# Genetic variation frequencies in Wilms' tumor: A meta-analysis and systematic review

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Over the last few decades, numerous biomarkers in Wilms' tumor have been confirmed and shown variations in prevalence. Most of these studies were based on small sample sizes. We carried out a meta-analysis of the research published from 1992 to 2015 to obtain more precise and comprehensive outcomes for genetic tests. In the present study, 70 out of 5175 published reports were eligible for the meta-analysis, which was carried out using Stata 12.0 software. Pooled prevalence for gene mutations *WT1*, *WTX*, *CTNNB1*, *TP53*, *MYCN*, *DROSHA*, and *DGCR8* was 0.141 (0.104, 0.178), 0.147 (0.110, 0.184), 0.140 (0.100, 0.190), 0.410 (0.214, 0.605), 0.071 (0.041, 0.100), 0.082 (0.048, 0.116), and 0.036 (0.026, 0.046), respectively. Pooled prevalence of loss of heterozygosity at 1p, 11p, 11q, 16q, and 22q was 0.109 (0.084, 0.133), 0.334 (0.295, 0.373), 0.199 (0.146, 0.252), 0.151 (0.129, 0.172), and 0.148 (0.108, 0.189), respectively. Pooled prevalence of 1q and chromosome 12 gain was 0.218 (0.161, 0.275) and 0.273 (0.195, 0.350), respectively. The limited prevalence of currently known genetic alterations in Wilms' tumors indicates that significant drivers of initiation and progression remain to be discovered. Subgroup analyses indicated that ethnicity may be one of the sources of heterogeneity. However, in meta-regression analyses, no study-level characteristics of indicators were found to be significant. In addition, the findings of our sensitivity analysis and possible publication bias remind us to interpret results with caution.

Wilms' tumor (WT) is the most common childhood renal malignancy that affects approximately 1 in 10 000 children in Europe and North America.[1] The median age of diagnosis is between 3 and 4 years and both kidneys are affected in ~5% of children.[2] Most cases of WT are sporadic, however, rare cases of familial WT have also been described.[3–5] Approximately 10% of WT cases are associated with germline mutations and/or congenital abnormalities, such as Denys–Drash syndrome,[6] Beckwith–Wiedemann syndrome,[7] Wilms–aniridia–genitourinary anomalies–mental retardation syndrome[8,9] and Perlman syndrome.[10,11] Wilms' tumor is a complex embryonal tumor with conventional triphasic histology (blastemic, epithelial, and stromal components). Although these three components are typically found, WT may also display heterologous elements such as cartilage, osteoid, and neural elements. This heterogeneity implies a complexity to the underlying causes of WT that has fascinated investigators for decades.

The *WT1* gene, located at chromosome 11p13, was first cloned in 1990 as one of the first tumor suppressor genes in WT.[8,9] Subsequently, *CTNNB1*[12] and *WTX*[13] have been identified in tumors. The combined frequency of *WT1*, *CTNNB1*, and *WTX* genetic alterations has been estimated to occur in roughly one-third of WT.[14,15] Furthermore, WT maintenance and disease progression are associated with the altered expression of many other genes, such as *TP53*, *MYCN*, *CITED1*, *SIX2*, *TOP2A*, and *CRABP2*.[16–25] Specifically, mutations in *TP53* appear to be a common finding in unfavorable histology (UH) WT and a notorious marker of treatment resistance.[16,17,26] A recent whole exome study has identified mutations in microRNA processing genes including *DROSHA* and *DGCR8*.[27–30] However, the frequency of alterations in *DROSHA* and *DGCR8* are similarly uncommon, leaving a significant fraction of cases without an identified "driver" genetic defect.[27]

Numerous recurrent copy number aberrations and loss of heterozygosity (LOH) events have been described, some of which affect known genes (e.g. 11p LOH and 17p loss),[26,31] but the critical genes with other regions (e.g. 1q gain, 1p loss and 16q loss) remain elusive.[32–34] Only a few of these aberrations have known associations with histology or outcome. The documented association between relapse and LOH for 1p and 16q is being used to stratify patients within the current Children's Oncology Group (COG) therapeutic protocols to warrant a more intensive drug regimen upfront for favorable histology (FH) WT.[32,35] Loss of genetic material at 4q, 11q, and 14q has also emerged as a feature of UHWT and poor prognosis.[20,36] Therefore, identification and characterization of these genes is of primary importance in understanding the onset and

progression of tumors, ultimately leading to recognition of potential markers and specific targets for prevention and individualized treatment of tumors. Over the last few decades, numerous markers, especially the novel ones, have been confirmed and shown variations in prevalence. However, the results of these studies were inconsistent, partly because the sample sizes were usually small, and the ethnic backgrounds and experimental techniques were varied. In order to overcome the limitation of individual studies, we carried out this meta-analysis to provide a more precise and comprehensive outcome for genetic tests and a basis for the prevention, early diagnosis, and treatment of WT.

## Methods

**Data sources.** Four English databases including PubMed, Web of Science, Embase, and Cochrane Library were electronically searched to retrieve studies on the gene mutations of WT published before 15 September 2015. The search was based on the following keywords: "genetic variation" or "mutation", combined with "Wilms tumor" or "Wilms' tumor" or "nephroblastoma". Furthermore, we checked the reference lists of retrieved reviews to identify more potential pertinent studies.

