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Expression profiling of *TP53, BLM, DIS3L2, GPC3, NSD1, PAX6* and *AMER1* genes in Wilms' tumor cases

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Wilms' tumor has been linked to a number of genes, but WT1 has been reported to be directly linked to the growth of this embryonic tumor. Due to mutations in additional genes occurring in conjunction with WT1, Wilms' tumor is linked to a variety of disorders that manifest as syndromic conditions, e.g., Li-Fraumeni syndrome, Bloom syndrome, Perlman syndrome, Simpson-Golabi-Behemel syndrome, Sotos syndrome, WAGR syndrome and X-chromosome syndrome, which are linked to oncogenes (OGs) and tumor suppressor genes (TGs), i.e., *TP53, BLM, DIS3L2, GPC3, NSD1, PAX6*, and *AMER1*, respectively. The study demonstrated the mRNA expression levels of the *TP53, BLM, DIS3L2, GPC3, NSD1, PAX6* and *AMER1* genes by code-set chemistry in 24 Wilms' tumor cases in comparison to their internal controls (adjacent to tumor tissues). Capture-and-reporter probe-based expression was carried out using NanoString technology. All the genes of interest were found to be significantly up- and downregulated according to the fold change expression study results. The mRNA expression of TP53 in 95.84%, BLM in 83.34%, DIS32 in 62.50%, NSD1 in 62.50%, AMER1 in 58.33%, GPC3 and PAX6 in 50% of Wilms' tumor cases were significantly upregulated. Hence, this study established that the *NSD1, DIS3L2, AMER1, TP53, BLM, PAX6*, and *GPC3* genes play roles in the development of these embryonic tumors and can be used as biological markers for Wilms' tumors. However, a larger sample size is needed to validate the above data.

Keywords: Wilms' tumor (WT), Li-Fraumeni syndrome, Bloom syndrome, Perlman syndrome, Simpson-Golabi-Behemel syndrome, Sotos syndrome, WAGR syndrome

Wilms' tumor or nephroblastoma is a common malignant kidney tumor in 3 to 4year-old children and is rare in adults. Wilms' tumor induces genetic changes in oncogenes (OGs) and tumor suppressor genes (TGs). Many OG & TG grafts have been associated with Wilms' tumor, but only the role of WT1 in the growth of this embryonic tumor has been reported[1,2]. Wilms' tumor is linked to a variety of disorders that contribute to a syndromic condition, i.e., Li-Fraumeni syndrome, Bloom syndrome, Perlman syndrome, Simpson-Golabi-Behemel syndrome, Sotos syndrome, WAGR syndrome and X-Chromosome syndrome are associated with OGs and TGs, i.e., *TP53, BLM, DIS3L2, GPC3, NSD1, PAX6*, and *AMER1*, respectively which need more extensive study[3,4].

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Studies have reported that 9-17% of Wilms' tumors are associated with a predisposing factor. These mutations are linked to a variety of disorders resulting in the development of a syndromic disorder caused by mutations in additional WT1-related genes, such as *WT2* and *PAX6*. These disorders lead to the progression of WAGR syndrome, Simpson-Golabi-Behemel syndrome, Sotos syndrome, Perlman syndrome, Li-Fraumeni syndrome, Denys-Drash syndrome, Bloom syndrome and Beckwith-Wiedemann syndrome etc[1,5]. At least 100 syndromes have been linked to WT, and the number of such syndromes is increasing[6]. Scott *et al*. classified the risk of various conditions. The syndromes associated with a high risk of developing Wilms' tumor (more than 20%) are Fanconi anemia/biallelic BRCA2, DDS, familial Wilms syndrome, Perlman syndrome, mosaic variegated aneuploidy and WAGR. The risk factors for developing Wilms tumor in 5-20% of patients were Frasier syndrome, BWS, and Simpson-Golabi-Behmel

syndrome. Similarly, Li-Fraumeni syndrome, Bloom syndrome, trisomy 18, hemi-hypertrophy, hereditary hyper-parathyroidism-jaw tumor syndrome, Mulbrey-Nanism, and 2q37 microdeletion syndrome are associated with a low risk of developing Wilms' tumor (less than 5% of patients)[5].

There is no clear characterization of the mRNA expression of genes linked to these syndromes, which could be useful genes for identifying Wilms' tumor cases. This report demonstrated the differential expression of these 7 genes and their association with this syndrome in Wilms' tumor patients, i.e., *TP53* (Li–Fraumen syndrome), *DIS3L2* (Perlman syndrome), *BLM* (Braom syndrome), *NSD1* (Sotos syndrome), *GPC3* (Simpson–Golabi–Behemel syndrome), *PAX6* (WAGR syndrome) and *AMER1* (WT in X chromosome), compared to its internal control (adjacent to tumor tissues).

Materials and Methods

Sample collection

The study was carried out at the Molecular Biology Unit, Center for Advanced Research, in collaboration with the Department of Pediatrics, King George's Medical University, Lucknow, with ethical clearance from the Institutional Ethics Committee of King George's Medical University, Lucknow, India. Before undergoing any study-related procedures, informed written consent was obtained from the parents of every donor in accordance with the regulations of CDSCO and DHR, ICMR, Government of India, to participate in this study.

