Association of *NUDT15* gene polymorphism with adverse reaction, treatment efficacy, and dose of 6-mercaptopurine in patients with acute lymphoblastic leukemia: a systematic review and meta-analysis

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Abstract

6-mercaptopurine (6-MP) serves as the backbone in the maintenance regimens of acute lymphoblastic leukemia (ALL). We aimed to evaluate the influence of *NUDT15* gene polymorphism on the risk of myelosupression, hepatotoxicity and interruption of 6-MP, as well as treatment efficacy and dose of 6-MP in ALL patients. A total of 24 studies with 3,374 patients were included in this meta-analysis. We found 9-fold higher risk of 6-MP induced leukopenia (odds ratio [OR] =9.00, 95% confidence interval [CI]: 3.73-21.74) and 2.5-fold higher risk of 6-MP-induced neutropenia (OR=2.52, 95% CI: 1.72-3.69) for *NUDT15* c.415C>T variant carriers in the dominant model. Moreover, we found that the dose intensity of 6-MP in ALL patients with one *NUDT15* c.415C>T variant alleles (CT) was 19% less than that in wild-type patients (CC) (mean differences: 19.43%, 95% CI: -25.36 to -13.51). The tolerable dose intensity of 6-MP in *NUDT15* c.415C>T homozygote variant (TT) and heterozygote variant (CT) carriers was 49% and 15% less than that in wild-type patients, respectively. The *NUDT15* c.415C>T variant group (CT+TT) had seven times (OR=6.98, 95% CI: 2.83-17.22) higher risk of developing 6-MP intolerance than the CC group. However, *NUDT15* c.415C>T polymorphism did not appear significantly associated with hepatotoxicity, treatment interruption or relapse incidence. We concluded that *NUDT15* c.415C>T was a good predictor for 6-MP-induced myelosuppression in ALL patients. The dose intensity of 6-MP in ALL patients with *NUDT15* c.415C>T variants was significantly lower than that in wild-type patients. This research provided a basis for further investigation into relations between *NUDT15* gene and adverse reaction, treatment efficacy and dose intensity of 6-MP.

Introduction

Acute lymphoblastic leukemia (ALL) is a malignant and heterogeneous condition in which uncontrolled proliferation of lymphoblasts (of B- or T-cell origin) occurs in the bone marrow, peripheral blood, and/or other organs. It affects both children and adults.[1,2] The overall survival (OS) of ALL has improved tremendously over the past few decades, and the 5-year OS exceeds 90% in children who receive contemporary therapy.[3,4] The thio-substituted purine analogue 6-mercaptopurine (6-MP), which can inhibit ALL cell DNA synthesis along with weekly methotrexate (MTX), is the backbone in the maintenance regimens of ALL.[5,6] However, due to the narrow therapeutic index, 6-MP can bring about dose-dependent toxicities such as myelosuppression and hepatotoxicity in ALL patient. These adverse effects can result in 6-MP dose reduction or therapy interruption, and even increase the risk of life-threatening infections and relapse.[7,8]

It was reported that 6-MP can be converted into active metabolites 6-thioguanine nucleotides (6-TGN) via a series of enzymes. 6-TGN consist of 6-thio(d)-GMP, 6-thio(d)-GDP,

and 6-thio(d)-GTP. 6-thio(d)-GTP are incorporated into DNA and RNA, which can cause inhibition of nucleotide and protein synthesis and lead to cytotoxicity typified by leukopenia.[9-11] Meanwhile, 6-MP and its metabolites can be methylated into inactive 6-methyl mercaptopurine (6-MMP) by thiopurine methyltransferase (TPMT). Therefore, TPMT activity is negatively related to 6-TGN content. Furthermore, TPMT deficiency caused by genetic variants is likely to increase the level of 6-TGN and therefore result in myelosuppression.[12-14] Given the formerly observed association between *TPMT* polymorphisms and thiopurines-induced leukopenia, US Food and Drug Administration has suggested preemptive *TPMT* testing for individualizing thiopurines dose and reducing the risk of drug-related adverse events.[15,16] However, compared with their Caucasians (10%) counterparts, Asians (3%) have lower frequency of *TPMT* variants and are more likely to be subject to 6-MP-induced leukopenia, dose intolerance, and dose reduction.[17] It was observed that hematotoxicity occurred in some patients with wild-type *TPMT*, which indicates that some other genetic variants are probably associated with 6-MP tolerance and related adverse reactions.[17-19]

Recently, a series of studies reported that genotype of nucleoside diphosphate-linked moiety X-type motif 15 (*NUDT15*), particularly *NUDT15* c.415C>T (rs116855232), had a strong association with 6-MP-induced myelosuppression and 6-MP intolerance in ALL.[20-22] Indeed, NUDT15 is hypothesized to dephosphorylate the 6-MP active metabolites 6-thio(d)-GTP to 6-thio(d)-GDP, which can prevent their incorporation into DNA and decrease the 6-MP cytotoxicity.[23] In other words, *NUDT15* variants are supposed to result in excessive levels of 6-thio(d)-GTP and increase host toxicity. Although the association between *NUDT15* c.415C>T variants and 6-MP-induced myelotoxicity is widely recognized, the pooled increased risk, by involving more newly published research, of myelosuppression in ALL patients with *NUDT15* c.415C>T variants has not been reported.[24-27] Moreover, the correlation between *NUDT15* polymorphism and risk for hepatotoxicity, treatment interruption, dose intensity of 6-MP or relapse of ALL were not evaluated in previous meta-analysis.[28-31] In order to fill this gap, we conducted a systematic review and meta-analysis aiming to assess the association between *NUDT15* gene polymorphism and adverse reaction, treatment efficacy and dose of 6-MP particularly in patients with ALL.

Methods

Search strategy and selection criteria

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Statement principles.[32] The protocol for this systematic review was registered on PROSPERO (registration number: CRD42022384698). PubMed, Web of Science, Embase, Cochrane Library, CNKI, China Biology Medicine, Wanfang and VIP databases were searched from their inception up to November 25, 2022 using the following key terms: (*NUDT15* OR MTH2 protein OR Nudix Hydrolase 15 OR nucleoside diphosphate-linked moiety X-type motif 15) AND (leukaemia OR leukemia). Eligible studies were included if they met the following inclusion criteria: i) investigating the association between at least one *NUDT15* gene polymorphism and adverse reactions (e.g., leukopenia, neutropenia, hepatotoxicity, treatment interruption), treatment efficacy of 6-MP or 6-MP dose (e.g., dose intensity, intolerant incidence, tolerable dose *et al.*); ii) patients are limited to those diagnosed with acute lymphoblastic leukemia; iii) the study type is a cohort study or case control; iv) literature language is English or Chinese. Exclusion criteria were as follows: i) review articles, editorials and comments; ii) duplicated studies; iii) case reports; iv) not an outcome of interest or not available. Two reviewers (SD, XFH) independently undertook the study selection according to prespecified inclusion and exclusion criteria, and any disagreement was resolved by discussion or by consulting a third author.

Data extraction

For each study included, two reviewers independently extracted and collected general information and outcome data in a standardized data extraction form. General information contained the first author's name, year of publication, ethnicity, study design, age, female/male, total sample size, 6-MP initial dose, phase of treatment, the outcome of study, SNP, the genotyping method, timing of follow-up and Hardy-Weinberg equilibrium. Outcome data included any available information about leukopenia, neutropenia, hepatotoxicity, relapse, treatment interruption, dose intensity, intolerant incidence, as well as tolerable dose. In addition, we e-mailed the corresponding author for detailed information when data from a study was unextractable and any discrepancies were resolved by consensus.

Quality assessment

Two researchers independently assessed the quality of eligible studies based on the Newcastle-Ottawa Scale (NOS). The NOS contains eight items, categorized in three domains: selection, comparability, and outcome (cohort studies) or exposure (case-control studies). The quality score of each study was rated from zero to nine.[33]

Statistical analysis

All outcomes were pooled using Review Manager (RevMan) version 5.4. We calculated odds ratios (OR) and their 95% confidence intervals (CI) to evaluate the association of *NUDT15* gene polymorphism and with the occurrence of 6-MP-induced adverse drug reactions, which include leukopenia, neutropenia, hepatotoxicity, treatment interruption along with relapse and intolerant incidence. Meanwhile,

we also analyzed mean differences (MD) and their 95% CI which presented potential influence of *NUDT15* gene polymorphism on 6-MP dose intensity and tolerable dose. Heterogeneity between studies was estimated by the χ^2 test and the I^2 statistic. If I^2 <50%, the fixed-effects model with the Mantel-Haenszel method was employed; otherwise, the random-effects model was adopted. It is noteworthy that sensitivity analysis by sequentially excluding each study and subgroup analysis were conducted according to specific circumstances.

Results

Retrieved results

Nine hundred and seven articles were retrieved among which 530 duplicates were removed. Through reading the titles and abstracts, 256 irrelevant studies were excluded, so a total of 121 studies qualified for full text screening. After screening, we further dropped eight reviews or comments, two editorial or letters, 36 conference abstracts, two studies written in neither English nor Chinese, three other gene or other disease studies, four potential duplicates, 19 without extractable data, as well as 23 without outcome of interest. So, 24 studies were finally included. The process of literature retrieval and study screening process are illustrated in Figure 1.

Study characteristics and quality assessment

Table 1 summarizes the characteristics of the included studies. They were published between 2015 and 2022. Ten of them came from China (including Hongkong and Taiwan),[25,26,34-41] three from Japan,[42-44] three from Korea,[45-47] three from Thailand,[48-50] two from India,[27,51] one from Colombia,[24] Lebanon,[52] and Kurdistan[52] respectively, as well as one multiracial study.[21] Their sample size ranged from 51 to 404. One of them was case control study[37] and 23 were cohort studies. The NOS score ranged from 6 to 8.

