence of protein expression was tabulated.

Next Generation Sequence analysis

To explore the biology of Iraqi WT further, genomic DNA was isolated from the 20 WT and 4 adjacent kidney specimens using QIAamp DNA FFPE Tissue kit according to the manufacturer's protocol (Qiagen, Valencia, CA). Briefly, 4 paraffin sections at 10 µm each were acquired from the highest quality tissue block of each patient (Vanderbilt Translational Pathology Shared Resource). After removal of wax in xylene, tissue sections were digested, and genomic DNA was isolated and purified. To evaluate the presence of mutations in the 10 target genes documented previously to promote Wilms tumorigenesis (*WT1*, *CTNNB1*, *AMER1* [i.e., *WTX*], and *IGF2*) or to facilitate treatment resistance and disease maintenance (p53, MYC-N, *CITED1*, *SIX2*, *CRABP2*, and *TOP2A*), genomic DNA was analyzed using Next Generation Sequencing (NGS) technology for single nucleotide variations, insertions, and deletions in these targeted loci. Briefly, multiplex amplicon sequencing libraries were prepared using an amplicon gene primer panel that targeted only coding regions of these 10 genes. Input DNA was quantified using the high sensitivity dsDNA assay on the Qubit fluorometer and normalized to 4 ng/µL. The multiplex PCR was performed in eight reactions per sample using a custom Qiagen GeneRead DNA-seq Panel following manufacturer's protocol without deviation (Qiagen).

Data Quality Control and Analysis

Variant calling was performed using the standard Genome Analysis Toolkit Haplotype Caller pipeline (GATK version 3.1–1, <http://www.broadinstitute.org/gatk/>) (17). Single nucleotide variant (SNV) mutation calls were made using the following threshold filters: 1) each had to pass GATK Variant Quality Score Recalibration filtering, 2) DP was greater than 10, 3) Genotype Quality was greater than 30, 4) SNV was not observed in the adjacent kidneys, and 5) the allele frequency in the 1000 Genomes Project was greater than 0.2%. For small insertions and deletions (Indels), we further manually inspected the alignment and removed calls close to the end of aligned reads. Additionally, we used MuTect software to call SNV mutations further to detect low allele fraction (AF) mutations (18). Given the low number of available adjacent kidney specimens that could be located, we combined these

four as a single sample and ran MuTect of individual tumors against this combined sample. We selected mutation calls with AF greater than 0.1 for further analysis.

Mutation calls are reported for those SNVs occurring only in the WT specimens, and were annotated using ANNOVAR (version 2014jul14) (19). Only non-synonymous SNV mutations with predicted deleteriousness in one of the algorithms implemented in ANNOVAR, and Indel mutations in exonic and UTR regions, are called as potentially functionally significant. This strict approach to mutation calls yielded the greatest possible confidence.

Results

WT Cohort

The median follow-up period for this WT cohort was 40.5 months (range: 6 to 78 months), and the frequency of unfavorable histology (UH) was typical for this childhood renal malignancy at 5%. Of the 20 WT patients included in this molecular analysis, only one child is confirmed to have died of progressive disease (Table I). That FHWT specimen interestingly contained a mutation in both *WT1* and *CTNNB1* (Table II). Initial resection of the primary WT was accomplished for 19 of the 20 patients before administration of chemotherapy. Remarkably, only two patients in this cohort were lost to follow-up (LTFU), one with no evidence of disease and having completed all therapy (presumed alive), and the other who was receiving treatment for local relapse in the nephrectomy bed and subsequently abandoned care (presumed dead). Advanced stage disease at presentation was prevalent in these 20 Iraqi children, as 9 presented with stage III and 5 with stage IV disease. Relapse rate was typical at 20%.

Immunohistochemistry

The immunohistochemical analysis, probing for expression of 6 proteins common to WT biology, revealed an expected pattern of detection between cellular elements and subcellular localization when compared with our prior studies (Figure 1; Table I) (7–9, 11, 16). In summary, this cohort of 20 WT specimens showed significant expression of 5 markers that label proliferating blastema, the putative WT stem cell. As expected, β -catenin was also detected in the nucleus of a majority of specimens harboring a mutation in this gene (Tables I and II). Furthermore, p53 was detected diffusely in the single UHWT specimen, which interestingly harbored a dual mutation in exon 4 of this marker of WT treatment resistance (16).