**Inclusion criteria.** Studies were included in the systematic review if they met all of the following criteria: (i) the publication explored the relationship between gene mutation and WT; (ii) the frequency of gene mutation and sample size was clearly documented, or other information was provided that assisted in interpreting the results; (iii) the sample size was 15 or more; and (iv) the publication language was confined to English.

**Exclusion criteria.** Studies that met any of the following exclusion criteria were excluded: (i) research based on animals or cells rather than general population; (ii) reviews, editorials, meeting abstracts, or commentaries; (iii) publications with no

target data or no relevant outcomes; (iv) multiple published reports. When there were several reports concerning the same cohort we included the high quality publication in our meta-analysis.

**Quality assessment.** A quality assessment of the included articles was carried out using the checklist for appraising studies of genotype prevalence proposed by Little *et al.*[37] It is a validated tool for appraising studies of genotype prevalence including four parts. They focused on the purpose of study (one item), analytic validity of genotyping (eight items), selection of study subjects (five items), and statistical issues (two items). Each item in the scale is scored as 0 (not reported or inadequate) or 1 (reported and adequate). The total ideal score is 16 for these studies. Details are shown in Table S1.

**Statistical analyses.** The combined rate and its 95% confidence interval were used to evaluate the strength of association between gene mutations and WT. Heterogeneity among included studies was checked by the $\chi^2$-based $Q$ test and $I^2$ test. If there was no heterogeneity between studies ($P > 0.10$, $I^2 = 0\%$), the fixed-effect model was used. Otherwise, the random-effects models was chosen (Table 1). All statistical tests were carried out with the meta-analysis software Stata 12.0 (Stata Statistical Software, College Station, TX, USA, www.stata.com). A *P*-value of 0.05 for any test or model was considered to be statistically significant unless otherwise specified.

**Sensitivity analyses.** Sensitivity analyses were carried out with Stata 12.0 to investigate the influence of every study on the overall effect.

**Subgroup analyses and meta-regression analyses.** Subgroup analyses were carried out according to ethnicity to explore the possible explanations for heterogeneity. We used the descriptions "Caucasian" and "non-Caucasian" to explore the ethnicity influence. Random-effect meta-regression analyses were

**Table 1. Results of a meta-analysis and publication bias in research regarding genetic mutation frequencies in Wilms' tumor, published 1992–2015**

| Gene mutation | R (95% CI) | Z | P-value | I²% | P_het | Begg's test P-value | Egger's test P-value | Gene models |
|---|---|---|---|---|---|---|---|---|
| DGCR8[27–29] | 0.036 (0.026, 0.046) | 7.190 | 0.000 | 22.8 | 0.274 | 1.000 | 0.309 | Random |
| MYCN[20,21,28,30,42,51,52] | 0.071 (0.041, 0.100) | 4.710 | 0.000 | 68.5 | 0.004 | 1.000 | 0.092 | Random |
| DROSHA[27–30] | 0.082 (0.048, 0.116) | 4.770 | 0.000 | 76.1 | 0.006 | 0.296 | 0.019 | Random |
| WT1[13,15,27,30,31,38,42,46,53–65] | 0.141 (0.104, 0.178) | 7.480 | 0.000 | 77.9 | 0.000 | 0.007 | 0.001 | Random |
| WTX[13–15,27,31,38,42,46,53–57,66] | 0.147 (0.110, 0.184) | 7.750 | 0.000 | 72.6 | 0.000 | 0.228 | 0.347 | Random |
| CTNNB1[13,15,27,30,38,42,46,53,54,56–58,60,61,67,68] | 0.140 (0.100, 0.190) | 7.870 | 0.000 | 70.9 | 0.000 | 0.010 | 0.010 | Random |
| TP53[16,26,30,38,39,41,42] | 0.410 (0.214, 0.605) | 4.110 | 0.000 | 0.8 | 0.000 | 1.000 | 0.300 | Random |
| Gain 1q[31,34,42,48,50,69] | 0.218 (0.161, 0.275) | 7.470 | 0.000 | 66.9 | 0.006 | 0.368 | 0.436 | Random |
| Gain 12[31,39,69,70] | 0.273 (0.195, 0.350) | 6.930 | 0.000 | 0.0 | 0.767 | 0.734 | 0.333 | Fixed |
| LOH 1p+16q[45,50,71] | 0.029 (0.017, 0.041) | 4.800 | 0.000 | 0.0 | 0.715 | 0.296 | 0.030 | Fixed |
| LOH 1p[32,42–45,50,51,71–74] | 0.109 (0.084, 0.133) | 8.600 | 0.000 | 66.3 | 0.001 | 0.640 | 0.586 | Random |
| LOH 22q[44,51,71,75] | 0.148 (0.108, 0.189) | 7.140 | 0.00 | 15.1 | 0.316 | 0.734 | 0.256 | Random |
| LOH 16q[32,36,40,42,43,45,48,50,71–73,76–79] | 0.151 (0.129, 0.172) | 13.510 | 0.00 | 50.3 | 0.110 | 0.499 | 0.098 | Random |
| LOH 7p[71,73,80,81] | 0.177 (0.126, 0.227) | 6.860 | 0.000 | 0.0 | 0.903 | 0.734 | 0.335 | Fixed |
| LOH 11q[36,42,51,59,71,73] | 0.199 (0.146, 0.252) | 7.380 | 0.000 | 57.0 | 0.040 | 0.707 | 0.322 | Random |
| LOH 11p15[31,44,46,68,73,76,82] | 0.286 (0.172, 0.399) | 4.920 | 0.000 | 84.9 | 0.000 | 0.548 | 0.049 | Random |
| LOH 11p13[31,40,42,44,61,76,82,83] | 0.319 (0.220, 0.417) | 6.340 | 0.000 | 69.5 | 0.002 | 0.108 | 0.040 | Random |
| LOH 11p[31,43,51,59,72,73,76] | 0.334 (0.295, 0.373) | 16.780 | 0.000 | 20.7 | 0.272 | 0.072 | 0.101 | Random |
| LOH 11p15.5[42,56,61] | 0.380 (0.140, 0.620) | 3.100 | 0.002 | 90.4 | 0.000 | 0.296 | 0.439 | Random |
| Loss 1p[34,39,84] | 0.167 (0.069, 0.265) | 3.340 | 0.001 | 80.7 | 0.006 | 1.000 | 0.855 | Random |
| Loss 11p[39,69,84] | 0.202 (0.022, 0.382) | 2.200 | 0.028 | 88.9 | 0.000 | 1.000 | 0.540 | Random |