Association between *NUDT15* gene polymorphism and adverse reactions of 6-MP

Association between NUDT15 c.415C>T or NUDT15 c.52G>A polymorphism and 6-MP induced leukopenia

In total, eight studies were included to compare the occurrence of leukopenia between 256 *NUDT15* c.415C>T variant (CT+TT) carriers and 820 wild-type patients (CC).[26,34,35,37,40,41,44,45] Five studies used white blood cell count (WBC) <2×10^3/μL as the definition of leukopenia,[26,35,37,40,44] Choi *et al.*[45] defined WBC <1.5×10^3/μL as leukopenia and the other two studies did not define it (*Online Supplementary Table S1*).[34,41] The result demonstrated that the CT+TT group had a nine times higher risk of leukopenia incidence than the CC group (OR=9.00, 95% CI: 3.73-21.74; *P*<0.00001) (Figure 2A). Sensitivity analysis with sequential omission of each study suggested that this effect was stable. The

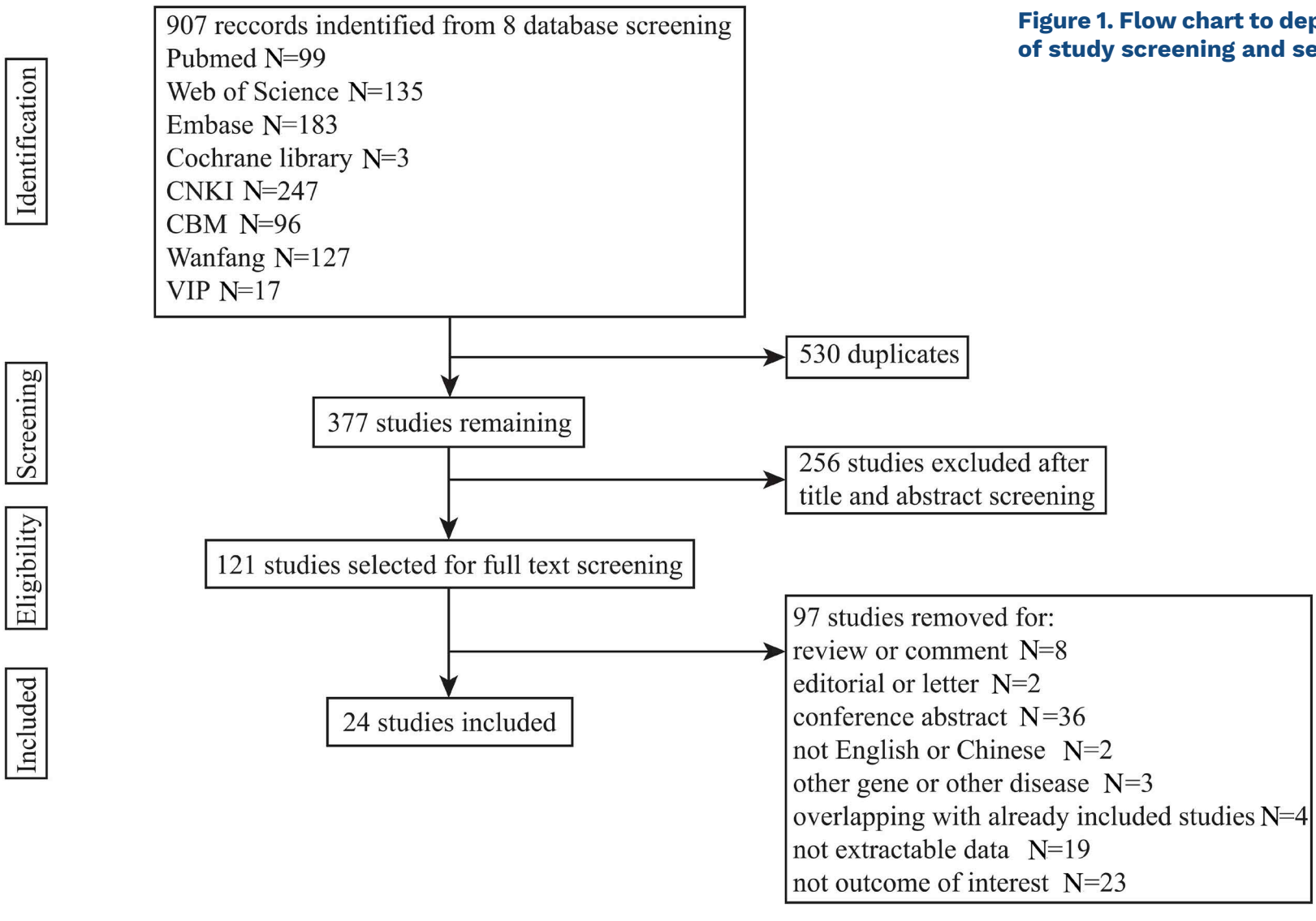


Figure 1. Flow chart to depict the process of study screening and selection.

Table 1. Characteristics of included studies.

Study	Ethnicity	Age in years*	Sample size N (F/M)	6-MP initial dose (mg/m²/d)	Outcome of study	*NUDT15* variant	Genotyping method	Follow-up time (range)	HWE	NOS score
Buaboonnam 2019[48]	Thai	5.2 (1.12-14.78)	102 (44/58)	maint (50)	neutr	c.415C>T	ASPCR	50.4 (12.3-84.0) months	NA	8
Cao 2020[34]	Chinese	leuk+: 4.8±3.02; leuk-: 4.55±3.18†	173 (85/88)	RI (60), cons (25), maint (50)	leuk	c.415> T	Microarray	NA	YES	8
Chiengthong 2016[49]	Thai	5.4 (1-15)	82 (NA)	maint (50)	neutr rel	c.415C>T	Pyrosequencing	8 (1-20) years	YES	7
Choi 2019[45]	Korean	5.1 (3.7-10.6)	139 (75/64)	maint (50)	leuk neutr TI	c.415C>T	Sanger sequencing	8 (1-20) years	YES	7
Correa-Jimenez 2021[24]	Colombians	6 (NA)	70 (35/35)	maint (50)	neutr TI rel	c.415C>T	TaqMan SNP genotyping	6 months	YES	7
Fan 2022[25]	Chinese	6.2 (NA)	145 (64/81)	maint (50)	HT	c.415C>T; c.52G>A	Sanger sequencing *3	6 months	NA	7
Khaeso 2022[50]	Thai	5 (1-15)	169 (79/90)	maint (50)	neutr	*2	*3, *5: TaqMan SNP genotyping; the rest: Sanger sequencing	6 months	YES	8
Khera 2019[51]	Indian	Risk SNP+: 5.81 (4.0-7.63) Risk SNP-: 5.24 (4.4-6.07)	58 (48/10)	maint (60)	neutr	c.415C>T	Sanger sequencing	36 weeks	YES	7
Kim 2018[46]	Korean	4.9 (1.1-17.3)	185 (75/110)	maint (50)	neutr	c.415C>T	Fluidigm SNP genotyping	74.2 (26.6-235.7) months	NA	8
Lee 2021[47]	Korean	8.3 (1.0-16.2)	83 (27/56)	maint (75)	DI TD	c.415C>T	Sanger sequencing	9.1 (0.9-16.6) years	NA	7
Li 2021[35]	Chinese	6.3±3.5	94 (46/48)	maint (50)	leuk neutr	c.415C>T	FISH	1-18 months	YES	7
Liang 2016[36]	Chinese	CC 4.6 (0.1-18) CT 5.0 (0.4-17.2) TT 4.5 (1.1-7.9)	404 (180/224)	maint (60)	rel TD	c.415C>T	Pyrosequencing	240 months	NA	7
Liu 2018[37]	Chinese	6.3 (0.67-13.4)	133 (57/76)	cons (50) maint (75)	leuk	c.415C>T	PCR-RFLP	NA	NA	6
Mao 2021[26]	Chinese	5.9 (0.63-13.75)	149 (64/85)	maint (50)	leuk HT TD	c.415C>T	FISH	18 (8-47) months	YES	7

Continued on following page.

Study	Ethnicity	Age in years*	Sample size N (F/M)	6-MP initial dose (mg/m²/d)	Outcome of study	*NUDT15* variant	Genotyping method	Follow-up time (range)	HWE	NOS score
Morad-veisi 2019[52]	Lebanese Kurdistani	Lebanese (6.63±4.93); Kurdistani (6.25±3.07)	Lebanese: 136 (59/77); Kurdistani: 74 (31/43)	maint (75)	HT	c.415C>T	TaqMan SNP genotyping	F:120 weeks M:143 weeks	YES	8
Pai 2021[27]	Indian	cohort 1: 16.5 (2-56) cohort 2: 17 (1-63)	cohort 1: 42 (7/35) cohort 2: 133 (45/88)	maint (50)	neutr TI int inc	c.415C>T	Sanger sequencing	18 (6-38) months	NA	8
Suzuki 2016[42]	Japanese	5.1 (1.6-15.8)	51 (28/23)	maint (40)	rel	c.415C>T	TaqMan SNP genotyping	8.3 (1.7-17.1) years	YES	8
Tanaka 2015[43]	Japanese	CC 5 (1-17) CT 5 (1-17) TT 8 (3-15)	92 (45/47)	maint (40)	leuk HT TI rel	c.415C>T	TaqMan SNP genotyping	3.6 (0.4-15.5) years	NA	8
Tanaka 2018[44#]	Japanese	4.9 (1-17)	95 (48/47)	maint (40)	leuk HT int inc	c.415C>T; c.52G>A	TaqMan SNP genotyping	3.6 (0.4-15.5) years	c.415C>T NO; c.52G>A. YES	8
Wang 2021[39]	Chinese	0.42 (6-16)	135 (59/76)	Init (50)	HT	c.415C>T	Sanger sequencing	NA	NA	7
Wang 2022[38]	Chinese	6.2±0.5	58 (22/36)	maint (50)	neutr	c.415C>T	Sanger sequencing	NA	NA	6
Yang 2015[21]	white, black, Hispanic, Asian	NA	371 (NA)	maint (75)	DI	c.415C>T	Illumina Human Exome BeadChip	6 months	YES	8
Zhou 2018[40]	Chinese	5.8 (1.1-14.0)	105 (39/66)	maint (50)	leuk HT TI	c.415C>T	MassArray platform	6 months	YES	8
Zhu 2018[41]	Chinese	1-15	188 (79/109)	RI (60), cons (25), maint (50)	leuk HT	c.415C>T; c.52G>A	NA	NA	NA	8

