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ORIGINAL ARTICLE

A systematic review and meta-analysis of the impact of *WT1* polymorphism rs16754 in the effectiveness of standard chemotherapy in patients with acute myeloid leukemia

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The polymorphism rs16754 of the *WT1* gene has been described as a possible prognostic marker in different acute myeloid leukemia (AML) cohorts; however, it is not supported by all the studies. We performed the first meta-analysis evaluating the effect of this polymorphism upon the effectiveness of standard AML therapy. Fourteen cohort studies were included (3618 patients). Patients with the variant allele showed a significant higher overall survival (OS) at 5 years (OR:1.24, 95% CI: 1.06–1.45, $P = 0.007$, with dominant model). *WT1* did not influence complete remission, but a higher disease-free survival was observed with the variant allele. In the subgroup analysis, Caucasians, pediatric and patients treated with idarubicin and etoposide carrying the variant allele showed consistent results in OS, whereas patients with cytogenetically normal AML did not show differences. To verify the effect of this polymorphism upon other outcomes, studies in larger and multiracial populations are needed.

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INTRODUCTION

Acute myeloid leukemia (AML) is a clinically and biologically heterogeneous disease characterized cytogenetically by recurrent abnormalities, which provide powerful prognostic information.[1] Cytogenetically normal AML (CN-AML) is the largest subgroup, representing ~45% of adult patients with AML.[2] In the last years, emerging data have indicated that somatic mutations are associated with treatment outcome and serve as a basis for molecularly guided risk assessment and treatment stratification.[3–7]

Mutations in the *Wilms tumor 1* gene (*WT1*) occur in ~10% of adults with CN-AML[8] and are potential markers in AML. Although these mutations are predicted to lead to loss of function of *WT1* with several studies reporting a worse outcome,[9–15] other authors found that these mutations have no prognostic impact.[16–18]

The *WT1* gene, located on chromosome band 11p13, encodes a zinc-finger transcription factor, which has emerged as an important regulator of normal and malignant hematopoiesis. Originally, *WT1* was identified as a tumor-suppressor gene isolated in Wilms tumor,[19] a pediatric kidney malignancy. However, accumulating data revealed that *WT1* appears to have an oncogenic rather than tumor-suppressor role.[20] Although the *WT1* role in hematopoiesis has not been clarified, expression was inversely associated with stem cell proliferation and differentiation.[21]

Almost all leukemia-associated *WT1* mutations occur within the zinc-finger domains; mainly within a hotspot in exon 7. This exon is the location of the synonymous single-nucleotide polymorphism (SNP) rs16754 that results in adenine (A) or guanine (G) containing alleles. The frequencies of these alleles vary between ethnic groups, so that G is the minor allele in Caucasians and the major allele in Asian populations.[22] *WT1* polymorphism does not seem to increase susceptibility to AML, with similar frequency between healthy volunteers and AML patients.[17] This synonymous SNP may affect to treatment outcome with different potential mechanisms, as alterations in RNA expression, stability, splicing and binding and changes in translational kinetics,[23] or on the other hand it could be in linkage disequilibrium with a functional SNP. As for *WT1* mutation, several publications have investigated the prognostic impact of *WT1* rs16754 SNP in AML cohorts producing non-conclusive or contradictory results.[17,24–37]

Considering that *WT1* rs16754 SNP may have a promising albeit inconclusive role in AML treatment, we carried out a meta-analysis on all eligible observational studies to estimate the effect of *WT1* polymorphism on AML patients and to quantify the potential between-study heterogeneity.

MATERIALS AND METHODS

Search strategy and selection of studies

This meta-analysis and systematic review was conducted and reported in accordance with the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-analyses)[38] by two independent authors (JM and MH).

We searched the following databases without restrictions: MEDLINE, Cochrane Central Register, EMBASE, Web of Science and Database of Abstracts of Reviews of Effects (DARE), ProQuest Medical Library, EBSCOhost Online Research Databases, WanFang and Chinese National Knowledge Infraestructure (CNKI) and LILACS. There were no limitations in

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Table 1. Characteristics of the studies included in the meta-analysis for *WT1* rs16754 polymorphism