CI, confidence interval; Fixed, fixed-effect model; LOH, loss of heterozygosity; Phet, *P*-value of heterogeneity; R, frequency of gene mutations; Random, random-effects models.

also carried out to identify study-level factors contributing to heterogeneity between studies according to experimental technology, types of sample, year of publication, and ethnicity.

**Publication bias.** Potential publication bias was assessed by Begg's rank correlation test and Egger's linear regression test using Stata 12.0.

**Data extraction.** Two investigators (C.D. and R.D.) independently identified the eligible studies for this systematic review. Any disagreement was resolved by the third party (F.L. and X.L.). Data was extracted and entered into an electronic form in Excel (Microsoft Corporation, WA, USA, www.microsoft.com) by C.D. and was independently checked by F.L. The following information was extracted from each eligible study: (i) gene variations, the name of the first author, and year of publication; (ii) race, study location and period, sample size, types of specimen; and (iii) other details of targeted indicators, including histopathology, preoperative chemotherapy, and bilateral or unilateral disease.

## Results

**Search of published works.** A total of 5174 potentially eligible publications were retrieved. Most of them were excluded after the initial screening of titles and abstracts. The main reasons for exclusion were not referring to our topic and duplication. A total of 114 relevant articles were included in our full-text selection and 69 eligible articles were included in our systematic review. The main reasons for exclusion were as follow: 34 studies lacked target data, population, or outcome; three studies were concerned with the same cohort; and 8 were case reports, reviews, or had a sample size less than 15 cases. Another relevant publication was found through reference screening. Finally, 70 articles met the criteria for our meta-analysis (Fig. 1).

**Characteristics of included studies.** The characteristics of the included studies are presented in Table S2. Studies were published from 1992 to 2015 involving more than 20 genetic variations. *WT1*, *WTX*, and *CTNNB1* were the genes most commonly studied. *DROSHA* and *DGCR8* alterations had been identified in microRNA processing genes in recent years. In addition, LOH principally tended to a few loci, at 1p, 7p, 11p, 11q, 16q, and 22q. More than half of the patients in our included reports were Caucasian; the studies were carried out in countries such as the USA, Germany, UK, and Italy. In addition, some non-Caucasian populations were analyzed in Kenya, China, and Japan. Patients were treated mainly according to either the International Society of Pediatric Oncology or COG protocols. The COG protocols were based on primary surgery followed by chemotherapy, whereas patients treated under the International Society of Pediatric Oncology protocols usually received preoperative chemotherapy, followed by surgery and adjusted postoperative chemotherapy and radiotherapy.