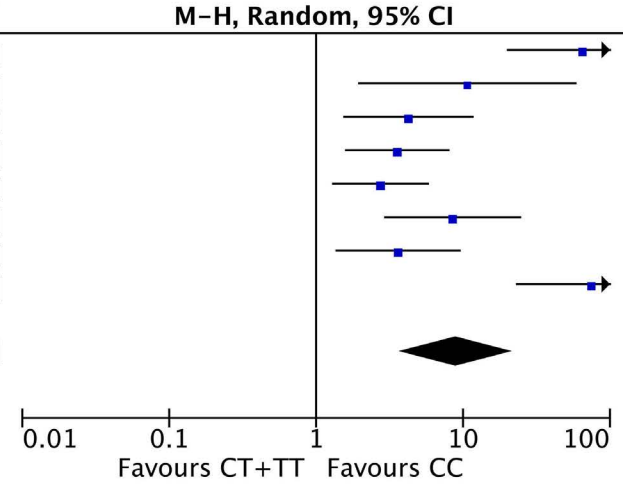
*Age in years was described as median (range) or mean ± standard deviation (SD) or range according to the original study. †Age at diagnosis. #Study Tanaka 2018 involved all of the patients in study Tanaka 2015 with 3 more patients, and both were included for they had different outcomes. Furthermore, as for the same outcomes from 2 studies, we only used the data with larger patient size in Tanaka 2018. 6-MP: 6-mercaptopurine; ASPCR: allele-specific polymerase chain reaction; cons: consolidation; DI: dose intensity; F: female; FISH: fluorescence *in situ* hybridization; d: day; HT: hepatotoxicity; HWE: Hardy–Weinberg equilibrium; init: initiation; int inc: intolerant incidence; leuk: leukopenia; M: male; maint: maintenance; NA: not available; neutr: neutropenia; NOS: Newcastle–Ottawa Scale; PCR: polymerase chain reaction; rel: relapse; RI: remission induction; TD: tolerable dose; TI: treatment interruption; SNP: single nucleotide polymorphism; CC: wild-type patients; CT: heterozygote variant carriers; TT: homozygote variant carriers.

study of Cao 2020[34] and Zhu 2018[41] contributed the biggest heterogeneity, probably due to involving the remission induction phase of treatment with the highest dose of 6-MP (60 mg/m/^{2}/d) among all the studies. The subgroup analysis using the same standard of leukopenia suggested that the CT+TT group developed a 3.9 times higher risk of leukopenia than the CC group (OR=3.89, 95% CI: 2.62-5.76; *P*<0.00001) with I^2 decreased from 83% to 0% (Figure 2B). Moreover, 927 patients in seven studies were included to evaluate the association of *NUDT15* c.415C>T gene polymorphism with leukopenia incidence in the recessive model (TT *vs.* CT+CC) [34,35,37,40,41,44,45] and the TT group had a significantly 15.9 times higher risk of leukopenia incidence than the CT+CC group (OR= 15.88, 95% CI: 5.80-43.48; *P*<0.00001) (Figure

A

Study or Subgroup	CT+TT Events	CT+TT Total	CC Events	CC Total	Weight	Odds Ratio M-H, Random, 95% CI
Cao 2020	32	36	15	137	12.1%	65.07 [20.20, 209.56]
Choi 2019	5	30	2	109	9.8%	10.70 [1.96, 58.37]
Li 2021	14	22	21	72	12.8%	4.25 [1.55, 11.63]
Liu 2018	21	35	29	98	13.7%	3.57 [1.60, 7.97]
Mao 2021	23	40	36	109	13.9%	2.74 [1.30, 5.77]
Tanaka 2018	19	25	19	70	12.6%	8.50 [2.95, 24.49]
Zhou 2018	12	31	11	74	13.0%	3.62 [1.38, 9.50]
Zhu 2018	33	37	15	151	12.1%	74.80 [23.29, 240.20]
Total (95% CI)		**256**		**820**	**100.0%**	**9.00 [3.73, 21.74]**
Total events	159		148			

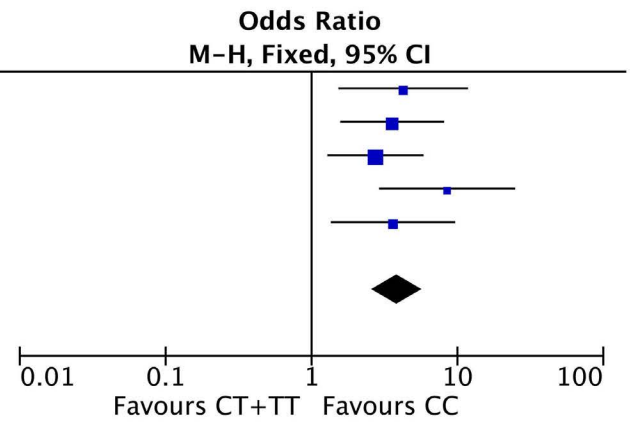
Heterogeneity: $Tau^2 = 1.31$; $\chi^2 = 41.91$, df = 7 ($P < 0.00001$); $I^2 = 83\%$
Test for overall effect: Z = 4.89 ($P < 0.00001$)



B

Study or Subgroup	CT+TT Events	CT+TT Total	CC Events	CC Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Li 2021	14	22	21	72	14.7%	4.25 [1.55, 11.63]
Liu 2018	21	35	29	98	25.1%	3.57 [1.60, 7.97]
Mao 2021	23	40	36	109	33.8%	2.74 [1.30, 5.77]
Tanaka 2018	19	25	19	70	9.9%	8.50 [2.95, 24.49]
Zhou 2018	12	31	11	74	16.4%	3.62 [1.38, 9.50]
Total (95% CI)		**153**		**423**	**100.0%**	**3.89 [2.62, 5.76]**
Total events	89		116			

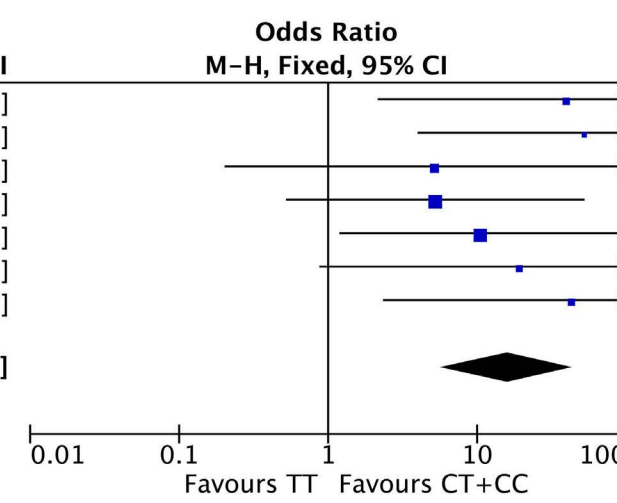
Heterogeneity: $\chi^2 = 3.04$, df = 4 ($P = 0.55$); $I^2 = 0\%$
Test for overall effect: Z = 6.75 ($P < 0.00001$)



C

Study or Subgroup	TT Events	TT Total	CT+CC Events	CT+CC Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Cao 2020	6	6	41	167	9.6%	39.63 [2.19, 718.55]
Choi 2019	2	3	5	136	2.9%	52.40 [4.05, 678.66]
Li 2021	1	1	34	93	14.5%	5.17 [0.21, 130.53]
Liu 2018	3	4	47	129	28.6%	5.23 [0.53, 51.76]
Tanaka 2018	6	7	32	88	27.2%	10.50 [1.21, 91.15]
Zhou 2018	2	2	21	103	8.1%	19.19 [0.89, 414.64]
Zhu 2018	6	6	42	182	9.0%	42.98 [2.37, 778.59]
Total (95% CI)		**29**		**898**	**100.0%**	**15.88 [5.80, 43.48]**
Total events	26		222			

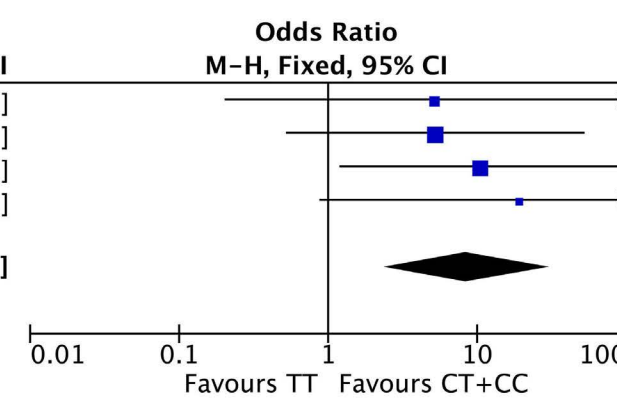
Heterogeneity: $\chi^2 = 3.19$, df = 6 ($P = 0.78$); $I^2 = 0\%$
Test for overall effect: Z = 5.38 ($P < 0.00001$)



D

Study or Subgroup	TT Events	TT Total	CT+CC Events	CT+CC Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Li 2021	1	1	34	93	18.5%	5.17 [0.21, 130.53]
Liu 2018	3	4	47	129	36.4%	5.23 [0.53, 51.76]
Tanaka 2018	6	7	32	88	34.7%	10.50 [1.21, 91.15]
Zhou 2018	2	2	21	103	10.4%	19.19 [0.89, 414.64]
Total (95% CI)		**14**		**413**	**100.0%**	**8.50 [2.38, 30.32]**
Total events	12		134			

Heterogeneity: $\chi^2 = 0.57$, df = 3 ($P = 0.90$); $I^2 = 0\%$
Test for overall effect: Z = 3.30 ($P = 0.0010$)