Study	n	Age, years (range)	Sex: male/female (%)	Ethnia	HWE	Genotype frequencies (%) of rs16754 A>G			AML status (%)		WBC count 10^9 per l (range)	FAB subtype	Cytogenetic risk (%)				Mutation status	Chemotherapy scheme	Clinical outcome
						AA	AG	GG	De novo	Secondary			Fav	Normal	Unfav	NR			
Ma *et al.*[24]	174	NR (adults)	NR	NR (Caucasian frequency)	Yes	70	26	4	100	0	NR	NR	NR	NR	NR	NR	NR	NR	OS at 7 years
Damm *et al.*[17]	249	46.8 (17–60)	51.8/48.2	Caucasian	Yes	74.3	24.1	4	91.6	8.4	26.2 (0.5–328)	Reported	0	100	0	0	*FLT3-ITD, NPM1, CEBPA, WT1, MLL-PTD*	Ara C+IDA +ETOP ± Others	CR, OS, RFS at 5 years
Hollink *et al.*[32]	232	9.2 (0.01–18.8)	57.3/42.7	Caucasian	Yes	72.8	25	2.2	100	0	40.6 (0.7–534.6)	Reported	22.8	53.9	0	23.3	*FLT3-ITD, NPM1, CEBPA, WT1, MLL-PTD*	Ara C+ANT	CR, OS, EFS at 5 years
Damm *et al.*[25] reply to Hollink	101	NR (adults)	NR	Caucasian	NR	75.2	24.8	NR	NR	NR	NR	100	0	0	0	NR	Ara C+IDA +ETOP ± Others	OS at 5 years	
Ho *et al.*[27]	790	10.2 (0.01–21.6)	53.5/46.5	Caucasian: 64 Hispanic: 16.5 African:10.5 Asian: 3.7	No	71	24.2	4.8	100	0	21.8 (0.3–860)	Reported	18.2	36.8	4.9	40	*FLT3-ITD, NPM1, CEBPA, WT1*	Ara C+IDA or DAUNO+6-TP+DEX +ETOP ± Others	CR, RR, OS, DFS,TRM at 5 years
Renneville *et al.*[33]	511	51 (15–71)	54/46	Caucasian	Yes	72.4	24	3.6	100	0	NR	Reported	10	56	20	14	*FLT3-ITD, NPM1, CEBPA, WT1*	Ara C+IDA or DAUNO or MIT ± Others	CR, RR, OS, at 5 years
Becker *et al.*[35]	433	62 (18–83)	50/50	Caucasian: 90 African: 5.5 Hispanic: 3 Asian: 0.7	Yes	71.4	25.9	2.8	100	0	26.5 (0.9–450)	Reported	0	100	0	0	*FLT3-ITD, NPM1, CEBPA, WT1, MLL-PTD*	Ara C +DAUNO ± Others	CR, OS, DFS at 5 years
Abalkhail *et al.*[29]	38[a]	8 (0.7–14)	NR	Arabs	Yes	53	42	5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	OS at 5 years
Choi *et al.*[36]	73	42 (15–78)	63/37	Asian[b]	Yes	6.8	39.7	53.4	100	0	23.1 (1–270)	NR	0	100	0	0	*FLT3-ITD, NPM1*	Ara C+IDA or DAUNO	CR, RR, OS, RFS, EFS at 10 years
Chen *et al.*[31]	86	6.7 (0.3–15)	51.2/48.8	Asian[b]	No	8.1	20.9	70.9	100	0	12.1 (0.9–723)	Reported	41.9	16.3	25.6	16.2	*FLT3-ITD, NPM1, CEBPA, WT1*	Ara C+DAUNO +ETOP[c]	CR, RR, OS, RFS at 3 years
Ho *et al.*[28]	466	NR (children)	NR	Caucasian: 65.4 Hispanic: 16.7 African: 9.6 Asian: 3.0	NR	68.9	27.3	100	0	NR	NR	NR	NR	NR	NR	NR	Ara C+IDA or DAUNO+6-TP +DEX +ETOP ± FLUDA	OS, TRM at 5 years	
Luna *et al.*[34]	138	62 (16–88)	57/43	Caucasian	Yes[d]	70.3	29.7	0	100	0	11.7 (0.6–396)	Reported	7.9	67.5	24.5	0	*FLT3-ITD, NPM1, CEBPA, WT1*	Ara C+IDA ± ETOP	CR, OS, DFS, RFS at 10 years
Luo *et al.*[30]	122	45 (16–72)	57/43	Asian	No	16.4	36.1	47.5	100	0	51.4 (0.5–300.5)	Reported	26.1	43.5	24.6	5.8	*FLT3-ITD, CEBPA, WT1, MLL-PTD*	Ara C+IDA or DAUNO	CR, RR, OS, DFS at 3 years
Zhang *et al.*[37]	205	40 (18–72)	54.2/45.9	Asian	Yes	7	40	53	100	0	18.8 (0.7–343.4)	Reported	9.3	0	61.9	28.8	NR	Ara C+DAUNO or MIT	CR, OS, RFS at 5 years