Using the parameters of 95% confidence, 0.1 precision, and a 7% expected incidence of Wilms' tumor cases, the sample size was determined to be n=24[7,8]. Twenty-four clinically and histo-pathologically confirmed Wilms' tumor (WT1 to WT17, WT21, and WT23 to WT28) and adjacent tumor tissue samples (WC1 to WC17, WC21, and WC23 to WC28) were collected immediately after surgery, frozen in liquid nitrogen and placed at 80°C for further use. Tumor tissue dissection was performed following standard protocol by the Department of Pediatric Surgery at King George's Medical University. 50 to 100 mg of viable tumor tissue was derived from solid pale gray to light pink or yellowish gray soft tissue, which was further confirmed by histopathology. Similarly, normal tissue was derived from adjacent tumor tissue as a control for each sample.

mRNA expression assay by capture and reporter probe-based chemistry

RNA was extracted from 50 to 100 mg of Wilms' tumor tissue and its internal control samples using the guanidium thiocyanate technique in TRIzol reagent. (Invitrogen, Carlsbad, CA)[9] DNase was used to screen for and eliminate DNA contamination from extracted RNA (NEB, Ipswich, MA) (NEB, Ipswich, MA). A 1% denaturing gel was used to evaluate the integrity of the RNA. The quantity and quality of the RNA in each preparation were examined using a Quawell-Q5000, and RNA with an A260/A280 absorbance ratio of 1.8 was used for further investigation. Using a Qubit 3.0 fluorometer, 150 ng of RNA from each sample was normalized in preparation for hybridization with the NanoString nCounter codeset, which contains various gene sets. The nCounter probe (capture and reporter) CodeSets for 7 genes, i.e., *TP53, BLM, DIS3L2, GPC3, NSD1, PAX6*, and *AMER1*, for Li-Fraumeni-Syndrome, Bloom syndrome, Perlman syndrome, Simpson-Golabi-Behemel syndrome, Sotos syndrome, WAGR syndromem and X-Chromosome syndrome and four housekeeping genes, i.e., *ACAD9, ACTB, GAPDH*, and *MRPS5*, were designed for the NanoString nCounter gene expression assay (Table 1).

Using two distinct probes (capture and reporter), the NanoString nCounter gene expression experiment was carried out for 48 samples (24 patients and 24 controls) from a panel of 7 gene sets. A total of 15 µL of the hybridization reaction master mix was prepared for each sample (12 samples per cartridge) at room temperature using 3 µL of Reporter CodeSet, 5 µL of hybridization buffer, 150 ng of sample RNA in a 5 µL volume, and 2 µL of Capture ProbeSet. This mixture was then incubated at 65°C for 17 h. For each patient, 30-35 µL of the hybrid master mix, including the colourcoded barcode capture and reporter probes, was placed in a cartridge for magnetic bead-based washing and scanning of the target gene copy number on the nCounter SprintTM profiler using the default parameters for background subtraction, spike-in-control normalization, and reference gene normalization.

Statistical analysis

The nSolver NanoString data analysis program was used to process the raw data for each sample, with the default quality control settings for tumor samples and proportions for comparison to the corresponding *P* values. In the nCounter panel, transcript counts

Table 1 — Genes (associated with different syndrome of Wilms' tumor)

Gene	Accession	Position	Target Sequence	Tm CP	Tm RP
TP53	NM_000546.2	1331-1430	GGGGAGCAGGGCTCACTCCAGCCACCTGAAGTCCAAAAAG GGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGAC AGAAGGGCCTGACTCAGAC	81	79
BLM	NM_000057.2	2136-2235	TGCTTGGTGAAGACTGTTTTATCCTGATGCCGACTGGAGGT GGTAAGAGTTTGTGTTACCAGCTCCCTGCCTGTGTTTCTCCT GGGGTCACTGTTGTCAT	81	81
DIS3L2	NR_046477.1	841-940	GGTGTTAAGAAACTCTCAGTTTGTGTTTCTGAGAAAGGAAG AGAGGATGGTGATGCACCGGTTACAAAAGATGAGACCACC TGCATTTCACAAGACACAA	80	82
GPC3	NM_001164617.1	1407-1506	GAAGAAACCTTATCCAGCCGAAGAAGGGAACTAATTCAGA AGTTGAAGTCTTTCATCAGCTTCTATAGTGCTTTGCCTGGCT ACATCTGCAGCCATAGCC	83	81
NSD1	NM_022455.4	10656-10755	ACCTGCCTGGGAAAACAGCTTCTGAGCCATTTTGGGGAGC AGTTCTTCATCTGAATGGATGGACATCTGGGCTTCCTTCAA GGGCCATTGAATGGGAACT	81	82
PAX6	NM_000280.3	1174-1273	GAACATCCTTTACCCAAGAGCAAATTGAGGCCCTGGAGAA AGAGTTTGAGAGAACCCATTATCCAGATGTGTTTGCCCGAG AAAGACTAGCAGCCAAAAT	84	83
AMER1	NM_152424.3	5756-5855	GTGCCCAAATTCCAACTTTCTGGCCTCTTACCAAAGCTGCT CTGGGATGGTGGGGCTGAAATTGGCCTGGCTATTTGGCAG GAAGTAAACACTCACCAAA	79	80

were standardized to 04 housekeeping genes and 06 positive controls when compared to control samples. Using normalized expression data for genes that differed significantly ($P > 0.05$), a heatmap and scatter plot were generated for nSolver. The data were subsequently examined using Origin graphic software.