E

Study or Subgroup	GA+AA Events	GA+AA Total	GG Events	GG Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Tanaka 2018	4	5	34	90	41.7%	6.59 [0.71, 61.41]
Zhu 2018	1	3	47	185	58.3%	1.47 [0.13, 16.56]
Total (95% CI)		**8**		**275**	**100.0%**	**3.60 [0.79, 16.38]**
Total events	5		81			

Heterogeneity: $\chi^2 = 0.81$, df = 1 ($P = 0.37$); $I^2 = 0\%$
Test for overall effect: Z = 1.66 ($P = 0.10$)

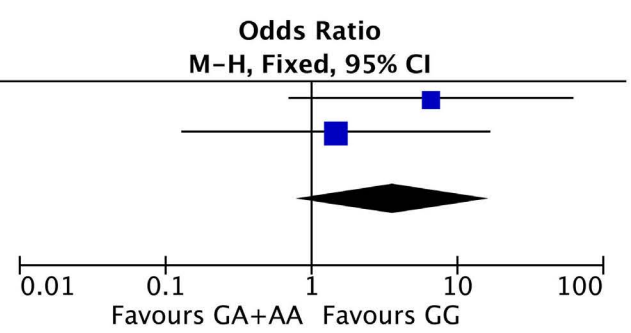


Figure 2. Forest plots for the meta-analysis of association of NUDT15 c.415C>T (rs116855232) (A-D) or c.52G>A (rs186364861) (E) with 6-MP induced leukopenia. (A) The dominant model (CT+TT *vs.* CC); (B) subgroup analysis in the dominant model; (C) the recessive model (TT *vs.* CT+CC); (D) subgroup analysis in the recessive model; (E) the dominant model (GA+AA *vs.* GG). CI: confidence interval.

2C). In the subgroup analysis using the same standard of leukopenia, the TT group had a 8.5 times higher risk of leukopenia incidence than the CT+CC group (OR=8.50, 95% CI: 2.38-30.32; *P*=0.0010) (Figure 2D).

Other than the correlation of *NUDT15* c.415C>T polymorphism with leukopenia being well investigated, some studies also focused on the association of *NUDT15* c.52G>A polymorphism with leukopenia in ALL patients. Two studies with 283 ALL patients in total were included to compare the events of leukopenia in *NUDT15* c.52G>A variant group (GA+AA) with the wild-type group (GG).[41,44] The difference between the two groups in the incidence of leukopenia was not statistically significant (OR=3.60, 95% CI: 0.79-16.38; *P*=0.10) (Figure 2E).

Association between NUDT15 c.415C>T polymorphism and 6-MP-induced neutropenia

We compared the events of neutropenia in *NUDT15* c.415C>T variant group and wild-type group based on ten studies comprising 1,082 patients with ALL.[24,27,35,38,45,46,48-51] Correa-Jimenez *et al.*[24] did not describe the definition of neutropenia, while three studies defined neutropenia as absolute neutrophil count (ANC) <1,500/μL, ANC <1,000/μL, and ANC <750/μL,[27,35,51] respectively, and the remaining six studies shared the same standard of neutropenia as ANC <500/μL (*Online Supplementary Table S1*).[38,45,46,48-50] The variant group (CT+TT) was proven to be significantly associated with a 2.5-fold higher risk of neutropenia in comparison to the wild-type group (OR=2.52, 95% CI: 1.72-3.69; *P*<0.00001) (Figure 3A). The subgroup analysis that defined ANC <500/μL as neutropenia showed that the CT+TT group had a 2.3 times higher risk of neutropenia incidence than the CC group (OR=2.28, 95% CI: 1.42-3.67; *P*=0.0007) (Figure 3B).

In addition, five studies with 552 patients were included to investigate the role of *NUDT15* c.415C>T gene polymorphism on the risk of neutropenia in the recessive model.[35,38,45,46,49] No significant difference between the two groups was found (OR=2.93, 95% CI: 0.92-9.37; *P*=0.07) (Figure 3C). However, the sensitivity analysis suggested that after excluding Li *et al.* 2021,[35] the homozygote variant of *NUDT15* c.415C>T (TT group) had a significantly 4.8 times higher risk of neutropenia incidence compared with the CT+CC group (OR=4.84, 95% CI: 1.21-19.35; *P*=0.03) (Figure 3D). A potential explanation was that only one patient with TT was in Li's analysis,[35] who did not even develop neutropenia at all, so the sample was too small to be representative.

Association between NUDT15 c.415C>T or NUDT15 c.52G>A polymorphism and 6-MP-induced hepatotoxicity

A total of 1,012 patients from seven studies were included for comparing the incidence of hepatotoxicity in *NUDT15* c.415C>T variant group with the wild-type group.[25,26,39-41,44,52] Two of the studies shared the same standard of hepatotoxicity as alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >5-fold of normal.[26,41] Three studies defined hepatotoxicity as ALT >500 U/L, ALT >700 U/L, and ALT or AST >500 U/L, respectively.[25,40,44] The remaining two defined hepatotoxicity as the highest direct bilirubin values ≥1.5 mg/dL, and as either ALT, AST, alkaline phosphatase or total bilirubin above 2-fold upper limit of normal, respectively.[39,52] All above-mentioned information are listed in *Online Supplementary Table S1*. The results revealed that *NUDT15* c.415C>T variants did not increase the incidence of hepatotoxicity (OR=1.27, 95% CI: 0.84-1.91; *P*=0.26) and the sensitivity analysis verified the stability of the results (Figure 4A). In addition, subgroup analysis of the two studies which defined ALT or AST >5-fold of normal as hepatotoxicity, did not show that the *NUDT15* c.415C>T variant group had an increased incidence of hepatotoxicity compared to the wild-type group (OR=1.06, 95% CI: 0.56-2.00; *P*=0.85) (Figure 4B). Moreover, no significant difference in the incidence of hepatotoxicity (OR=2.43, 95% CI: 0.95-6.20; *P*=0.06) (Figure 4C) was observed in the recessive model either.[25,39-41,44] Three studies comprising 426 ALL patients were included to compare the events of hepatotoxicity in *NUDT15* c.52 G>A variant carriers (GA+AA) *versus* wild-type patients (GG) and the result indicated that there was no significant difference in hepatotoxicity incidence in either (OR=1.09, 95% CI: 0.22-5.31; *P*=0.91) (Figure 4D).[25,41,44]

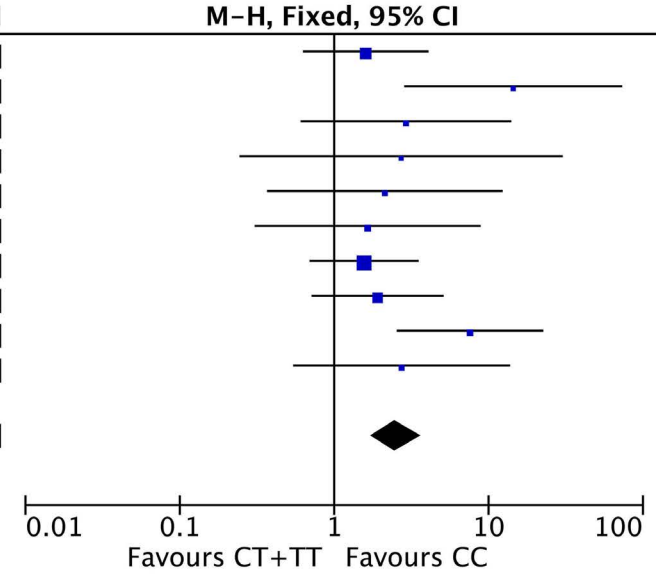
Association between NUDT15 c.415C>T polymorphism and 6-MP treatment interruption

Five studies with 537 ALL patients were included to compare the events of treatment interruption in the *NUDT15* c.415C>T variant group (CT+TT) and the wild-type group (CC).[24,27,40,43,45] The definition of treatment interruption of these included studies is listed in *Online Supplementary Table S1*. Specifically, treatment interruption was defined as the cessation of medicine administration caused by adverse events such as cytopenia, infections or hepatotoxicity. The result suggested that there was no significant difference in the incidence of treatment interruption between two groups (OR=1.36, 95% CI: 0.41-4.46; *P*=0.62) (Figure 5A). However, the sensitivity analysis did not support the result - with one study excluded,[40] the *NUDT15* c.415C>T variant carriers developed a 2.5-fold higher risk of treatment interruption than wild-type patients (OR=2.51, 95% CI: 1.23-5.15; *P*=0.01) (*Online Supplementary Figure S1*). Considering that patients with high-risk ALL were excluded in this study, and it defined interruption as the cessation of 6-MP resulting from infections or hepatotoxicity, this study had a lower interruption incidence than other studies. This might be a reason for its unstable results. Although there is a difference in the initial dose of 6-MP in these studies, the subgroup analysis of the four studies with the same initial dose still did not confirm a significant difference in the treatment interruption incidence between *NUDT15* c.415C>T variant group and the wild-type group (OR=1.48, 95% CI: 0.23-9.32; *P*=0.68) (*Online Supplementary Figure S2*). Nor did a significant difference exist between the

A

Study or Subgroup	CT+TT Events	CT+TT Total	CC Events	CC Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Buaboonnam 2019	12	24	30	78	20.9%	1.60 [0.64, 4.02]
Chiengthong 2016	10	12	18	70	2.6%	14.44 [2.89, 72.26]
Choi 2019	3	30	4	109	4.6%	2.92 [0.62, 13.82]
Correa-Jimenez 2021	1	4	7	64	1.8%	2.71 [0.25, 29.78]
Khaeso 2022	2	33	4	136	4.3%	2.13 [0.37, 12.15]
Khera 2019	14	16	34	42	6.9%	1.65 [0.31, 8.75]
Kim 2018	29	39	91	140	30.1%	1.56 [0.70, 3.47]
Li 2021	13	22	31	72	17.6%	1.91 [0.72, 5.04]
Pai 2021	12	18	24	115	6.4%	7.58 [2.58, 22.29]
Wang 2022	3	12	5	46	4.6%	2.73 [0.55, 13.58]
Total (95% CI)		**210**		**872**	**100.0%**	**2.52 [1.72, 3.69]**
Total events	99		248			