Abbreviations: AML, acute myeloid leukemia; AMSA, Amsacrine; ANT, Anthracycline; Ara C, Cytarabine; *CEBPA,* CCAAT/enhancer-binding protein alpha; CR, complete remission; DAUNO, daunorubicin; DEX, dexamethasone; DFS, disease-free survival; EFS, event-free survival; ETOP, etoposide; FAB, French–American–British; Fav, favorable; *FLT3-ITD,* FMS-related tyrosine kinase 3 internal tandem duplication; FLUDA, Fludarabine; HWE, Hardy–Weinberg equilibrium; IDA, Idarubicin; MIT, mitoxantrone; *MLL-PTD*, mixed lineage leukemia protein partial tandem duplications; *NPM1*, nucleophosmin; NR, not reported; OS, overall survival; RFS, relapse-free survival; RR, rate of relapse; 6-TP, 6-Thioguanine; TRM, treatment-related mortality; Unfav, unfavorable; WBC, white blood cell; *WT1*, Wilms tumor 1. [a]The abstract studied 86 patients (38 pediatric and 48 adults) but it only included survival data of pediatric population. [b]Frequencies in Asian population are opposite than Caucasians, GG is the homozygous wild type and AA the homozygous mutant genotype. [c]Twenty of the included patients did not meet all the inclusion criteria (FAB-subtype M3). [d]Only reported AA and AG genotypes. The GG genotype was no found.

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Table 2. Methodological quality of studies

Study	*Selection*				*Comparability*		*Outcome*		
	Representativeness of exposed individuals in the community	*Cohorts drawn from the same community*	*Standard method for measure of effectiveness outcomes (OS, CR)*	*Demonstration that outcome was not present at start of study*	*Comparability of cohorts (age, gender)*	*Control of confounders (ethnicity, different baseline pathologies, other therapies[a])*	*Assessment of outcome with bone marrow aspirates or biopsies*	*Follow up long enough for outcomes to occur (⩾24 months)*	*Adequacy of follow-up of cohorts*
High quality									
Damm *et al.*[17]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hollink *et al.*[32]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Zhang *et al.*[37]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Moderate quality									
Ho *et al.*[27]	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Renneville *et al.*[33]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Becker *et al.*[35]	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Choi *et al.*[36]	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Chen *et al.*[31]	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Ho *et al.*[28]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Luna *et al.*[34]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Luo *et al.*[30]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Low quality									
Ma *et al.*[24]	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Abalkhail *et al.*[29]	No	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Damm *et al.*[25] reply to Hollink	No	Yes	Yes	Yes	No	No	Yes	Yes	Yes

Abbreviations: CR, complete remission; OS, Overall survival. [a]Other therapies: transplantation, radiotherapy, different induction scheme.

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language, date or status publication restrictions. Additional studies were identified by manual search of the following journals: *Leukemia*, *Cancer*, *British Journal of Haematology*, *Blood*, *Lancet*, *Lancet Oncology*, *Journal of Clinical Oncology*, *Pharmacogenomics*, *Pharmacogenomics Journal and Pharmacogenetics and Genomics*. Congress abstracts of the American Society of Hematology (ASH), the European Hematology Association (EHA) and the Spanish Society of Hematology and Hemotherapy (SEHH) were reviewed. We also hand searched the reference lists of important studies and reviews. The literature last search was on 4 June 2015.

Similar keywords were used in different databases: *WT1* (or *wilms tumor gen* or rs16754), AML and polymorphism (or single-nucleotide or SNP or genetic polymorphisms or pharmacogenetics).

Study selection was conducted by both authors independently. In case of disagreement a third reviewer (LR) was contacted. Studies that fulfilled the following criteria were included: (1) AML studies using standard induction (including cytarabine, anthracyclines and/or etoposide); (2) studies containing useful genotype frequencies of *WT1* rs16754 polymorphism; (3) studies evaluating the association of the *WT1* polymorphism and AML outcomes; (4) *in vivo* studies. Studies that included patients with promyelocytic leukemias (FAB subtype M3) were excluded since these leukemias were treated with different regimens.