Results

The *TP53* (Li–Fraumen syndrome), *GPC3* (Simpson–Golabi–Behemel syndrome), *BLM* (Bloom syndrome), *DIS3L2* (Perlman syndrome), *NSD1* (Sotos syndrome), *PAX6* (WAGR syndrome), and *AMER1* (WT in X chromosome) genes were classified into four distinct groups, i.e., Cluster 1 (WT1, WT14, WT3, WT11 and WT28 cases); Cluster 2 (WT4, WT24, WT12, WT5, WT15, WT2, WT23, WT26, and WT25 cases); Cluster 3 (WT6, WT27, WT17, WT10, WT13 and WT7 cases); and Cluster 4 (WT16, WT21, WT8 and WT9 cases), based on the fold change in expression (Table 2 & Fig. 1-4). The number of mRNAs was 295.32, 281.96, 889.58, 4532.86, 885.64, 339.73 and 1623.38 for the *AMER1, BLM, DIS3L2, GPC3, NSD1, PAX6* and *TP53* genes, respectively (Fig. 4).

Expression profile of *TP53* genes (associated with Li-Fraumeni syndrome)

The expression profile of the *TP53* gene (associated with Li-Fraumeni syndrome) was upregulated in 95.84% of the patients in Clusters 1, 2 and 4 and downregulated in only 4.16% of the patients in Cluster 3 (Table 2 & Fig. 1-4).

Expression profile of *BLM* genes (associated with Bloom syndrome)

The expression profile of the *BLM* gene (Bloom syndrome) was upregulated in 83.34% of patients from Clusters 1, 2 and 4 (except samples WT5, WT 8, and WT 9) and downregulated in 16.66% of patients from Cluster 3. The BLM gene was upregulated in 15 Wilms' tumor samples, out of these highly upregulated in WT1 (9.39), WT2 (6.13), WT3 (10.43), WT4 (6.2), WT7 (15.34), WT14 (97.24), WT16 (3.63) and WT21 (6.06) where as in WT5, WT8 and WT9 BLM were downregulated by -1.11, -1.05, and -1.63-fold, respectively (Table 2 and Fig. 1-4).

Expression profile of *DIS3L2* genes (associated with Perlman syndrome)

The *DIS3L2* gene (Perlman syndrome) was upregulated in 62.50% of the patients in Clusters 1, 2 and 4 (except for samples WT26, WT 7, and WT 21) and downregulated in 37.50% of the patients in Cluster 3 (Table 2 and Fig. 1-4).

Expression profile of *GPC3* genes (associated with Simpson–Golabi–Behemel syndrome)

The expression profile of *GPC3* genes (Simpson-Golabi-Behemel syndrome) was upregulated in 50% of patients from Clusters 1 and 2 and downregulated

Table 2 — Differential expression of the Wilms' Tumor Syndrome related genes on the basis of fold change.

Samples	*TP53* Li-Fraumeni Syndrome	*BLM* Bloom Syndrome	*DIS3L2* Perlman Syndrome	*GPC3* Simpson-Golabi-Behemel syndrome	*NSD1* Sotos Syndrome	*PAX6* WAGR Syndrome	*AMER1* WT in X Chromosome
WT 1 *vs* Control	2.08	8.09	1.33	4.53	-1.15	-1.27	1.1
WT 2 *vs* Control	1.58	5.28	1.04	1.23	1.37	1.29	1.41
WT 3*vs* Control	1.59	8.98	1.11	1.9	-1.37	-1.3	1.08
WT 4 *vs* Control	2.47	5.34	1.21	3.01	1.47	1.36	-1.07
WT 5 *vs* Control	1.89	-1.29	1.5	2.81	1.5	2.16	1.03
WT 6 *vs* Control	1.11	1.26	-1.18	-1.95	-1.51	1.05	-1.77
WT 7 *vs* Control	2.19	13.21	-3.31	2.1	1.7	-2.57	1.01
WT 8 *vs* Control	1.61	-1.22	2.33	-32.13	1.36	610.75	1.79
WT 9 *vs* Control	1.54	-1.89	2.17	-35.65	1.06	584.3	1.79
WT 10 *vs* Control	1.14	1.13	-1.09	-1.6	-1.12	-2.94	-3.51
WT 11 *vs* Control	1.69	3.31	1.13	-1	-1.05	-3.32	-1.46
WT 12 *vs* Control	2.18	2.18	1.15	3.43	1.36	-2.59	1.6
WT 13 *vs* Control	-1.12	-1.04	-1.4	-6.06	-1.16	1.27	-1.92
WT 14 *vs* Control	1.87	6.24	1.24	1.73	1.04	-3.32	1.21
WT 15 *vs* Control	1.23	2.15	1.08	-1.19	1.49	-2.6	1.45
WT 16 *vs* Control	2.56	3.13	1.16	-1.69	-1.19	352.64	1.45
WT 17 *vs* Control	1.3	1.42	-1.32	-2.14	-1.15	-2.34	-1.81
WT 21 *vs* Control	3.01	5.22	-1.94	-58.89	1.1	784.44	1.03
WT 23 *vs* Control	1.67	9.61	1.09	2.06	1.44	-3.32	-7.88
WT 24 *vs* Control	2.21	4.83	1.32	3.16	1.85	4.25	-1.07
WT 25 *vs* Control	1.39	5.2	1.11	1.07	1.4	3.02	-3.55
WT 26 *vs* Control	1.56	5.24	-1.59	1.82	1.27	-2.35	-8.86
WT 27 *vs* Control	1.51	1.26	-1.2	-1.14	-1.08	-2.68	-1.83
WT 28 *vs* Control	2.22	11.18	-1.17	-1.43	1.18	-2.09	1.1
Up regulated	95.84%	83.34%	62.50%	50%	62.50%	50%	58.33%
Down regulated	4.16%	16.66%	37.50%	50%	37.50%	50%	41.66%