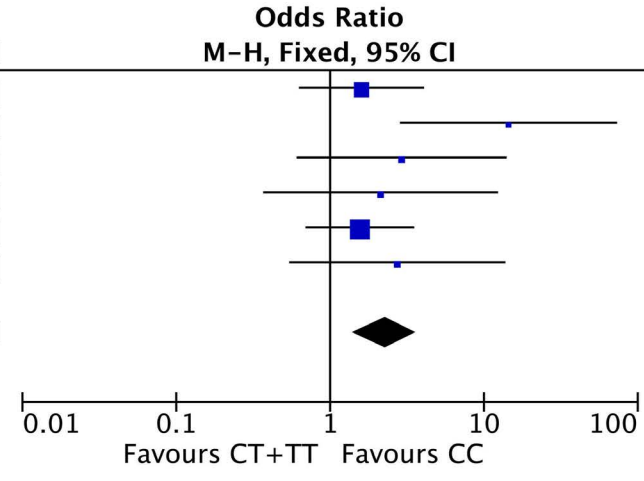
Heterogeneity: X^2 = 11.49, df = 9 (P= 0.24); I^2 = 22%
Test for overall effect: Z = 4.77 (P< 0.00001)



B

Study or Subgroup	CT+TT Events	CT+TT Total	CC Events	CC Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Buaboonnam 2019	12	24	30	78	31.1%	1.60 [0.64, 4.02]
Chiengthong 2016	10	12	18	70	3.9%	14.44 [2.89, 72.26]
Choi 2019	3	30	4	109	6.9%	2.92 [0.62, 13.82]
Khaeso 2022	2	33	4	136	6.5%	2.13 [0.37, 12.15]
Kim 2018	29	39	91	140	44.8%	1.56 [0.70, 3.47]
Wang 2022	3	12	5	46	6.8%	2.73 [0.55, 13.58]
Total (95% CI)		**150**		**579**	**100.0%**	**2.28 [1.42, 3.67]**
Total events	59		152			

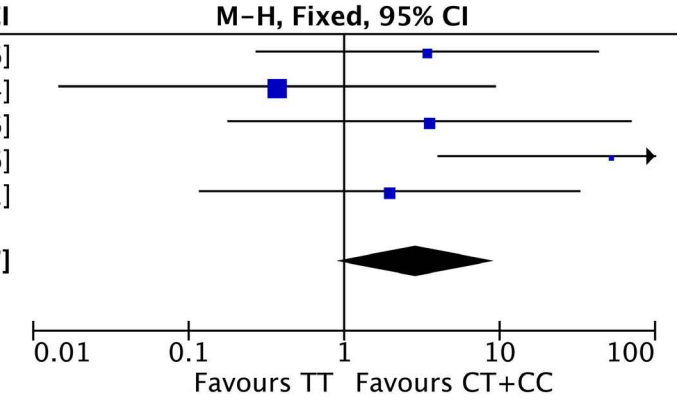
Heterogeneity: X^2 = 6.63, df = 5 (P= 0.25); I^2 = 25%
Test for overall effect: Z = 3.40 (P= 0.0007)



C

Study or Subgroup	TT Events	TT Total	CT+CC Events	CT+CC Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Wang 2022	1	3	7	55	14.8%	3.43 [0.27, 42.96]
Li 2021	0	1	44	93	42.7%	0.37 [0.01, 9.34]
Kim 2018	3	3	117	176	20.0%	3.54 [0.18, 69.76]
Choi 2019	2	3	5	136	2.2%	52.40 [4.05, 678.66]
Chiengthong 2016	1	2	27	80	20.2%	1.96 [0.12, 32.61]
Total (95% CI)		**12**		**540**	**100.0%**	**2.93 [0.92, 9.37]**
Total events	7		200			

Heterogeneity: X^2 = 6.56, df = 4 (P= 0.16); I^2 = 39%
Test for overall effect: Z = 1.81 (P= 0.07)



D

Study or Subgroup	TT Events	TT Total	CT+CC Events	CT+CC Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Chiengthong 2016	1	2	27	80	35.4%	1.96 [0.12, 32.61]
Choi 2019	2	3	5	136	3.9%	52.40 [4.05, 678.66]
Kim 2018	3	3	117	176	34.9%	3.54 [0.18, 69.76]
Wang 2022	1	3	7	55	25.9%	3.43 [0.27, 42.96]
Total (95% CI)		**11**		**447**	**100.0%**	**4.84 [1.21, 19.35]**
Total events	7		156			

Heterogeneity: X^2 = 3.83, df = 3 (P= 0.28); I^2 = 22%
Test for overall effect: Z = 2.23 (P= 0.03)

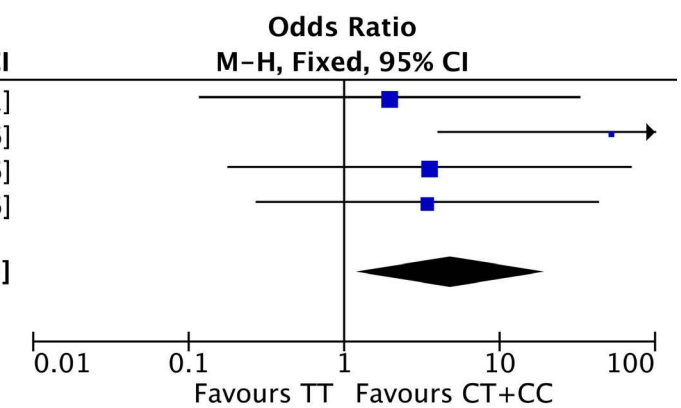


Figure 3. Forest plots for the meta-analysis of association of *NUDT15* c.415C>T (rs116855232) with 6-mercaptopurine-induced neutropenia. (A) The dominant model (CT+TT *vs.* CC); (B) subgroup analysis in the dominant model; (C) the recessive model (TT *vs.* CT+CC); (D) subgroup analysis in the recessive model. CI: confidence interval.

association of *NUDT15* c.415C>T polymorphism with occurrence of treatment interruption in the recessive model (OR=1.47, 95% CI: 0.39-5.52; P=0.57) (Figure 5B),[40,43,45] which was supported by sensitivity analysis.

Association between *NUDT15* gene polymorphism and efficacy of 6-MP

In addition to the above-listed adverse events, we were also concerned about whether the efficacy of 6-MP was

influenced by *NUDT15* gene polymorphism. The reported outcomes related to 6-MP's efficacy included event-free survival (EFS), overall survival (OS), relapse, and death. However, due to the limited studies and unavailable data, we could not evaluate EFS or OS using quantitative meta-analysis. Therefore our meta-analysis was limited to relapse of ALL patients after treatment with 6-MP as an efficacy outcome.

We did not find a pre-existing meta-analysis about the influence of gene polymorphism on the relapse of ALL patients after treatment with 6-MP. In order to fill this gap, in our research, 603 patients in five studies were involved to compare the events of relapse in *NUDT15* c.415C>T variant carriers with wild-type patients.[24,36,42,43,49] No significant difference was found in the relapse incidence between two groups (OR=1.20, 95% CI: 0.63-2.27; *P*=0.58) (Figure 6A), and the sensitivity analysis supported this finding. In addition, two studies with 402 patients