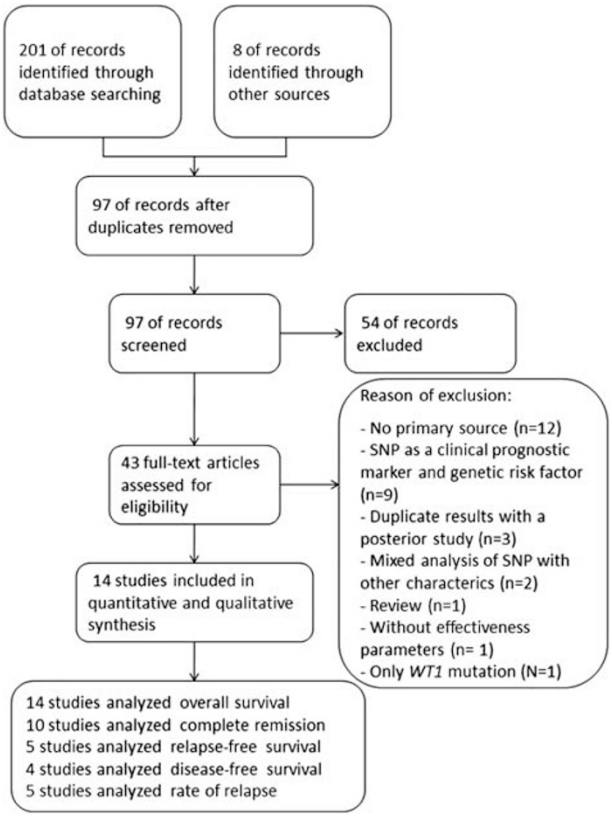

Figure 1. Summary of evidence search and selection.

Data extraction

Information was extracted independently by two reviewers. From included studies the following data was extracted (summarized in Table 1): characteristics of the study (language, publication status and methodological quality), patients baseline characteristics (sex, median age, ethnic origen, AML status, FAB subtype, diagnosis white blood cells (WBC), cytogenetics and main mutations), chemotherapy scheme and polymorphism frequencies of rs16754 SNP (A > G) and if they are in accordance with Hardy–Weinberg equilibrium, as well as genotyping method. We also collected effectiveness outcomes: mean overall survival (OS), complete remission (CR) and others.

Three authors were contacted to request missing information regarding outcomes, but the response was not received and we inferred the effectiveness data with the available information (like Kaplan–Meier graphs or number of events), when possible.

Methodological quality

Two reviewers (JM and MH) independently assessed the methodological quality of the included studies. Disagreements were recorded and resolved

Table 3. Summary of ORs with 95% CIs for ordinary genetic contrasts of the association between the *WT1* rs16754 polymorphism and effectiveness variables with fixed effects

Contrast	*Overall subgroup*	n	*OR (95% CI)*	I^2 *(%)*	P-*value*[b]
OS at 5 years (Figure 2)					
AA vs **AG/GG**	All studies[17,24,25,27–37]	3618	1.24 (1.06–1.45)	67[a]	**0.007**
AA/AG vs **GG**	10 studies[17,24,27,30–33,35–37]	2875	1.12 (0.84–1.49)	41	0.44
OS at 3 years					
AA vs **AG/GG**	12 studies[17,24,25,27,29–31,33–37]	2920	1.11 (0.93–1.32)	69	0.24
AA/AG vs **GG**	9 studies[17,24,27,30,31,34–37]	2643	1.02 (0.77–1.35)	53	0.91
CR at 5 years					
AA vs **AG/GG**	10 studies[17,27,30–37]	2793	1.10 (0.89–1.37)	0	0.38
AA/AG vs **GG**	8 studies[17,27,30–33,35,36]	2655	0.80 (0.58–1.11)	7	0.18
RFS at 5 years					
AA vs **AG/GG**	5 studies[17,31,34,36,37]	703	1.19 (0.81–1.76)	71	0.38
AA/AG vs **GG**	4 studies[17,31,36,37]	565	0.79 (0.51–1.22)	27	0.28
DFS at 5 years					
AA vs **AG/GG**	4 studies[27,30,34,35]	1303	1.03 (0.80–1.32)	52	0.84
AA/AG vs **GG**	3 studies[28,30,35]	1165	1.77 (1.09–2.86)	5	**0.02**
RR at 5 years					
AA vs **AG/GG**	5 studies[27,30,31,33,36]	1387	0.85 (0.66–1.09)	0	0.21
AA/AG vs **GG**	5 studies[27,30,31,33,36]	1387	0.88 (0.58–1.33)	0	0.54