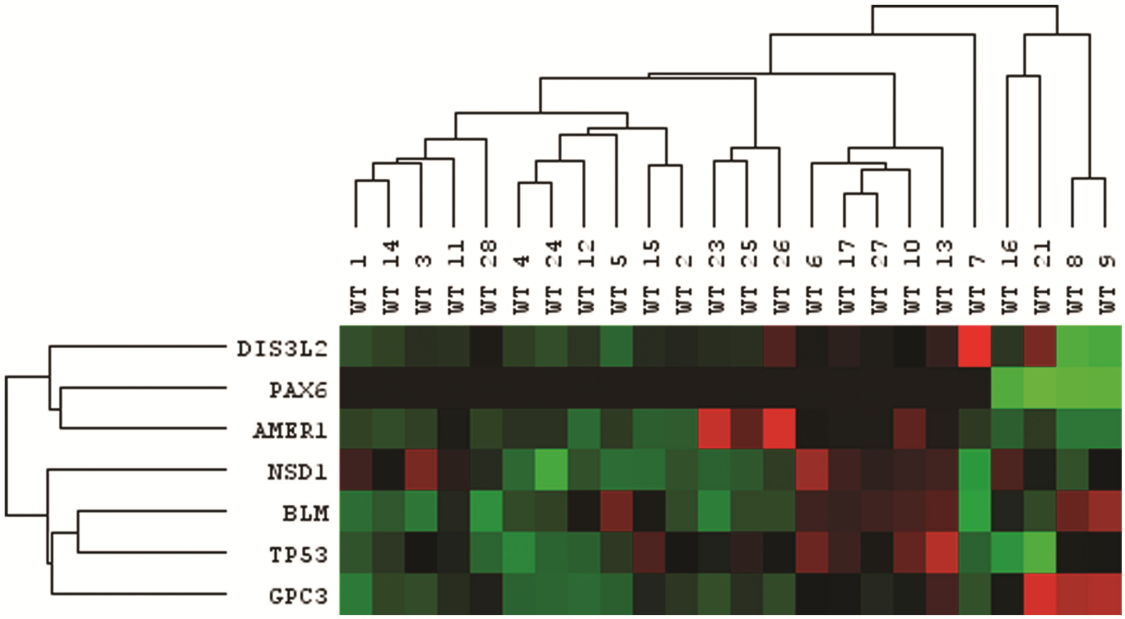

Fig. 1 — Differential expression of TP53-Li-Fraumeni syndrome, BLM-Bloom syndrome, DIS3L2 syndrome, DIS3 syndrome, GPC3-Simpson syndrome, NSD1-Sotos syndrome, PAX6-WAGR Syndrome, syndrome, and AMER1-X-chromosome in comparison to four housekeeping genes, *ACAD9, ACTB, GAPDH*, and *MRPS5*, in 24 Wilms′ tumor cases, namely, WT1 to WT17, WT21, and WT23 to WT28, their internal control samples (normal tissue adjacent to tumor tissue of the same cases), namely, WC1 to WC17, WC21, and WC23 to WC28, on the basis of fold change.

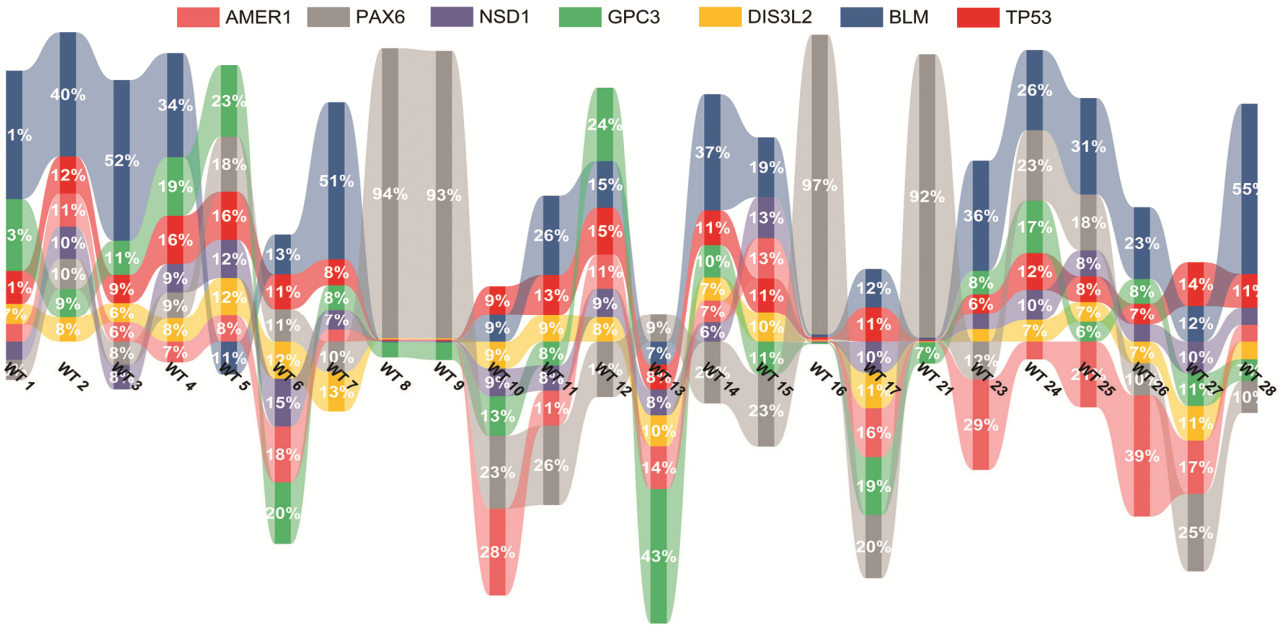

Fig. 2 — Chord diagram of the TP53-Li-Fraumeni syndrome, BLM-Bloom syndrome, DIS3L2 syndrome, DIS3-Syndrome, GPC3-Simpson syndrome, NSD1-Sotos syndrome, PAX6-WAGR syndrome, and AMER1-X-chromosome genes on the basis of fold changes in comparison to four housekeeping genes, *ACAD9, ACTB*, and *GAPDH; MRPS5*, of 24 wilms′ tumor cases, namely, WT1 to WT17 WT 21,and WT21; and WT23 to WT28, their internal control samples (normal tissue adjacent to tumor tissue of the same cases), namely, WC1 to WC17 and WC21; and WC23 to WC28, 06 positive controls and 04 housekeeping genes.