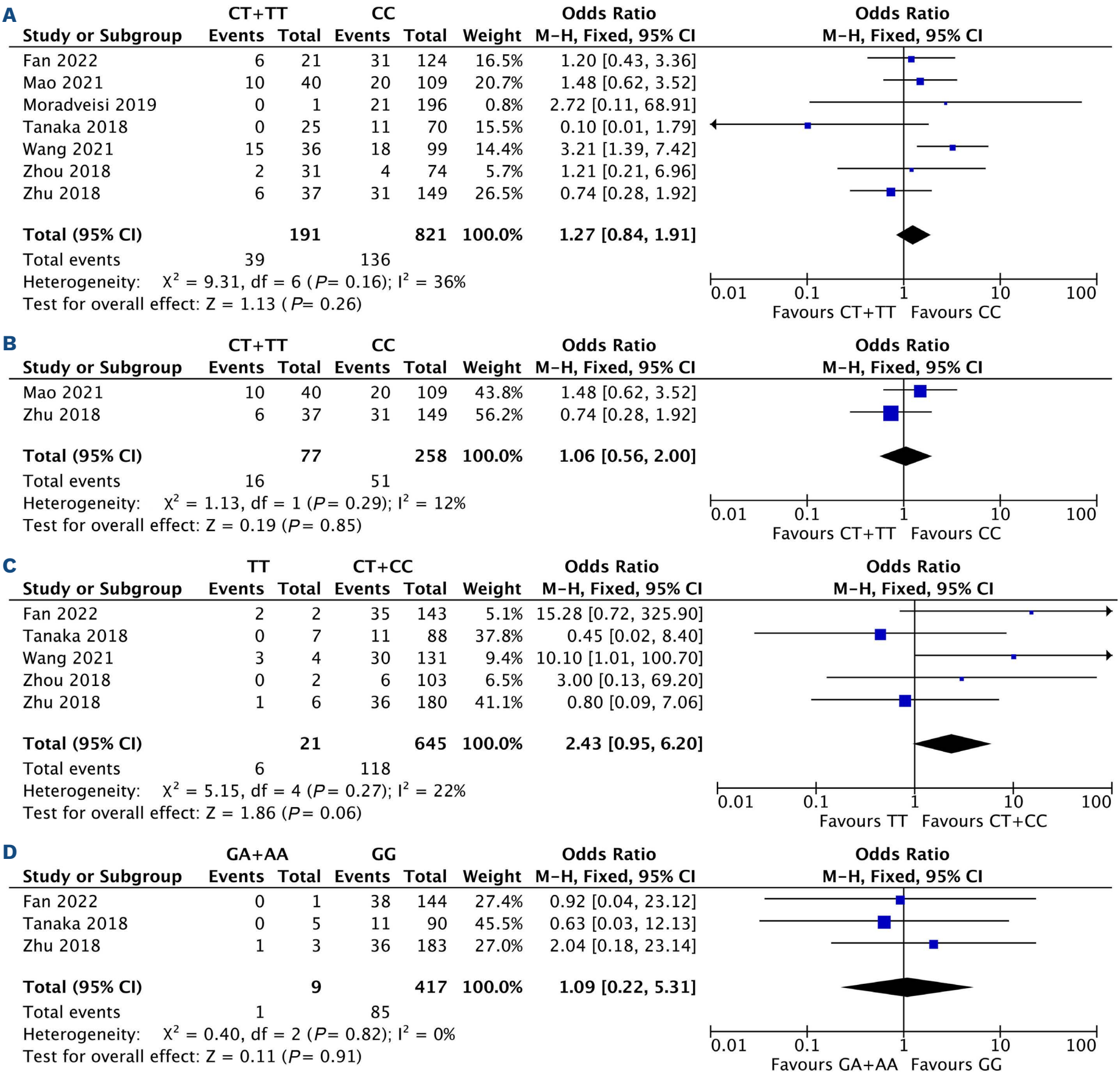


Figure 4. Forest plots for the meta-analysis of association of *NUDT15* c.415C>T (rs116855232) (A-C) or *NUDT15* c.52G>A (rs186364861) (D) with 6-mercaptopurine-induced hepatotoxicity. (A) The dominant model (CT+TT *vs.* CC); (B) subgroup analysis in the dominant model; (C) the recessive model (TT *vs.* CT+CC); (D) the dominant model (GA+AA *vs.* GG). CI: confidence interval.

were available for comparing the incidence of relapse in *NUDT15* c.415C>T recessive model, and similarly, no significant difference was observed (OR=2.29, 95% CI: 0.44-11.84; *P*=0.32) (Figure 6B).[36,43]

Association between *NUDT15* c.415C>T polymorphism and dose of 6-MP

The variants in *NUDT15* that can influence 6-MP tolerance in ALL patients have been identified. Nevertheless, there

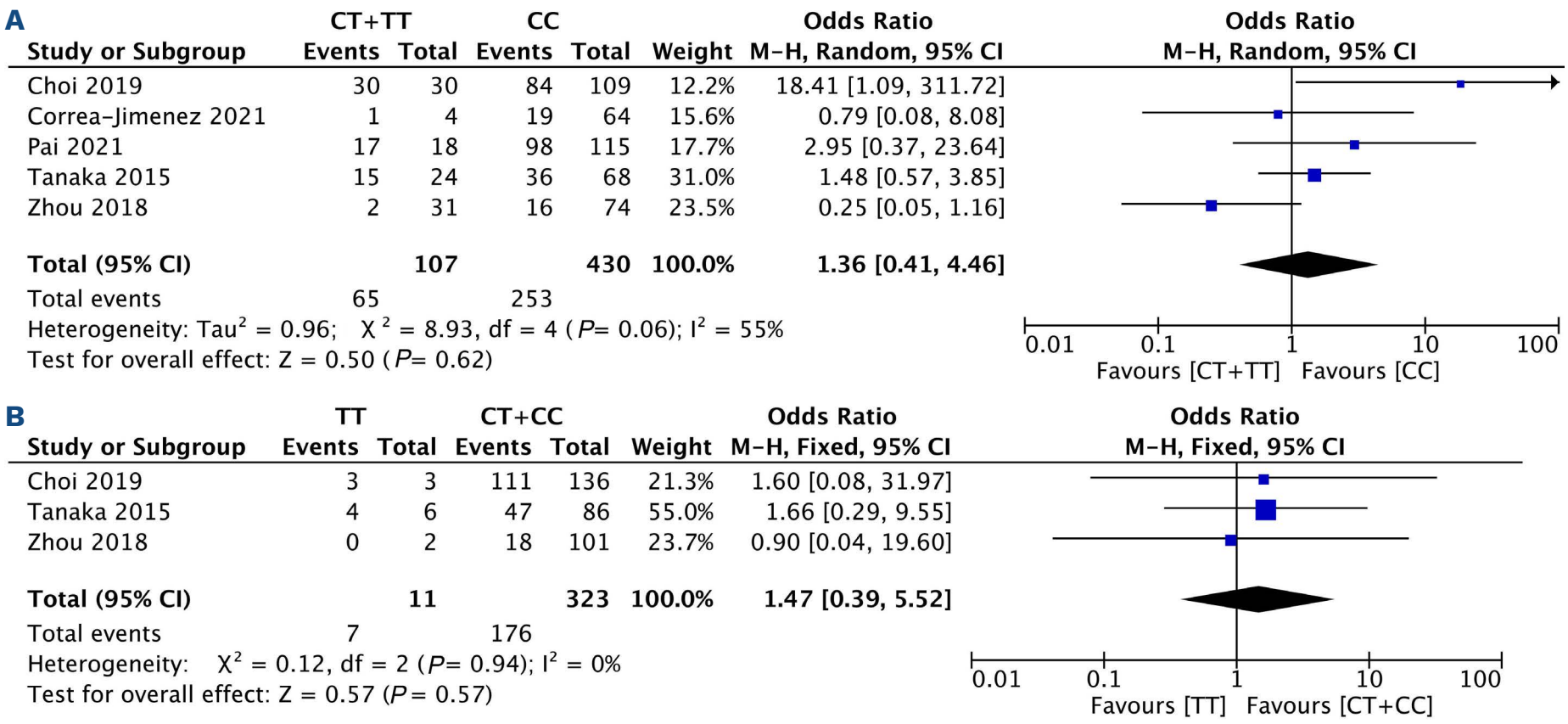


Figure 5. Forest plots for the meta-analysis of association of *NUDT15* c.415C>T (rs116855232) with 6-mercaptopurine treatment interruption. (A) The dominant model (CT+TT *vs.* CC); (B) the recessive model (TT *vs.* CT+CC). CI: confidence interval.

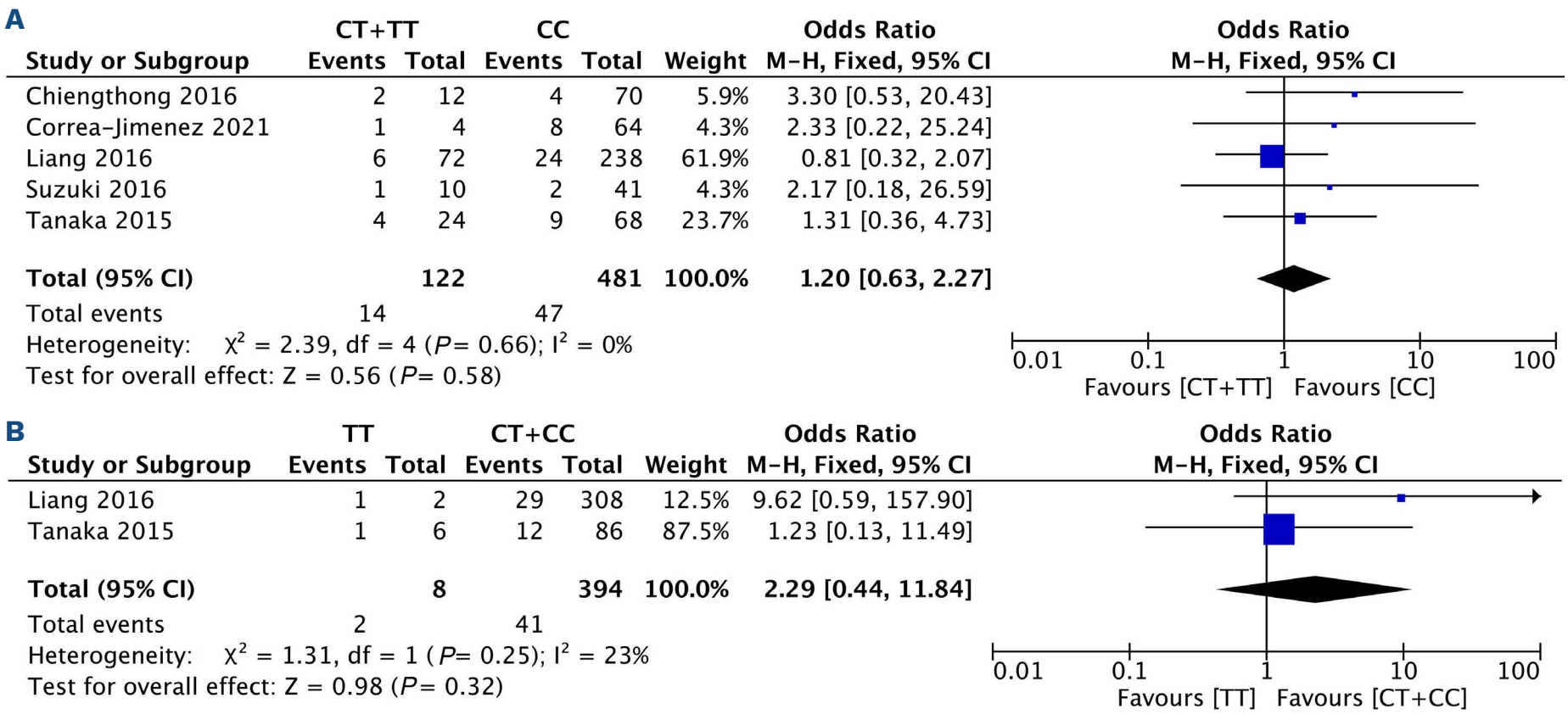


Figure 6. Forest plots for the meta-analysis of association of *NUDT15* c.415C>T (rs116855232) with relapse of patients after treatment with 6-mercaptopurine. (A) The dominant model (CT+TT *vs.* CC); (B) the recessive model (TT *vs.* CT+CC). CI: confidence interval.

has been no meta-analysis concerning the influence of gene polymorphism on the dosing of 6-MP in ALL. We did a quantitative analysis of the outcome regarding dosing of 6-MP (e.g., dose intensity, tolerable dose) in ALL to make a recommendation for the individualized therapy of 6-MP in ALL in the future.