**Quantitative synthesis for gene variations of WT.** The pooled frequencies of gene variations ranged from 0.03 (0.02, 0.04) to 0.41 (0.21, 0.61) including some gene mutations (*WT1*, *WTX*, *CTNNB1*, *TP53*, *MYCN*, *DROSHA*, and *DGCR8*), LOH (1p,
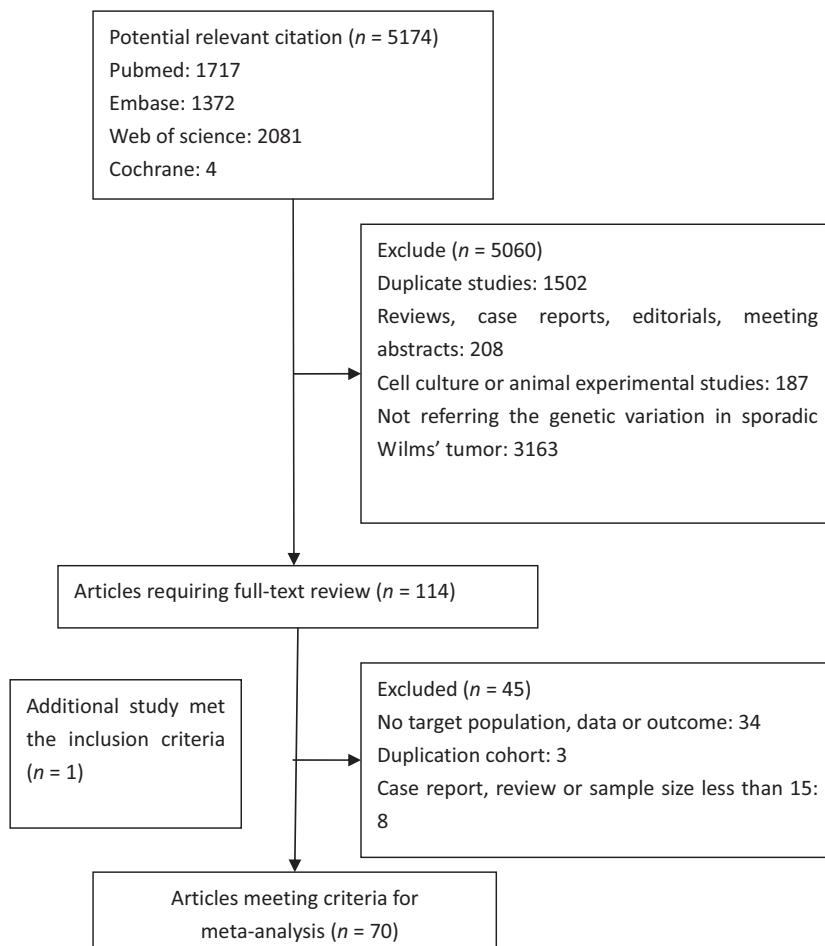
Potential relevant citation (*n* = 5174)
Pubmed: 1717
Embase: 1372
Web of science: 2081
Cochrane: 4

Exclude (*n* = 5060)
Duplicate studies: 1502
Reviews, case reports, editorials, meeting abstracts: 208
Cell culture or animal experimental studies: 187
Not referring the genetic variation in sporadic Wilms' tumor: 3163

Articles requiring full-text review (*n* = 114)

Additional study met the inclusion criteria (*n* = 1)

Excluded (*n* = 45)
No target population, data or outcome: 34
Duplication cohort: 3
Case report, review or sample size less than 15: 8

Articles meeting criteria for meta-analysis (*n* = 70)

**Fig. 1.** Flow chart of the selection of relevant published works regarding genetic variation frequencies in Wilms' tumor. Of 5174 potentially eligible publications, 5060 were excluded after screening of titles and abstracts. Sixty-nine eligible articles were included from 114 included in our full-text selection. The reasons for exclusion were: lack of target data, population, or outcome (34 studies), analysis of the same cohort (three studies), and case reports, reviews, or sample size <15 cases (eight studies). One additional publication was found through reference screening. Finally, 70 articles met the criteria for our meta-analysis.

11p, 11q, 16q, and 22q), and copy number gain (1q and chromosome 12) (Table 1). Copy number loss was not pooled due to too few original studies.[26,34,38–41] The forest plots of *WT1*, *WTX*, and *CTNNB1* are provided in Figures 2, 3, and 4, respectively. Other forest plots are shown in Figures S1–S16.

**Sensitivity analysis.** In a few genetic variations, the sensitivity analysis indicated that some results were unstable. See Figures S17–S35 for details.

**Subgroup analysis and meta-regression analyses.** Based on the subgroup analysis, our results indicated that the pooled prevalence of mutations for Caucasian and non-Caucasian populations in *WT1*, *WTX*, and *CTNNB1* were: 0.14 (0.10, 0.19) and 0.16 (0.10, 0.22); 0.15 (0.10, 0.19) and 0.17 (0.13, 0.21); and 0.16 (0.11, 0.22) and 0.11 (0.05, 0.18), respectively. The forest plots of *WT1*, *WTX*, and *CTNNB1* are shown in Figures 5, 6, and 7, respectively. No study-level characteristics of these

indicators were found to be significant in meta-regression analyses (Figs S36–S38).

**Publication bias.** Begg's rank correlation test and Egger's linear regression test in some of our results indicated that some publication bias might exist (Table 1).

**Quality assessment.** The scores of included studies ranged from 5 to 15. Twelve studies scored less than or equal to 7, 10 studies scored more than or equal to 12, and 48 studies scored between 8 and 11 (Table S2). Details are provided in Table S3.

## Discussion

Based on our systematic review and meta-analysis, we found that the pooled prevalence of *WT1*, *WTX*, and *CTNNB1* mutation in patients with WT was 0.141 (0.104, 0.178), 0.147