WT-specific gene mutations

The NGS analysis, targeting 10 WT-associated genes that contribute to its tumorigenesis, self-perpetuation, and disease progression, detected a number of both expected and novel mutations that appear to be functionally relevant (Table II). Specific to the four genes commonly analyzed for alterations in WT, *WT1* was mutated in 4 specimens (20%; typical frequency reported in other populations). *CTNNB1* was mutated in 7 WT specimens (35%; higher frequency than reported in other populations) and in 3 of the 4 specimens harboring a *WT1* mutation, which is a typical association for these two genes in other series analyzing

WT genetic alterations. *AMER1* (i.e., *WTX*) was mutated in 3 WT specimens (15%; typical frequency). Finally, in the one UHWT, p53 was mutated twice in exon 4 (Table III). Of the remaining 6 genes that are of interest to different aspects of WT biology, 4 (*SIX2*, *IGF2*, *TOP2A*, and *CITED1*) were mutated in a single WT specimen each included in this cohort (frequency of 5% each). Neither *MYC-N* nor *CRABP2* was found to have a mutation in this analysis.

Discussion

This report is the first to characterize the demographics and biology of a recent WT cohort from the war-torn and resource-constrained city of Baghdad, Iraq. The principal findings from this work suggest that Iraqi children having WT present largely with advanced-stage disease yet within the typical age spectrum, raising concern for a potentially more aggressive biology. Next Generation Sequencing (NGS) was used to investigate for a population-specific biology. A largely expected pattern of alterations in 10 WT-associated genes was observed using targeted techniques, while several unique mutations were revealed as well. Although the follow up period of slightly more than 3 years is relatively short for a WT cohort, survival was remarkable. This survival rate approaches that documented in the Western world and improves upon that reported from the CWTH in Baghdad during the 2005 to 2009 era, which was alarmingly only 51% (3). Once validated in other Iraqi WT specimens that are being collected prospectively, the results of this NGS analysis may one day guide future therapies to more or less intensive regimens according to patient- and tumor-specific biological criteria.

WT biology recapitulates the growth properties of the developing kidney, but shows an imbalance towards progenitor maintenance and symmetric cell division. The Iraqi WTs analyzed in this study commonly showed cellular predominance of blastema, its putative cancer stem cell and the malignant analogue of the multipotent nephron progenitor in the embryonic metanephric mesenchyme (MM) and an ominous feature of aggressive behavior (25–27). Indeed, these Iraqi WT specimens showed robust activation of 5 markers of WT blastema and MM, which likely accounts for the more commonly advanced stage of disease at presentation. Using strict filters for true mutations in the 10 genes analyzed, *CTNNB1* showed tumor-specific alterations in 35% of these WT specimens, which is higher than the typical frequency of 15% in Western populations studied. This proto-oncogene was mutated in 3 of the 4 specimens also harboring a *WT1* alteration, which is an expected association (4, 28, 29).

The authors acknowledge several limitations with this pilot study that center around the challenges of conducting molecular research on specimens originating from patients being treated in low-income countries. First, the patient and specimen numbers are low due to the significant constraints on resources available to Iraqi physicians to document patient outcomes and to archive WT specimens during the previous suppressive regime and recent war. Incorrect diagnoses and tissue necrosis further contributed to the need to exclude specimens from the final analysis. Further, tumor specimen availability may indicate that a patient was more thoroughly integrated into the Iraqi healthcare system, which might introduce a selection bias to this analysis. Greater adherence and/or follow-up among

patients with available tumor specimens could help to explain the excellent survival rates observed in this cohort. Second, loss of heterozygosity (LOH) at 1p and 16q and copy number gain at 1q have been determined as poor prognostic indicators in multiple series from the Western world and are used to risk stratify FHWT patients who have had initial resection of the primary lesion (i.e., have not received neoadjuvant therapy), but these important biological factors could not be assessed rigorously in these current studies given the rare occurrence of these chromosomal aberrations (~5% of WT). To address these study limitations and to validate the current findings, plans are underway to optimize patient follow up and to expand the molecular analysis to additional Iraqi patients having tissues archived at international institutions or with parents and those being treated prospectively from the time of this study.