Dose intensity and intolerant incidence of 6-MP

Dose intensity refers to the ratio of the actual prescribed 6-MP dose by physician to the protocol dose, for the dose of 6-MP would be adjusted because of adverse events or not achieving the target. We assessed potential effects of gene polymorphism on the dose adjustment of 6-MP in ALL. Two articles with 1,109 patients qualified for a meta-analysis about the association between *NUDT15* c.415C>T gene polymorphism and dose intensity of 6-MP in ALL,[21,47] and one provided two groups of data (1 represents AALL03N1 cohort, another 1 represents St Jude cohorts).[21] The dose intensity of 6-MP in ALL patients with one *NUDT15* c.415C>T variant alleles (CT) was 19% less than wild-type patients (CC) (MD: 19.43%, 95% CI: -25.36 to -13.51; *P*<0.00001) (Figure 7A). Only one study included the dose intensity of patients with two *NUDT15* c.415C>T variant alleles (TT) (8.3±7%, N=2),[21] so a quantitative meta-analysis of the model (TT *vs.* CC) was not feasible. Additionally, given that some studies defined 6-MP intolerance as dose intensity <50%, two studies were utilized to compare the risk of 6-MP intolerance between *NUDT15* c.415C>T variant carriers and wild-type patients.[27,44] We found that the *NUDT15* c.415C>T variant group (CT+TT) had a seven-times higher risk of developing 6-MP intolerance compared with the wild-type group (CC) (OR=6.98, 95% CI: 2.83-17.22; *P*<0.0001) (Figure 7B).

Tolerable dose of 6-MP

Two studies were included in the meta-analysis to compare the tolerable dose of 6-MP in 112 *NUDT15* c.415C>T variant carriers (5 homozygotes and 107 heterozygotes) with 347 wild-type patients.[26,36] The initial dose of 6-MP in these

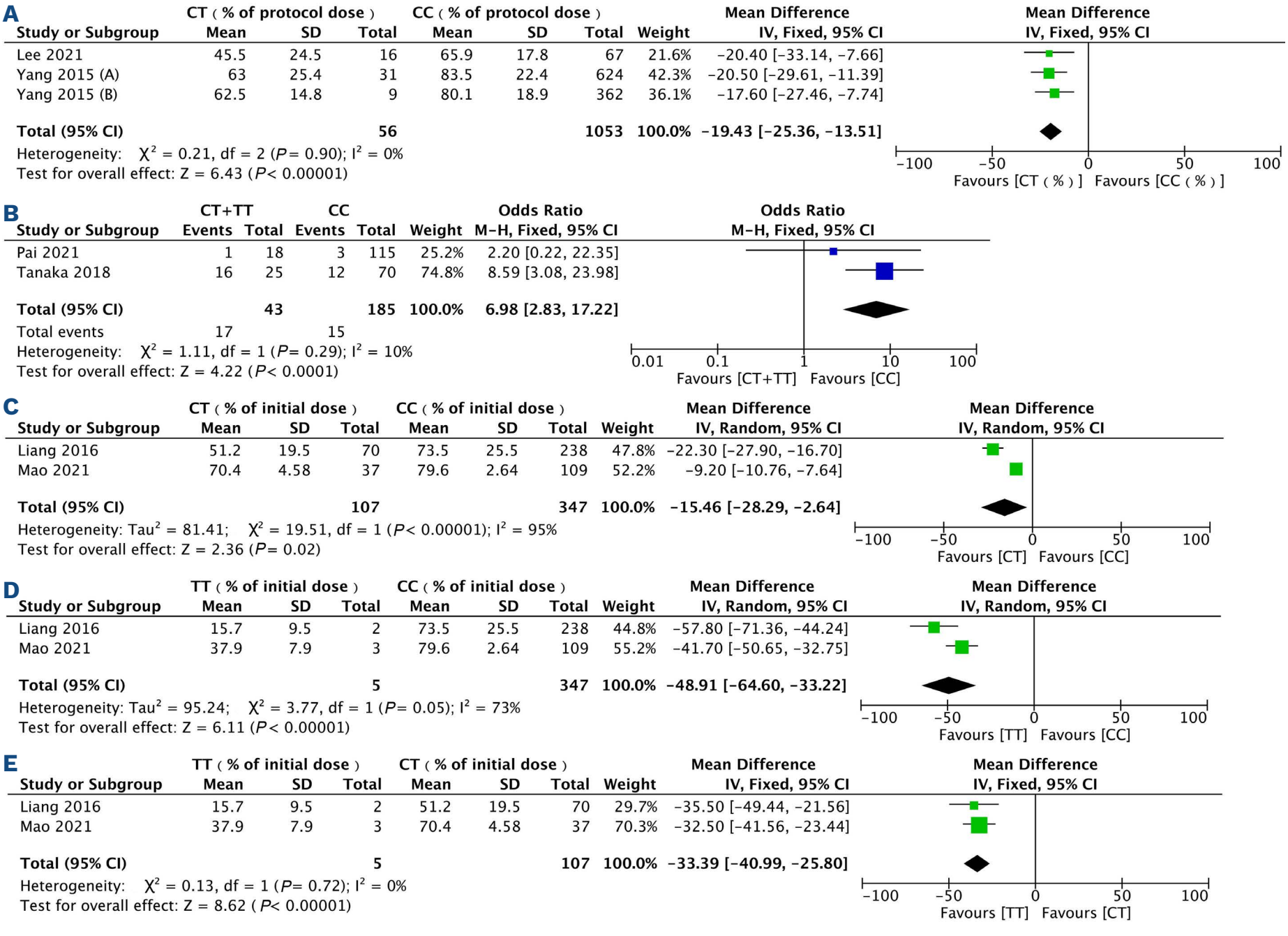


Figure 7. Forest plots for the meta-analysis of association of *NUDT15* c.415C>T (rs116855232) with dose intensity (A) or intolerant incidence of 6-mercaptopurine (B) or tolerable dose intensity (C-E). (A) CT *versus* CC; (B) CT+TT *versus* CC; (C) CT *versus* CC; (D) TT *versus* CC; (E) TT *versus* CT. CI: confidence interval.

two studies were 50 mg/m^2/d and 60 mg/m^2/d, respectively. We calculated the ratio of tolerable dose by initial dose as tolerable dose intensity for the purpose of avoiding the influence of the variation in initial dose between the two studies on the pooled results. The tolerable dose intensity of 6-MP in the *NUDT15* c.415C>T CC, CT and TT group was 80%, 70% and 38% respectively in Mao's research,[26] while in Liang's,[36] the tolerable dose intensity of 6-MP in the *NUDT15* c.415C>T CC, CT and TT group was 74%, 51%, 16% respectively. The meta-analysis suggested that the pooled tolerable dose intensity of 6-MP in the CT group was 15% less than that in the CC group (MD: -15.46%, 95% CI: -28.29 to -2.64; *P*=0.02) (Figure 7C). Furthermore, the tolerable dose intensity of 6-MP in the TT group was 49% less than the CC group (MD: -48.91%, 95% CI: -64.60 to -33.22; *P*<0.00001) (Figure 7D). Additionally, we found the tolerable dose intensity of 6-MP in the TT group was 33% less than that in the CT group (MD: -33.39%, 95% CI: -40.99 to -25.80; *P*<0.00001) (Figure 7E).

Discussion

6-MP is a commonly used drug for the treatment of ALL, especially in the maintenance phase.[53] As known to us, the typical adverse reactions of thiopurines include myelosuppression, hepatotoxicity, hair loss and pancreatitis *et al*.[54] Variants in *TPMT* gene, as the most well-known genetic predictor for myelosuppression of thiopurine, can be responsible for merely 25% of the leukopenia cases.[55] *NUDT15* was identified through a genome-wide association study (GWAS) as a predictor for thiopurine intolerance in children with ALL[21] and patients with IBD.[22]

Our systematic review suggested that *NUDT15* c.415C>T polymorphism had a strong relationship with 6-MP induced leukopenia and neutropenia in ALL patients but not with 6-MP induced hepatotoxicity. No strong correlation between *NUDT15* c.52G>A polymorphism and 6-MP induced leukopenia or hepatotoxicity was observed. It was demonstrated that *NUDT15* c.415C>T was less likely to be a good predictor for the relapse of ALL patients who were treated with 6-MP. Moreover, it was found that the dose intensity and tolerable dose of 6-MP in ALL patients with *NUDT15* c.415C>T variant were greatly reduced.

NUDT15 c.415C>T was demonstrated to be a genetic predictor for thiopurine-induced myelosuppression in a variety of diseases such as IBD,[56] ALL,[21] rheumatoid arthritis,[57] neurological diseases,[58] and autoimmune hepatitis,[59] which was supported by our study as well in ALL patients through meta-analysis. In our review, *NUDT15* c.415C>T variant carriers resulted in a 9-fold and 2.5-fold higher risk for leukopenia and neutropenia, respectively. In addition to *NUDT15* c.415C>T, 20 variants of *NUDT15* gene such as c.52G>A, c.36_37insGGAGTC *et al*. have been reported so far. However, the correlation between *NUDT15*5* and *NUDT15*6* with thiopurine-related myelosuppression remain contested.[50] Our study suggested that the difference in the risk for 6-MP-associated leukopenia in *NUDT15* c.52G>A variant carriers and wild-type patients was not statistically significant. The influence of *NUDT15* c.52G>A gene polymorphism on 6-MP-associated leukopenia was less than *NUDT15* c.415C>T in our study. This is consistent with the analysis of IBD patients,[60] and substantiates *NUDT15*5* being classified as uncertain function alleles in the Clinical Implementation Consortium (CPIC) Guidelines.[61] Associations of other variants of the *NUDT15* gene with 6-MP-induced hematotoxicity were not evaluated in the meta-analysis due to lack of enough studies (N<2).