**Fig. 2.** Forest plot for frequency of *WT1* gene mutation in Wilms' tumor. Studies are plotted according to the first author's name and publication year. Horizontal lines represent 95% confidence interval (CI). Each square represents the prevalence point estimate and its size is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with confidence interval given by its width. The unbroken vertical line is at the null value (prevalence = 0). ES, effect size.
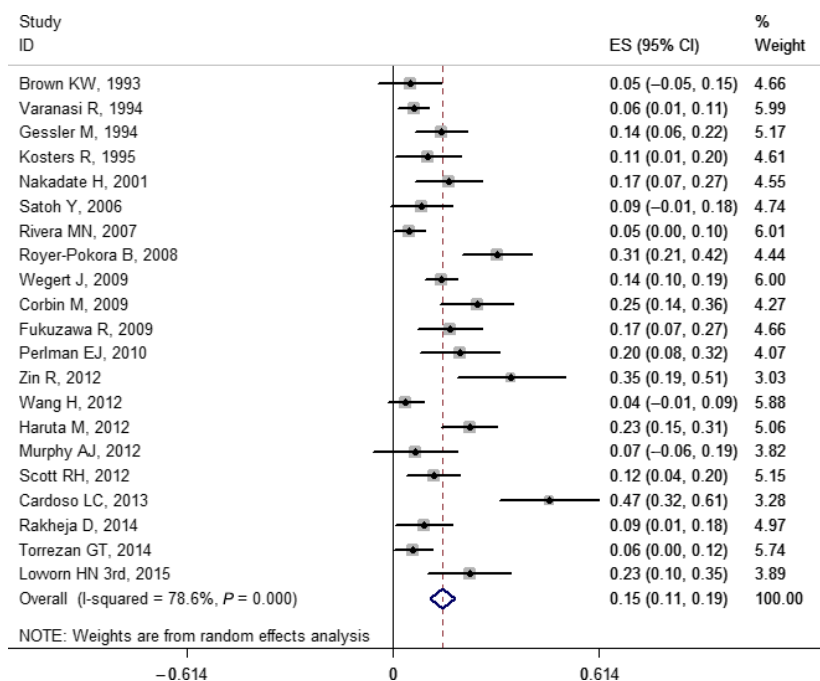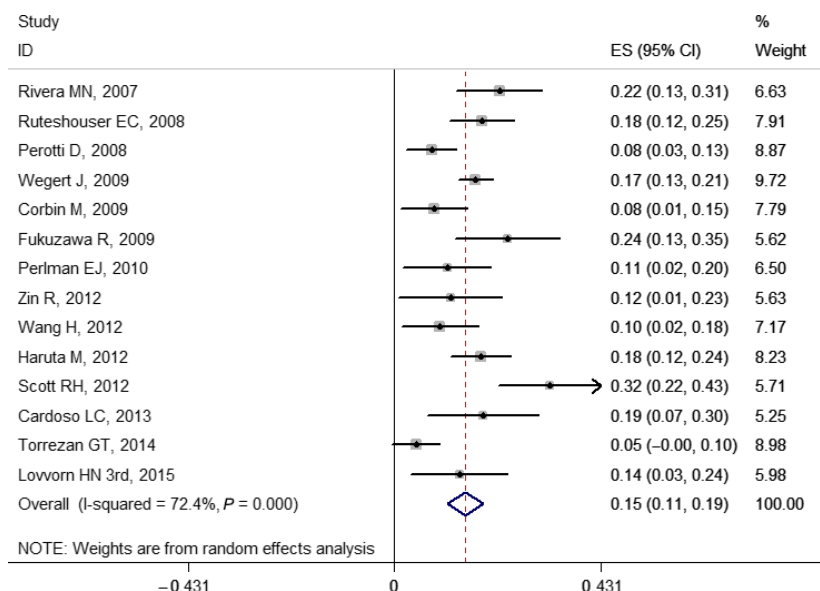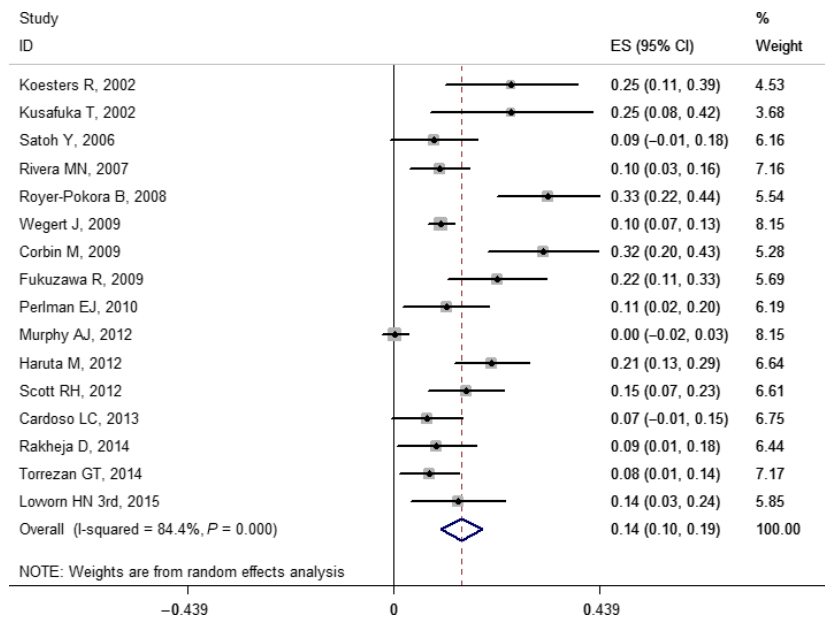