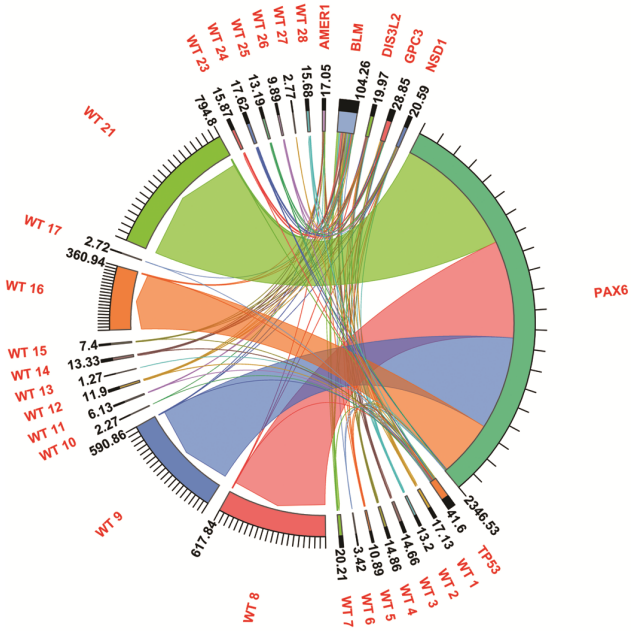

Fig. 3 — Ribbon chart of the TP53-Li-Fraumeni syndrome, BLM-Bloom syndrome, DIS3L2 syndrome, DIS3 syndrome, GPC3-Simpson syndrome, NSD1-Sotos syndrome, PAX6-WAGR syndrome, and AMER1-X-chromosome genes on the basis of fold change in comparison to four housekeeping genes, *ACAD9, ACTB*, and *GAPDH; MRPS5*, of 24 Wilms′ tumor cases, namely, WT1 to WT17 and WT21; and WT23 to WT28, their internal control samples (normal tissue adjacent to tumor tissue of the same cases), namely, WC1 to WC17, , and WC23 to WC28; 06 positive controls; and 04 housekeeping genes.

in 50% of patients from Clusters 3 and 4. A total of 32.13, 35.65, 58.89 and 6.06-fold lower expression was detected in the WT8, WT9, WT21 and WT13 patients, respectively (Table 2 and Fig. 1-4).

Expression profile of the *NSD1* gene (associated with Sotos syndrome)

The expression profile of the *NSD1* gene (Sotos syndrome) was upregulated in 62.50% of the patients in Clusters 1, 2 and 4 (excluding samples WT1, WT3 and WT16) and downregulated in 37.50% of the patients in Cluster 3 (Table 2 and Fig. 1-4).

Expression profile of the *PAX6* gene (associated with WAGR syndrome)

The expression profile of the *PAX6* gene (WAGR syndrome) was upregulated in 50% of patients from Clusters 2 and 4, where WT8 exhibited 610.75-fold upregulation, WT9 exhibited 584.3-fold upregulation, WT16 exhibited 352.64-fold upregulation, and WT21 exhibited 784.44-fold upregulation. The profile of genes downregulated in 50% of patients was from Clusters 1 and 3 (Table 2 and Fig. 1-4).

Expression profile of the *AMER1* gene (associated with the WT on the X chromosome)

The *AMER1* gene (WT on the X chromosome) was upregulated in 58.33% of the patients in Clusters 1, 2 and 4 (excluding WT23 and WT26) and