In conclusion, this seminal study provides an initial glimpse into the biology of WT arising in Baghdad, Iraq that shares biological features with specimens treated in other regions of the world, yet also carries potentially unique, population-specific characteristics. Although current conditions in Iraq do not support high throughput genomic screening to prognosticate WT outcome and to personalize therapy, simple immunohistochemical staining for p53 accumulation, for example, may be a useful starting point to risk-stratify potentially treatment resistant tumors. Nevertheless, efforts to help patients overcome the barriers to health care in a war zone and to personalize therapy according to specific biological factors will one day improve outcomes for these patients living in a significantly challenged environment.

Acknowledgments:

The authors would like to thank Travis Clark and Holli Dicks for their technical assistance to conduct the Next Generation Sequencing analysis of these specimens.

Funding Details: This work was supported in part by the Carolyn Perot Rathjen Chair in Pediatrics at Vanderbilt (HF), the NIH/NCRR (Grant number G20 RR030956 - VANTAGE Shared Resource at Vanderbilt), and the Vanderbilt Ingram Cancer Center Support Grant number 5P30 CA068485 (Translational Pathology Shared Resource).

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Fig.1.

Representative photomicrographs showing the expression domain, both between cellular elements (i.e., blastema and epithelia) and subcellular compartments, of 6 markers of WT biology in this Iraqi cohort. (A) Low and (B) high power images show β -catenin detection between cellular elements (blastema and epithelia positive; stroma negative) and subcellular compartments. This tumor (Iraq WT-13) showed robust nuclear localization consistent with its activating mutation in exon 3 (Table I; bars, 200 and 50 μ m, respectively). (C) CITED1 is visualized most robustly in the nucleus of blastema and less intensely in epithelia, as also

shown in this IraqWT-13 specimen (bar, 200 μ m). (D) NCAM is further detected almost exclusively in blastema of WT, as further shown in IraqWT-13. Epithelia are typically and stroma exclusively negative for NCAM detection (bar, 200 μ m). (E) WT1 and (F) SIX2 are visualized in a typical pattern labelling predominantly blastema and rare epithelia in IraqWT-9 (bar, 50 μ m). (G, H) These photomicrographs show wide spread nuclear accumulation of p53 in IraqWT-21 cells, which is a diffusely anaplastic specimen and carries a dual mutation in exon 4 of this gene. Arrowheads show the pathognomonic cellular features of anaplasia:

Table 1

Demographic and disease characteristics of patients with Wilms tumor

IraqWT#	Age at diagnosis, mon	Gender	Histology	Stage	Adjuvant therapy	Cellular Predominance	β-catenin	WT1	p53	CITED1	SIX2	NCAM
1	69	M	FH	IV	C-D-E-C	Blastema	B-cy; E-cy	B-nu		B-cy, nu; E-cy, nu	B-nu	B-cy; E-cy
2	11	F	FH	II	V-A-D	Blastema	B-cy; E-cy	B-nu; E-nu		B-cy, nu; E-cy, nu	B-nu; E-cy	B-cy; E-cy
3	42	M	FH	IV	C-D-E-C	Triphasic	B-cy; E-cy	B-nu	Focal: B-nu; E-nu	B-cy, nu; E-cy, nu	B-nu; E-cy	B-cy; E-cy
4	49	F	FH	III	V-A-D	Triphasic	B-cy; E-cy	B-nu; E-nu		B-cy, nu; E-cy, nu	B-nu; E-nu	B-cy; E-cy
5	13	F	FH	III	V-A	Triphasic	B-cy, nu; E-cy	B-nu; E-nu		B-cy, nu; E-cy, nu	B-cy, nu; E-cy, nu	B-cy; E-cy
6	51	F	FH	III	V-A-D	Triphasic	B-cy, nu; E-cy	B-nu; E-nu	B-nu	B-cy, nu; E-cy, nu	B-cy, nu; E-cy, nu	B-cy; E-cy
7	39	M	FH	Relapse	C-D-E-C	Blastema	B-cy; E-cy	B-nu; E-nu		B-cy, nu; E-cy, nu	B-nu; E-nu	B-cy; E-cy
8	13	M	FH	III	V-A	Epithelial	B-cy; E-cy	E-nu		B-cy, nu; E-cy, nu	B-nu; E-nu	B-cy; E-cy
9	14	F	FH	III	V-A-D	Epithelial	B-cy; E-cy	E-nu		B-cy, nu; E-cy, nu	B-cy, nu; E-cy, nu	B-cy; E-cy
10	15	M	FH	II	V-A-D	Triphasic	B-cy; E-cy	B-nu; E-nu		B-cy, nu; E-cy, nu	B-cy, nu; E-cy, nu	B-cy; E-cy
11	9	M	FH	III-Relapse	V-A : C-D-E-C	Triphasic	B-cy, nu; E-cy		B-nu	B-cy, nu; E-cy, nu	B-nu; E-cy, nu	B-cy; E-cy
12	61	F	FH	III	V-A-D	Blastema	B-cy; E-cy	B-nu; E-nu		B-cy, nu; E-cy, nu	B-nu; E-nu	B-cy; E-cy
13	64	M	FH	IV-Relapse	C-D-E-C	Blastema	B-cy; E-cy			B-nu	B-cy, nu	B-cy
14	89	F	FH	III	V-A-D	Triphasic	B-cy; E-cy	B-nu		B-cy, nu; E-cy, nu	B-cy; E-cy, nu	B-cy; E-cy
15	28	F	FH	Relapse	C-D-E-C	Triphasic	B-cy; E-cy	B-nu; E-nu		B-cy, nu; E-cy, nu	B-cy; E-cy	B-cy, nu; E-cy, nu
16	61	F	FH	IV	C-D-E-C	Triphasic	B-cy, nu; E-cy	B-nu; E-nu		B-cy, nu; E-cy, nu	B-cy, nu; E-cy, nu	B-cy; E-cy
17	79	F	UH	IV	C-D-E-C	Blastema	B-cy; E-cy	B-nu; E-nu	B-nu; E-nu	B-cy, nu; E-cy, nu	B-cy; E-cy	B-cy; E-cy