**Fig. 3.** Forest plot for frequency of *WTX* gene mutation in Wilms' tumor. Studies are plotted according to the first author's name and publication year. Horizontal lines represent 95% confidence interval (CI). Each square represents the prevalence point estimate and its size is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with confidence interval given by its width. The unbroken vertical line is at the null value (prevalence = 0). ES, effect size.
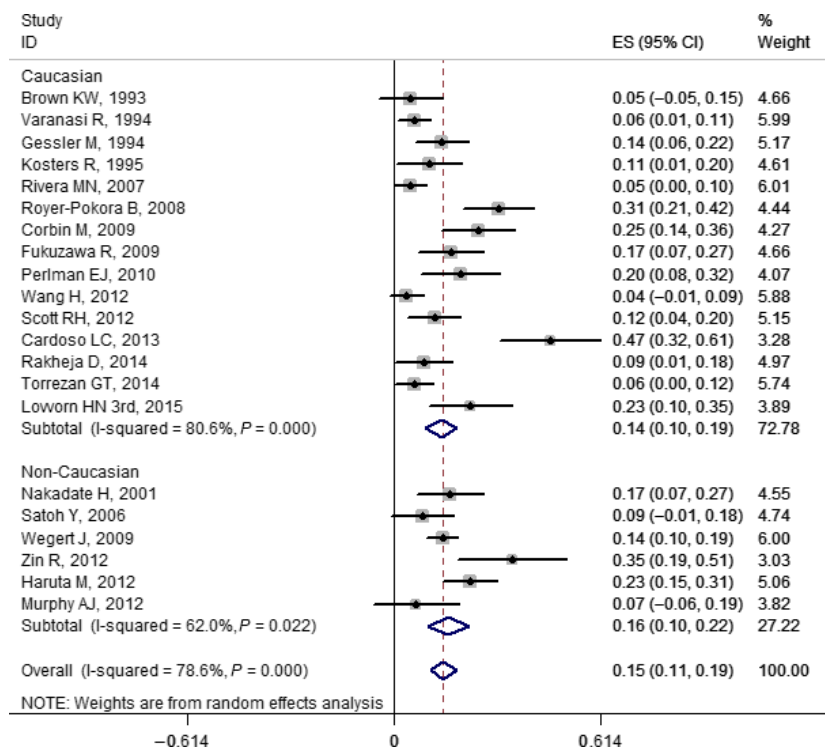
**Fig. 4.** Forest plot for frequency of *CTNNB1* gene mutation in Wilms' tumor. Studies are plotted according to the first author's name and publication year. Horizontal lines represent 95% confidence interval (CI). Each square represents the prevalence point estimate and its size is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with confidence interval given by its width. The unbroken vertical line is at the null value (prevalence = 0). ES, effect size.



**Fig. 5.** Forest plot for frequency of *WT1* gene mutation in Wilms' tumor stratified by ethnicity. Studies are plotted according to the first author's name and publication year. Horizontal lines represent 95% confidence interval (CI). Each square represents the prevalence point estimate and its size is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with CI given by its width. The unbroken vertical line is at the null value (prevalence = 0). ES, effect size.

(0.110, 0.184), and 0.140 (0.100, 0.190), respectively. The incidence of *WT1* and *CTNNB1* combined was 28.1%, and *WT1* and *WTX* combined was 28.8%. Our findings were basically consistent with Ruteshouser *et al.*'s report, which indicated that *WT1* and *WTX* mutations occur with similar frequency, and that mutations in *WT1*, *WTX*, and *CTNNB1* account for only approximately one-third of tumors.[14] However, the reported mutations in these three genes were mostly inconsistent in WT. Reasons for the difference were the relatively small number of investigated cases, and that reports on mutations in different ethnicities have been published. For example, one study reported that WT poses a significant cancer health disparity to black children of sub-Saharan African ancestry, not only because of its more common occurrence among black populations worldwide, but also because of its persistently high lethality in resource-constrained nations on the African continent, such as Kenya.[42] Our analysis also found that there may be statistical differences between ethnicities across these genes.