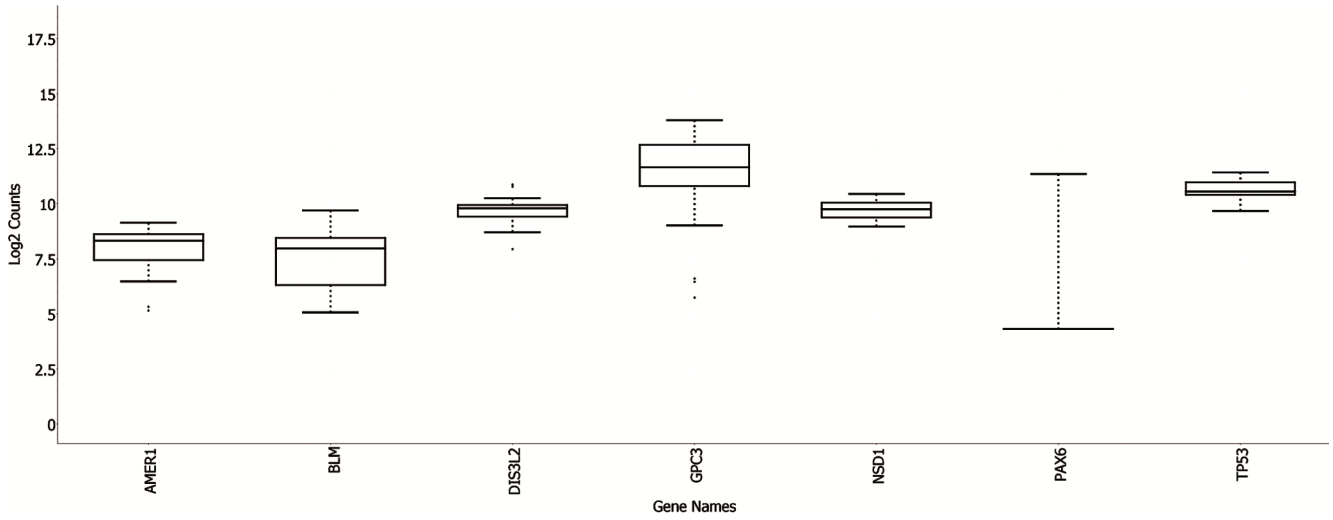

Fig. 4 — The number of mRNAs was 295.32 for AMER1, 281.96 for BLM, 889.58 for DIS3L2, 4532.86 for NSD1, 339.73 for PAX6 and TP53 1623.38 for TP53 (the median value is represented by a horizontal line within the box defined by the first and third quartiles). The talis represents 1.5× the interquartile range. Values outside of this range are displayed as individual points.)

downregulated in 41.66% of the patients in Cluster 3 (Table 2 and Fig. 1-4).

Discussion

Wilms' tumor is the most common renal malignancy in pediatric populations. *Segars et al.,*reported that 9–17% of Wilms' tumor cases are associated with a predisposing syndrome[6]. Here, compared with those in internal controls, the differential expression of 7 genes associated with Wilms' tumor syndrome, i.e., *TP53* (Li–Fraumeni syndrome), *DIS3L2* (Perlman syndrome), *BLM* (Bloom syndrome), *GPC3* (Simpson–Golabi–Behemel syndrome), *NSD1* (Sotos syndrome), PAX6 (WAGR syndrome), and *AMER1* (WT in the X chromosome), was observed in Wilms' tumor cases; moreover, the role of these genes in tumor progression was assessed (Fig. 5).

A novel digital molecular "colour-coded barcode" probe (capture and reporter) technology called the NanoString nCounter gene expression assay was used in the study. By characterizing the multiplexing of hundreds of different transcripts into a single reaction, even a single copy of an mRNA transcript can be identified. Here, compared with other mRNA expression assays, this method was demonstrated to be cost effective and highly accurate.

Previously in our laboratory it was demonstrated that in Wilms' tumor cases, gain of chromosomes 3p13.0-q29, 4p16.3-14.0, 7 and 12p13.33-q24.33 loss of chromosomes 1p36.11-q44, 11p15.5-q25, 21q 22.2-22.3 and 22q11.21-13.2 as well as discrete deletions of chromosomes 1p and 1q. These data indicate that the roles of other onco and tumor suppressor genes in the progression of disease are distinct. These genes included 16 oncogenes (*PTPN11, BRAF, KRAS, HRAS, BCL2, U2AF1, PIK3CA, SMO, GATA2, EGFR, MET, CBL, ALK, ERBB2, FGFR3* and *RET*) and 20 tumor suppressor genes (*MEN1, MLL2, MLL3, PBRM1, PRDM1, SMARCB1, SETD2, WT1, NF1, NF2 CDH1, APC, RB1, FUBP1, BCOR, BRCA1, PTEN, FBXW7, EP300* and *GATA1*). Nonsense mutations p.R306*:c.916CNT, p.R196*:c.586CNT and p.R213*:c.637CNT, and missense mutations p.R282W:c.844CNT, p.R273H/L:c.818GNA/T, p.R273C/S:c.817CNT/A, p.R249S:c.747GNT, p.R248Q/L:c.743GNA/T, p.R248W:c.742CNT, p.G245S/C:c.733GNA/T, p.Y220C:c.659ANG, p.H179R:c.536ANG, p.C176F:c.527GNT, p.R175H:c.524GNA, p.Y163C:c.488ANG and p.V157F:c.469GNT in *TP53* genes and amplification of GPC3 and *AMER1* genes in Wilms' tumor cases were detected. The expression profiles of the above genes were studied, and the roles of these genes and their associations with tumor progression in Wilms' tumor patients were established[3,4].

The *TP53* gene (a guardian of the genome) encodes the tumor suppressor protein TP53, which controls cellular proliferation. Mutations in the *TP53* gene permit cells to divide uncontrollably, which fosters the growth of tumors. As a result of changes in the