Hepatotoxicity is another common adverse reaction which always leads to the reduction or interruption of thiopurine. Different studies have inconsistent findings regarding association of *NUDT15* polymorphism with 6-MP-induced hepatotoxicity,[25,26,39-41,44,52] and the majority of studies fail to find *NUDT15* c.415C>T to be a predictor for hepatotoxicity of 6-MP. Some researchers explain that *NUDT15* might not affect 6MMPN (6-methyl mercaptopurine nucleotide), which is known as a metabolite of thiopurine associated with hepatotoxicity.[62] Other than *NUDT15*, *TPMT*3C* rs1142345,[63] *COMT* rs4680,[34] and *MTHFR* rs1801133 variants *et al*.[64] are identified as genetic predictors of thiopurine-associated hepatoxicity. Possible varying effect magnitudes of these genes in inducing hepatotoxicity leave room for future's research. The influence of combinational drug, methotrexate (MTX), which is also hepatoxic, should be considered as a confounding factor as well. Though interruption of 6-MP is supposed to be an indication of incidence of serious adverse reaction, our meta-analysis failed to find a significant relationship between *NUDT15* c.415C>T polymorphism and interruption of 6-MP. Nevertheless, the high heterogeneity between included studies and the significant greater risk for therapy interruption in *NUDT15* c.415C>T variant carriers after excluding Zhou's research[40] showed that the pooled results are subject to change by adding high quality studies in future. We speculated that the cause of this situation may be exclusion of high-risk ALL individuals and different definition of "interruption" in Zhou's research. Bhatia *et al.*[65] once demonstrated that 6-MP non-adherence led to a high risk of relapse, and variability in TGN levels also contributed to the relapse incidence. The interruption of 6-MP should be avoided to minimize the relapse of ALL, and using *NUDT15* genetic testing-guided 6-MP dosing to avoid the treatment interruption of 6-MP is worthy of research.

In addition to investigating the influence of *NUDT15* polymorphism on 6-MP-associated adverse reaction, we also wonder if gene polymorphism plays a role on the efficacy of 6-MP in ALL patients. The reported efficacy outcomes of 6-MP in ALL include EFS, OS, relapse, and death.[26,27,36,43,48] The rate of relapse in ALL patients appears high in developing countries,[66] which relates to a poor prognosis and acts as one of major causes for death.[67] Therefore, elucidating the predictor for relapse and preventing its occurrence is important. The higher level of DNA-TG, the downstream metabolite of

MTX/6-MP combination chemotherapy, is found to be correlated with a lower relapse hazard.[6,68] As a pharmacogenetic predictor for relapse of ALL, phosphoribosyl pyrophosphate synthetase 1 gene (*PRPS1*)[67,69] and cytosolic 5'-nucleotidase II gene (*NT5C2*)[70,71] are identified as relapse-specific mutations in recent research. In our meta-analysis, no association between *NUDT15* c.415C>T polymorphism with relapse risk of ALL was observed. Nishii *et al.*[72] found that DNA-TG was positively correlated with 6-MP dosage regardless of *NUDT15* genotype. In their research, 6-MP dose was reduced in *NUDT15*-deficient patients to achieve a level of DNA-TG comparable to that in wild-type patients and thereby mitigate *NUDT15* deficiency-mediated toxicity. Many studies have reported that patients with *NUDT15* variants receive lower dose of 6-MP than those harboring wild-type *NUDT15* during ALL treatment, and this was supported by our meta-analysis.[21,40,43] In conclusion, the similar relapse rate in the *NUDT15* group in this study was probably because the 6-MP dose during treatment was adjusted to optimize the exposure of DNA-TG in expectation of preventing 6-MP toxicity. However, this study is limited by the relatively small sample size and short follow-up period, so research with large samples and long follow-up is expected in the future to confirm the effect of *NUDT15* on relapse risk. Because of the limited number of relevant studies and some unavailable data, we did not perform a meta-analysis about the association of *NUDT15* polymorphism with EFS and OS in the present review. Although all of the above-mentioned studies found no significant difference for OS or EFS among *NUDT15* c.415C>T genotypes,[26,27,36,43,48] Tanaka *et al.*[43] pointed out a tendency for a worse EFS rate in their study in carriers of *NUDT15* c.415C>T variant allele, though explanations were not given. Therefore, further investigating this potential correlation is recommended. Death cases were reported only in two studies, and no significant difference in the incidence of death between the *NUDT15* c.415C>T variant group and the wild-type group was observed (*Online Supplementary Figure S3*).

Pharmacogenomics studies can not only help us identify pharmacogenetic predictors for efficacy and safety of medical treatment, but also aim to provide references for individualizing therapy. *NUDT15* variants carriers have a greater risk for developing leukopenia than wild-type patients, and accordingly, dosing of 6-MP based on *NUDT15* genotype to avoid the severe adverse reaction and maintain the efficacy is a critical issue. Although CPIC Guidelines gave a recommendation for thiopurine dosing based on *TPMT* and *NUDT15* genotypes, the dosing of 6-MP is not always adjusted according to the guideline in the current clinical practice.[73] In addition, the efficacy and safety of 6-MP regimen adjustment also have not yet been verified in clinical use. In this article, we conducted a meta-analysis to compare the pooled dose intensity, tolerable dose of 6-MP in the clinical study of ALL patients who carry *NUDT15* variants with wild-type patients, providing a reference for dose adjustment for *NUDT15* variants carriers in clinical practice. Due to the variance in the initial dose of 6-MP in the included studies, it would be preferable to use dose intensity for the dose adjustment of 6-MP, that will give a better idea on how much the dose was reduced. The tolerable dose intensity of 6-MP in ALL patients with *NUDT15* c.415C>T homozygote variant (TT) and heterozygote variant (CT) was 49% and 15% less than that of wild-type patients, reminding us patients with *NUDT15* c.415C>T homozygote variant (TT) should be much more carefully monitored and 6-MP dose should be greatly reduced.

Former reported meta-analyses seldomly evaluated the association of *NUDT15* polymorphism with 6-MP-induced leukopenia in a specific disease,[28,29,31,74] except for one which found increased risk for thiopurine-induced leukopenia in IBD patients for *NUDT15* c.415C>T (OR=6.90, 95% CI: 5.2-9.1),[30] and another one which conducted a subgroup analysis in different diseases and reported a significant association between *NUDT15* c.415C>T and leukopenia in ALL patients with only four studies included (OR=13.13, 95% CI:3.43-50.23 for the dominant model).[75] The increased risk for 6-MP-induced leukopenia in ALL patients with *NUDT15* c.415C>T variants was 9-fold higher than wild-type ones according to our meta-analysis of eight studies with large sample sizes. This increases the reliability of the results in ALL patients. Furthermore, association between *NUDT15* polymorphism with neutropenia, hepatotoxicity, treatment interruption, treatment efficacy and dose of 6-MP in ALL patients were also for the first time reported in our meta-analysis.

However, there are some limitations in our systematic review. First of all, the majority of included studies are retrospective and the studies regarding dose intensity of 6-MP and tolerable dose of 6-MP are few. Secondly, there is a high heterogeneity between different studies concerning incidence of leukopenia and interruption. This is probably ascribed to inconsistent definition of the outcome such as leukopenia or the difference in the baseline such as the initial dose of 6-MP. Moreover, low representation of a small sample for patients with *NUDT15* variants in several studies also contributes to the heterogeneity. Thus, more studies with a larger sample size are expected to further confirm the reliability of the present results. Thirdly, some outcomes were presented in different forms, further limiting the number of comparable studies. For example, a study reported 5-year EFS,[48] while another one provided 3-year progression-free survival.[27]

In conclusion, our meta-analysis showed that *NUDT15* c.415C>T was a good predictor for 6-MP-induced myelosuppression in ALL patients, and the dose intensity of 6-MP in ALL patients with *NUDT15* c.415C>T variants was much less than in wild-type patients. Although *NUDT15* c.415C>T variants carriers might have a higher risk of experiencing treatment interruption and relapse of ALL than wild-type patients, the correlation is not significant in this meta-analysis. Further large prospective studies, which can better evaluate

the association between *NUDT15* gene, efficacy, adverse reaction and dose of 6-MP in ALL patients, are encouraged. Additionally, the influence of confounding factors such as other genes (*TPMT*, inosine triphosphatase [*ITPA*] *et al.*) or the combinational drug on efficacy and adverse reactions of 6-MP requires further investigation. The economic stake of pretreatment genetic testing is an important clinical concern. So far, only a few studies have reported cost effectiveness analysis of pretreatment screening for *NUDT15*-defective alleles when using thiopurines in ALL or IBD patients.[73,76] Further prospective studies of genotype-guided dosing of 6-MP with large sample size are needed to assess the efficacy, safety and economic benefits of pretreatment *NUDT15* gene testing. It is also of great significance to compare the cost effectiveness of pretreatment *NUDT15* c.415C>T testing alone or in combination with other *NUDT15* risk alleles or with other genes such as *TPMT* and *ITPA*.

Conclusion

Through this systematic review, we provided the following revelations for the clinical practice. Firstly, *NUDT15* c.415C>T variants are likely to increase the risk of toxicity of 6-MP in ALL patients. Secondly, the dose intensity and tolerable dose of 6-MP in *NUDT15* c.415C>T variant carriers were significantly less than that in wild-type patients, so the dose adjustment of 6-MP during the treatment should be individualized among different *NUDT15* genotypes. Thirdly, *NUDT15* genotype did not affect the relapse of ALL based on the current research.

Disclosures

No conflicts of interest to disclose.

Contributions

SD and JLC conceived the study. SD, XFH and XH performed literature search. SD, XFH, XXL and JLC performed article selection, data extraction and analysis. SD and XFH wrote the manuscript draft. JLC, XXL, MM, MC, XH and HKS provided guidance on the methodology. SD, XFH, JLC, XXL, XH, MM, MC, RZ and ZYL edited the manuscript. SD, JLC and XXL funded the study. All authors approved final version of the article.

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Data-sharing statement

All original data of our study are included in the manuscript and Online Supplementary Material. The Online Supplementary Material can be found with the online version of this article. The protocol for this systematic review was registered on PROSPERO.

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