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IraqWT#	Age at diagnosis, mon	Gender	Histology	Stage	Adjuvant therapy	Cellular Predominance	β-catenin	WT1	p53	CITED1	SIX2	NCAM
22	14	F	FH	III	V-A-D	Blastema	B-cy; E-cy	B-nu; E-nu		B-cy, nu; E-cy, nu	B-nu; E-nu	B-cy; E-cy
23	23	M	FH	II	V-A-D	Triphasic	B-cy, nu; E-cy	B-nu; E-nu		B-cy, nu; E-cy, nu	B-cy, nu; E-cy, nu	B-cy; E-cy
24	8	M	FH	II	V-A	Triphasic	B-cy; E-cy	B-nu; E-nu		B-cy, nu; E-cy, nu	B-cy, nu; E-cy	B-cy; E-cy

U unfavorable histology, *UH* unfavorable histology, *V* vincristine, *A* actinomycin-D, *D* doxorubicin, *C* cyclophosphamide, *E* etoposide, *C* carboplatin, *B* blastema, *E* epithelia, *cy* cytosol, *nu* nucleus
Patient is deceased from disease progression

Table 2

Genetic alterations in this Iraqi Wilms tumor cohort

Chromosome	REF	ALT	Function	Exonic function	Gene	Amino acid change	Tumor allelic fraction	CHROM:position:tumor
Single-nucleotide mutation								
2	C	A	exonic	nonsynonymous	<i>SIX2</i>	NM_016932:exon1:c.G389T;p.C130F	0.285143	2:45235861:IraqWT-19
3	A	G	exonic	nonsynonymous	<i>CTNNB1</i>	NM_001098209:exon3:c.A121G;p.T41A [#]	0.4060150	3:41266124:IraqWT-23
3	C	G	exonic	nonsynonymous	<i>CTNNB1</i>	NM_001098209:exon3:c.C134G;p.S45C [#]	0.514989	3:41266137:IraqWT-13 [*]
3	C	T	exonic	nonsynonymous	<i>CTNNB1</i>	NM_001098209:exon3:c.C155T;p.P52L	0.16	3:41266158:IraqWT-12
3	T	C	exonic	nonsynonymous	<i>CTNNB1</i>	NM_001098209:exon4:c.T331C;p.S111P	0.52459	3:41266534:IraqWT-10
3	T	C	exonic	nonsynonymous	<i>CTNNB1</i>	NM_001098209:exon7:c.T980C;p.I327T	0.642857	3:41268742:IraqWT-7
3	A	T	exonic	nonsynonymous	<i>CTNNB1</i>	NM_001098209:exon7:c.A1004T;p.K335I	0.2461538	3:41268766:IraqWT-7
3	T	G	exonic	nonsynonymous	<i>CTNNB1</i>	NM_001098209:exon8:c.T1147G;p.W383G [#]	0.147577	3:41274897:IraqWT-20
3	G	A	exonic	nonsynonymous	<i>CTNNB1</i>	NM_001098209:exon9:c.G1432A;p.A478T	0.6	3:41275266:IraqWT-19
11	C	T	exonic	nonsynonymous	<i>WT1</i>	NM_000378:exon1:c.G557A;p.S186N	0.13889	11:32456335:IraqWT-12
11	G	A	exonic	nonsynonymous	<i>WT1</i>	NM_000378:exon1:c.C398T;p.P133L	1	11:32456494:IraqWT-24
11	G	A	exonic	stopgain	<i>WT1</i>	NM_000378:exon7:c.C1237T;p.R413X	0.974592	11:32414263:IraqWT-23
11	G	A	exonic	nonsynonymous	<i>IGF2</i>	NM_001042376:exon3:c.C389T;p.A130V	0.5026178	11:2170374:IraqWT-6