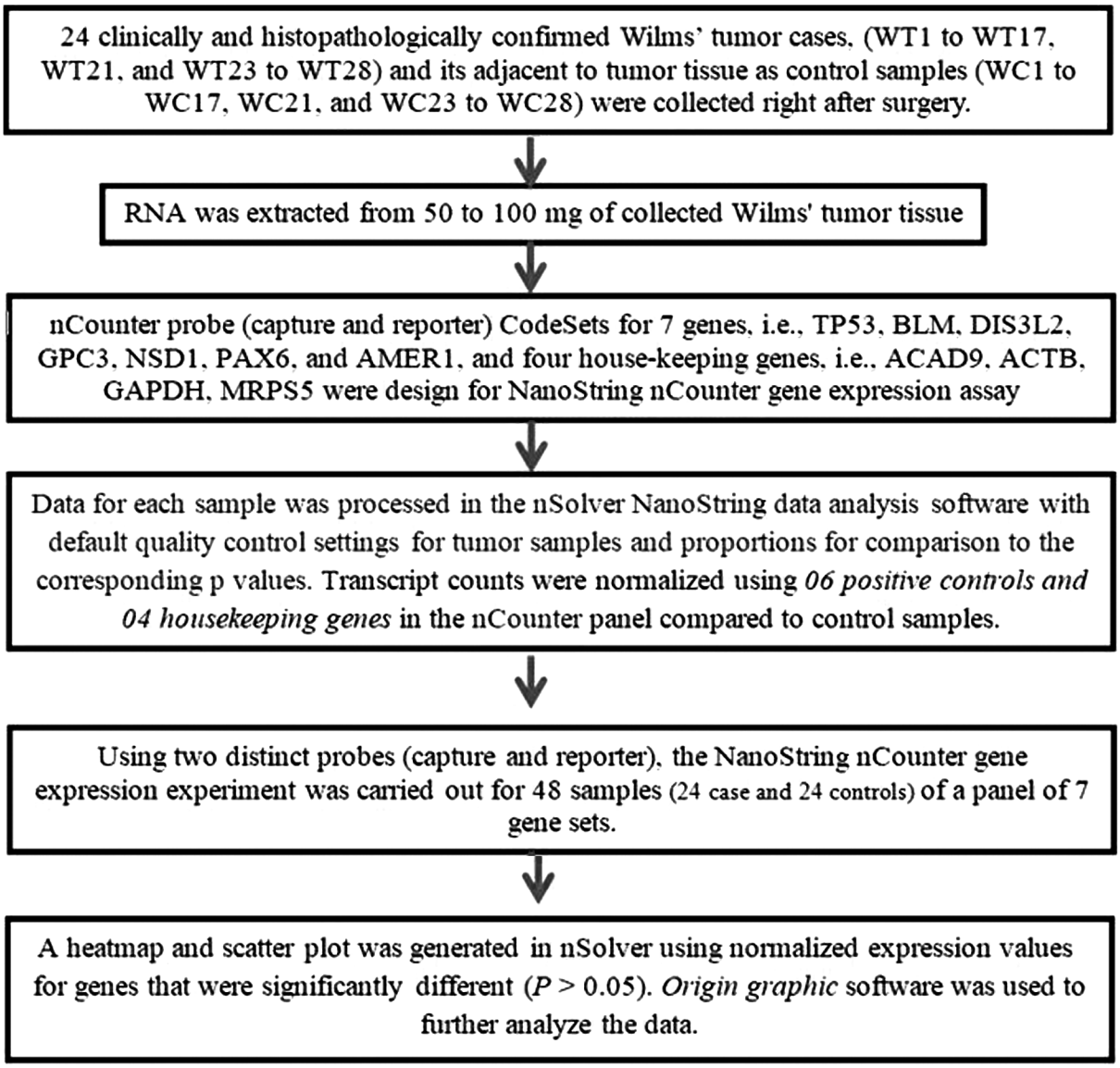

Fig. 5 — Flow diagram for the expression profiling of the *TP53, BLM, DIS3L2, GPC3, NSD1, PAX6 and AMER1* genes in Wilms' tumor patients

microenvironment of geneto-environmental factors, a heterozygous autosomal dominant mutation in the *TP53* gene on chromosome 17p13 results in Li-Fraumeni syndrome. A cancer cell develops Li-Fraumeni syndrome when there is a mutation in one copy of the *TP53* gene, which implies that cells with two altered copies of this gene do not harbor the functional TP53 protein. This results in disruption of the microenvironment of cell growth and initiates tumorigenesis[10,11]. Varley *et al*. first reported a case of TP53 splicing mutation in a family with Li–Fraumeni syndrome and Wilms' tumor. In Li-Fraumeni syndrome, there are germline mutations in the *TP53* gene, and germline mutations are usually inherited and are present in essentially every cell of the body[12]. Somatic and germline mutations predispose patients to various early-onset cancers via the tumor suppressor gene *TP53*, the most frequently altered gene in human malignancies. However, TP53 mutations in Wilms' tumor patients appear to be limited to tumors of the anaplastic subtype, and these polymorphisms are not clearly associated with the condition[13-20]. It has not yet been shown that pediatric anaplastic histology Wilms' tumor may regulate TP53 signaling. Hence, future research should concentrate on the molecular mechanism of TP53 in Wilms' tumor[21].

In our study, it was observed that most of the genes were upregulated in 95.84% of Wilms' tumor patients,

and its overexpression indicates uncontrolled cell growth, which is typically observed in most cancers. However, no studies have been reported on the expression of the *TP53* gene in Wilms tumor patients with Li-Fraumeni syndrome.

Small stature, microcephaly, light sensitivity, insulin resistance, immunodeficiency and an elevated risk of cancer are all features of Bloom syndrome and caused by an autosomal recessive, homozygous or compound heterozygous mutation in the *BLM* gene on chromosome 15q26[22]. According to Scott *et al*., Bloom syndrome is a low-risk syndrome that manifests in only 5% of Wilms' tumor cases. However, the role of BLM in the mechanisms of DNA replication and repair is unclear[5]. To understand the role of BLM in hematopoietic cells, its role in immunoglobulin rearrangement, and the cause of immunodeficiency in Bloom's syndrome, the expression of *BLM* genes in various human tissues and hematopoietic cell lines was analyzed. BLM was found to be strongly expressed in the testis, thymus B and T, and myelomonocytic and megakaryocytic cell lines[23,24]. BLM expression was mostly upregulated in 83.34% of the WT tumors, as observed in our study; however, no BLM expression was detected in Wilms' tumor cases. Accordingly, germline mutations in *BLM* genes have been reported in Wilms' tumor *cases*[25].