Accumulation of the TP53 protein in WT specimens has been associated with unfavorable histology and treatment resistance.[16,17,26] It has been further postulated that the *TP53* mutation in WT is a late occurrence in its disease sequence and progression.[33] There is a clear relationship between *TP53* mutations and anaplastic WT, the histologic subtype with poorer prognosis. This indicates that these mutations are
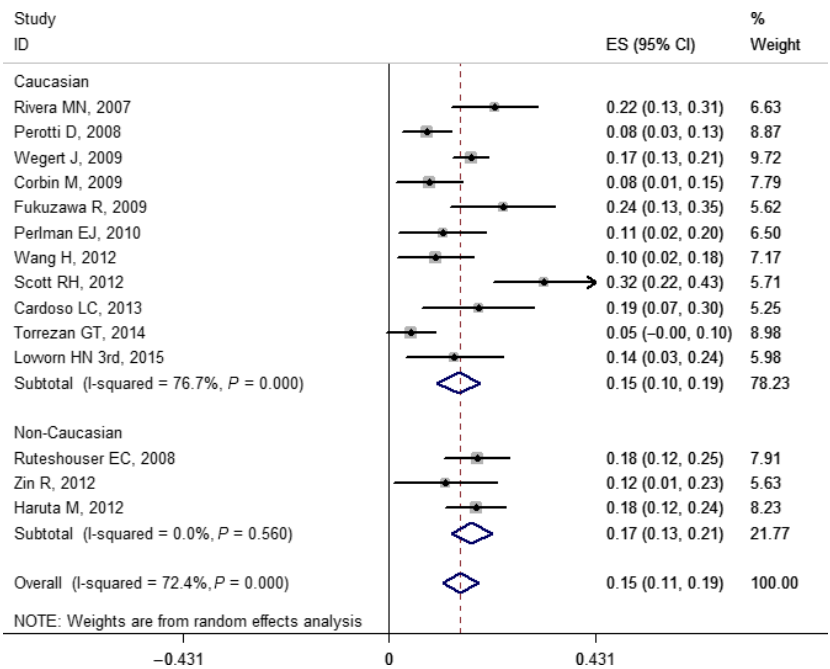
**Fig. 6.** Forest plot for frequency of *WTX* gene mutation in Wilms' tumor stratified by ethnicity. Studies are plotted according to the first author's name and publication year. Horizontal lines represent 95% confidence interval (CI). Each square represents the prevalence point estimate and its size is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with confidence interval given by its width. The unbroken vertical line is at the null value (prevalence = 0). ES, effect size.
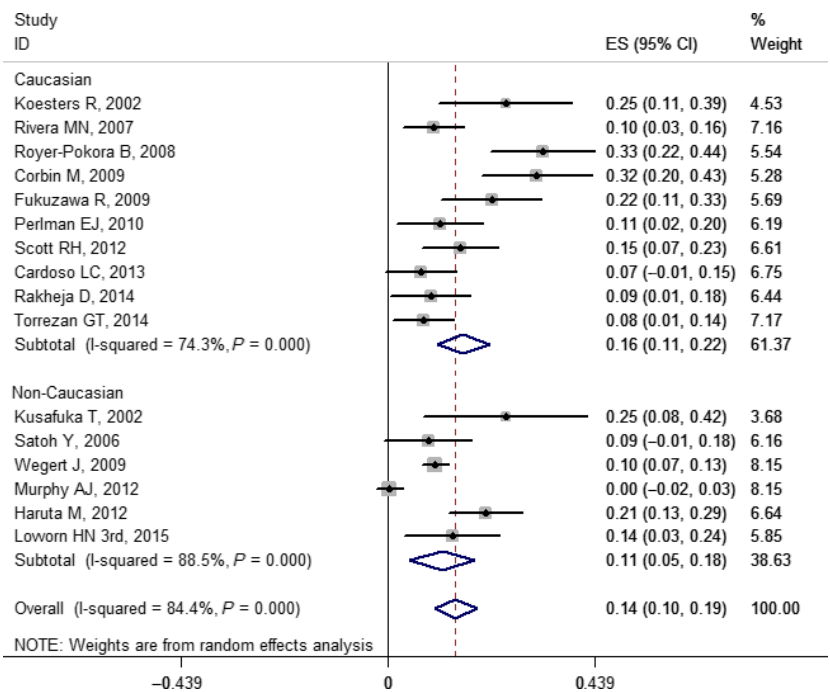


**Fig. 7.** Forest plot for frequency of *CTNNB1* gene mutation in Wilms' tumor stratified by ethnicity. Studies are plotted according to the first author's name and publication year. Horizontal lines represent 95% confidence interval CI. Each square represents the prevalence point estimate and its size is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with confidence interval given by its width. The unbroken vertical line is at the null value (prevalence = 0). ES, effect size.

related to tumor progression and associated with a more aggressive type of disease.[16,26,41] In anaplastic WT, the pooled frequency of *TP53* mutation was 0.410 (0.214, 0.605) based on our systemic review. Therefore, this indicates that screening for such alterations may be advisable in routine diagnosis, especially if there is any hint of anaplasia.

The *MYCN* gene has been observed in several previous WT studies.[20,21,28] Originally, Williams *et al.*[20] reported that 4/54 anaplastic (7.4%) and 27/272 non-anaplastic (9.9%) tumors in this series had *MYCN* gain. Overall, the results indicated that *MYCN* copy number gain was not restricted to the anaplastic series, but was relatively common in tumors of both histologies. Recently, Williams *et al.*

described that copy number gains that included the *MYCN* locus were detected in 37/292 (12.7%) of tumors overall and in 7/23 (30.4%) of diffuse anaplastic WTs. Their finding of an association between *MYCN* gain and anaplasia, as well as outcome, makes the *MYCN* pathway an attractive target for further research into new approaches to treatment.[21] However, the frequency of alterations in *MYCN* was merely 0.071 (0.041, 0.100) in our study. It indicates that the frequency of *MYCN* mutations are similarly uncommon, leaving a significant fraction of cases without an identified "driver" genetic defect.