17	C	T	exonic	nonsynonymous	<i>TOP2A</i>	NM_001067:exon26:c.G3289A;p.V1097I	0.375	17:38555189:IraqWT-11
17	C	T	exonic	nonsynonymous	<i>TP53</i>	NM_001126115:exon4:c.G422A;p.R141H	0.11609	17:7577120:IraqWT-21
17	G	A	exonic	nonsynonymous	<i>TP53</i>	NM_001126115:exon4:c.C448T;p.R150W	0.804	17:7577094:IraqWT-21
X	A	C	exonic	nonsynonymous	<i>AMER1</i>	NM_152424:exon2:c.T477G;p.F159L [#]	0.360465	X:63412690:IraqWT-11
X	A	C	exonic	nonsynonymous	<i>AMER1</i>	NM_152424:exon2:c.T477G;p.F159L [#]	0.896364	X:63412690:IraqWT-5
X	T	C	exonic	nonsynonymous	<i>AMER1</i>	NM_152424:exon2:c.A3331G;p.K1111E	0.531842	X:63409836:IraqWT-15
X	G	A	exonic	nonsynonymous	<i>CITED1</i>	NM_001144886:exon3:c.C449T;p.S150L	0.105263	X:71521706:IraqWT-2
X	G	A	exonic	nonsynonymous	<i>CITED1</i>	NM_001144885:exon3:c.C32T;p.A111V	0.111111	X:71522766:IraqWT-2
Chromosome	REF	ALT	Function	Exonic function	Gene	Amino acid change	Tumor allelic fraction	Tumor Sample
Insertion/deletion								
11	A	AC	exonic	frameshift	<i>WT1</i>	NM_000378:exon8:c.1316dupG;p.C439fs (5)	1.00	IraqWT-13 [*]
X	A	AG	UTR3	N/A	<i>AMER1</i>	N/A	0.111111; 0.1961722	IraqWT-11; IraqWT-5

Previously reported mutations in Wilms tumor.

* Patient is deceased from disease progression.

N/A not available

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Table 3

Mutation rate for 10 target Wilms tumor (WT)-associated genes

Gene	Mutation rate % (No.)	Reported frequency*	Notes	References
<i>WT1</i>	20% (4)	0.15 (0.11, 0.19)		[20]
<i>CTNNB1</i>	35% (7)	0.14 (0.10, 0.19)	<i>CTNNB1</i> mutated in 3 of 4 specimens with <i>WT1</i> mutation	[20]
<i>AMER1 (WTX)</i>	15% (3)	0.15 (0.11, 0.19)		[20]
p53	5% (1)	0.41 (0.21, 0.60) [‡]	P53 mutation detected in the single UHWT, consistent with previous reports indicating p53 mutation exists in 50 – 86% of anaplastic WT [21]	[20]
<i>SIX2</i>	5% (1)	0.01 – 0.03		[22, 23]
<i>IGF2</i>	5% (1)	0.07		[24]
<i>TOP2A</i>	5% (1)	0.09		[24]
<i>CITED1</i>	5% (1)	Not available		
<i>MYC-N</i>	0	0.07 (0.04, 0.10)		[20]
<i>CRABP2</i>	0	Not available		

* Frequency provided by meta-analysis when available, reported as effect size (95% confidence interval) [20]. For genes not included in the meta-analysis, range of frequencies reported in the literature is provided.

[‡] Note that the majority of the studies reported here analyzed UHWT only. Overall incidence of p53 mutation in WT is ~5% [24]