PLS is a high-risk syndrome characterized by a rare, autosomal recessive homozygous or compound heterozygous mutation in the *DIS3L2* gene on chromosome 2q37, where 75% of patients have nephro-blastomatosis, macrosomia, nephromegaly, hepatomegaly, hypotonia, characteristic facets and cryptorchidism. One study suggested that 28% of children with Perlman syndrome ultimately develop Wilms' tumor; two-thirds of those diagnosed with malignancy and 55% of those with bilateral WT[11,26-28]. *Studies have demonstrated that* patients with Wilms' tumor have germline mutations in Wilms′ tumor predisposition genes, e.g., *DIS3L2, GPC3*, and *TP53*, which paves the way for increased risk for developing adult cancers[29].

In the present study, the DIS3L2 gene (Perlman syndrome) was upregulated in 62.50% of Wilms' tumor cases and downregulated in 37.50% of the WT cases. No expression studies have been reported on Wilms' tumor cases to date.

Simpson–Golabi–Behmel syndrome is an X-linked multiple congenital anomaly caused by mutations in the gene encoding glypican-3 on chromosome Xq26, although GPC3 mutations have been reported in fewer cases[27].

In this study, it was observed the expression of the *GPC3* gene (Simpson–Golabi–Behmel syndrome) was down regulation of 50% of Wilms' tumor cases. One major outcome of the study is demonstrated that interestingly─58.89 to 32.13- fold downregulation. Hence, the expression pattern of the *GPC3* genes may be used as maker candidate for Wilms' tumor. Tanya *et al.* reported mutation analysis of 64 Wilms' tumor cases and expression profile of 36 Wilms' tumor cases, and reported 29/36 expressed GPC3[27].

Heterozygous mutations in the *NSD1* gene and deletions in the 5q35 region are both responsible for Sotos syndrome (SOTOS)[30]. *The NSD1* gene, which is responsible for chromatin and protein interactions, has been related to Wilms' tumor and developmental kidney abnormalities[30,33]. The expression profile of the *NSD1* gene (Sotos syndrome), in this study, was upregulated in 62.50% of cases and downregulated in 37.50% of Wilms' tumor cases. Boer *et al*., observed 3.5–5 times higher mRNA expression of IGFBP-3 in basal conditions was found in NSD1þ/2fibroblasts in compared to controls[34].

WAGR (Wilms-aniridia-genitourinary-mental retardation) syndrome is heterozygous constitutional microdeletions at 11p13 encompassing both *WT1* and *PAX*6 genes. Renal failure is a serious risk that affects approximately 40% of people by the age of 20[5,35]. As reported earlier, PAX6 mutations are correlated with a higher risk of Wilms' tumor development[36].

The expression profile of *PAX6* gene (WAGR Syndrome) was upregulated in 50% of cases. A maximum of 784.44 to 352.64-fold upregulations were observed, however no expression on WT cases was reported. Koroma *et al*., reported PAX6 protein expression continues in the adult retina, lens, and cornea and may help maintain good ocular health[37,38].

15% to 20% of Wilms′ tumor cases have the germline mutation WTX, also known as *AMER1*, a novel putative tumor suppressor gene, which is situated on the X chromosome at Xq11.1[5,39-42]. The majority of Wilms′ tumor cases with WTX mutations had abnormalities on the 11p15 epigenome[43]. Mutations in *AMER1* genes (somatic type) altered the protein structure, which amplified Wnt signaling, promoted the uncontrolled proliferation of kidney cells and promoted tumor growth[44-49].

The expression and function of WTX, in kidney Wilms′ tumor, were not reported in other types of

human cancer. The expression profile of *AMER1* gene in our study samples it was demonstrated upregulated in 58.33% of cases and downregulated in 41.66% of cases. Miguel *et al.* reported the *WTX* gene, was inactivated in approximately one-third of wilms′ tumor (15 of 51 tumors)[41]. Yao-Yao *et al.* was reported higher expression in normal tissues when compared to corresponding cancers in 459 cases[50]. These findings suggest that WTX can serve as a biological marker for Wilms′ tumor.

Conclusion

The study demonstrated that there are significant differences in the expression profiles of the genes *TP53* (associated with Li-Fraumeni syndrome), *BLM* (associated with Bloom syndrome), *DIS3L2* (associated with Perlman syndrome), *GPC3* (associated with Simpson-Golabi-Behemel syndrome), *NSD1* (associated with Sotos syndrome), *PAX6* (associated with WAGR syndrome), and *AMER1* (associated with WT on the X chromosome) in Wilms' tumor cases. The study established that the genes *NSD1, DIS3L2, AMER1, TP53, BLM, PAX6*, and *GPC3* are biological markers for Wilms' tumor and play key roles in the development of this embryonic tumor.

Author contributions

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conceptualization, DKS, NS.; Methodology, DKS.; Investigation, DKS.; Formal Analysis, DKS.; Resources JR, NS; Writing - Original Draft, T DKS, MD.; Writing - Review & Editing,: MD AS and DKG.; Visualization, NS.; Supervision, DKS, NS.; Funding Acquisition, DKS.

Ethical statement

This study was performed in accordance with the principles of the Declaration of Helsinki. Approval was granted by the Central Drugs Standard Control Organization (CDSCO), Government of India approved (Registration No; ECR/2621/Ins/ UP/2013/RR/19) Institutional Ethics Committee of King George's Medical University, Lucknow, India File no 1899/Ethics/2021 (Ref. Code: 85th ECM II A/P6).Informed written consent was obtained from the parents of every donor in accordance with the regulations of CDSCO and ICMR, Government of India, to participate in this study.

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Conflict of interest

The authors have no conflicts of interest to declare.

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