Similar mutations of *DROSHA* and *DGCR8*, as well as a small number of mutations in *MYCN*, have recently been

reported together with altered microRNA patterns in mutant tumors by whole exome study.[27–30] Their results provide evidence that *DROSHA*, *DGCR8*, and the microRNA biogenesis pathway may have a crucial role in WT tumorigenesis. *DROSHA* encodes a nuclear RNase III protein. One COG study reported that *DROSHA* E1147K is a recurrent mutation and that *DROSHA* and other microRNA-processing genes are mutated in approximately 33% (22/66) of WT samples, implying that defective microRNA biogenesis may contribute to WT development.[27] However, based on our meta-analysis, we found that the general mutation prevalence of *DROSHA* and *DGCR8* was 0.082 (0.048, 0.116) and 0.036 (0.026, 0.046), respectively, in WT. Therefore, as the COG cohort study includes WT samples of predominant blastemal histology, the frequency of mutations in this pathway in a broad unbiased WT series still remains to be determined.[27]

Use of LOH assays to determine areas of allele loss has shown that the majority of WT have few or no changes, and that these tend to be restricted to a few loci, principally at 1p, 11p, 11q, 16q, and 22q.[28,32,43,44] The National Wilms Tumor Study Group has prospectively investigated the influence of tumor-specific LOH at chromosomes 1p and/or 16q on outcome, and reported that losses involving 1p and 16q correlated positively with a poor prognosis.[32] Other studies have revealed an association of LOH at chromosomes 1p, 11q, 16q, and 22q with an increased risk of relapse.[36,44,45] Although LOH on 11p is not associated with any difference in outcome in general, children younger than 2 years with small tumors and allele loss on 11p were recently found to be at greater risk for relapse when treated with minimal therapy (chemo-naive).[46] Our results indicate that the LOH signatures lie in the low sensitivity. This observation supports the need to combine biomarkers based on LOH analyses with other clinical, molecular, and histological prognostic factors but in larger series of patients.

In addition, characteristic chromosomal aberrations include gain at 1q and chromosome 12. The hallmark 1q gain has been reported by early chromosomal as well as array-based studies.[47–49] Furthermore, Hing *et al.* reported high frequency gain of 1q in 27 of 46 (59%) relapse versus 5 of 21 (24%) non-relapse cases of WT.[49] Otherwise, the results of one study have confirmed a strong association between 1q gain and 1p and 16q loss.[50] As a result of our meta-analysis, the pooled prevalence of 1q and chromosome 12 gain was 0.218 (0.161, 0.275) and 0.273 (0.195, 0.350). However, the important gene or genes on 1p, 1q, chromosome 12, and 16q that contribute to tumorigenesis remain an enigma.

In subgroup analyses of *WT1*, *WTX*, and *CTNNB1*, the heterogeneity of the non-Caucasian group in *WTX* was 0, indicating that ethnicity might be one of the sources of heterogeneity. However, in meta-regression analyses, no study-level characteristics of those indicators were found to be significant. This limitation was unavoidable as we had no access to primary data. It is possible that unidentified study-level factors were not observed and the results could be exaggerated or narrowed. So our results suggest that we must pay attention to the report specification in future research.

## Limitations

This meta-analysis still has some limitations. Because information regarding subclasses (blastemal, epithelial, and stromal), bilateral/unilateral, and FHWT/UHWT were not given in most of the included studies, we could not carry out more detailed subgroup analysis to explore heterogeneity in this meta-analysis. Moreover, the sensitivity analysis indicated that some of the results of our study were unstable. Begg's rank correlation test and Egger's linear regression test in some of our results also suggested that there might exist publication bias. In addition, some of the included studies were focused on germline mutations, but we did not distinguish between somatic and germline mutations, which may have affected the results. Because of the publication biases in *WT1* and *CTNNB1*, we must interpret those results with caution.

## Disclosure Statement

The authors have no conflict of interest.

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## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Fig. S1.** Forest plot for frequency of *TP53* gene mutation.

**Fig. S2.** Forest plot for frequency of *MYCN* gene mutation.

**Fig. S3.** Forest plot for frequency of *DROSHA* gene mutation.

**Fig. S4.** Forest plot for frequency of *DGCR8* gene mutation.

**Fig. S5.** Forest plot for frequency of loss of heterozygosity at 1p.

**Fig. S6.** Forest plot for frequency of loss of heterozygosity at 1p and 16q.

**Fig. S7.** Forest plot for frequency of loss of heterozygosity at 7p.

**Fig. S8.** Forest plot for frequency of loss of heterozygosity at 11p.

**Fig. S9.** Forest plot for frequency of loss of heterozygosity at 11p13.

**Fig. S10.** Forest plot for frequency of loss of heterozygosity at 11p15.15.

**Fig. S11.** Forest plot for frequency of loss of heterozygosity at 11p15.

**Fig. S12.** Forest plot for frequency of loss of heterozygosity at 11q.

**Fig. S13.** Forest plot for frequency of loss of heterozygosity at 16q.

**Fig. S14.** Forest plot for frequency of loss of heterozygosity at chromosome 22.

**Fig. S15.** Forest plot for frequency of gain at 1q.

**Fig. S16.** Forest plot for frequency of gain at chromosome 12.

**Fig. S17.** Sensitivity analysis for *WT1*.

**Fig. S18.** Sensitivity analysis for *WTX*.

**Fig. S19.** Sensitivity analysis for *CTNNB1*.

**Fig. S20.** Sensitivity analysis for*TP53*.

**Fig. S21.** Sensitivity analysis for *MYCN*.

**Fig. S22.** Sensitivity analysis for *DGCR8*.

**Fig. S23.** Sensitivity analysis for *DORSHA*.

**Fig. S24.** Sensitivity analysis for gain at 1q.

**Fig. S25.** Sensitivity analysis for gain at 12.

**Fig. S26.** Sensitivity analysis for loss of heterozygosity at 1p.