Abbreviations: CN-AML, cytogenetically normal acute myeloid leukemia; CR, complete remission; DFS, disease-free survival; OR, odds ratio; OS, overall survival; RFS, relapse-free survival; RR, rate of relapse; *WT1*, Wilms tumor 1. [a]Significant heterogeneity using the fixed-effect model. We calculated with random effects (OR: 1.19, 95% CI: 0.86–1.63, I^2: 67%, *P*: 0.30). [b]*P*-value of test of overall effect. Results with statistical significance are in bold.

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by a third reviewer (LR). Kappa statistics were used to evaluate reviewers agreement. Criteria used were showed in Table 2.

We define the risk of bias with the following criteria: low risk if all criteria were met, moderate risk if only one was not met, and high risk if the number of unmet criteria were two or more.

Analysis

The influence of the *WT1* rs16754 genotypes and effectiveness variables was evaluated by pooled odds ratios (OR) and 95% confidence interval (95% CI) using the fixed effects (Mantel–Haenszel method). The statistical significance of pooled OR were determined with *Z* test (values of $P < 0.05$ were considered statistically significant). RevMan software (version 5.2 The Cochrane Collaboration, The Nordic Cochrane Center, Copenhagen, Denmark) was used to conduct this meta-analysis. The association between AML outcomes and *WT1* rs16754 genotypes was performed using dominant (AA vs AG/GG) and recessive models (AA/AG vs GG).

Statistical heterogeneity across studies was tested using the χ^2-test (heterogeneity if $P < 0.1$) and the I^2 statistic (significant heterogeneity when $I^2 > 50\%$). If heterogeneity was present, the meta-analysis was repeated using the random effects model.

Subgroup analyses were performed for effectiveness variables (OS and CR) based on the ethnicity, age and chemotherapy scheme of the patients. Other predefined variables (included in Table 1) were not analyzed because the information provided by authors was not related with the genotype frequencies and effectiveness variables. We also analyzed OS and CR in patients with CN-AML. Differences between subgroups were evaluated with interaction test (chi-squared).

The possibility of publication bias was conducted evaluating the funnel plots symmetry and with Egger's test (statistical significance if $P < 0.1$).

RESULTS

Systematic search obtained 201 citations from databases and journals and 8 records identified through other sources (Figure 1). Of the 43 citations selected for full reading, only 14 fulfilled the inclusion criteria and were included (all in English). Reviewers showed an excellent agreement in study selection (kappa = 0.91).

The OS at some of the analyzed times was inferred from the Kaplan–Meier plots in eight studies.[24,25,29–31,34,37]

Study and patient characteristics

Fourteen cohort studies were included (3618 patients).[17,24,25,27–37] The characteristics of the individual studies included are provided in Table 1. Patients' mean age was 35.4 years (range of 1–88 years) and they were males in 53.5% of the cases. Three studies did not include age or gender data.[24,25,28] The most abundant ethnic group was Caucasian (72%), followed by Asian (14.7%). Concerning AML, 95.5% were *de novo* AML and the predominant FAB subtypes represented were M2 (30.9%), M4 (24.7%) and M1 (19.6%). Normal cytogenetic risk was present in 70.4% of patients, whereas 18% had favorable risk and 11.5% had unfavorable one. Mutations were evaluated in nine studies[17,27,30–36] and they are summarized in Table 1.

Genotype distributions were in accordance with the Hardy–Weinberg equilibrium in most studies, with three exceptions[27,30,31] and there were two studies without genotype frequencies.[24,25] The method used for genotyping consisted of PCR and direct sequencing in eight studies,[24,28–31,33,35,36] real-time reverse transcriptase PCR in four studies,[17,25,32] mass spectrometry and PCR-restriction fragment length polymorphism to validate in one study[37] and two different methods in the last study.[34] The baselines characteristics and evaluable AML outcomes for included studies concerning *WT1* rs16754 polymorphism are listed in Table 1.

Risk of bias

Only three of the included studies met all 9 criteria and were classified as low risk of bias.[17,32,37] Eight studies met 8 criteria (intermediate risk of bias).[27,28,30,31,33–36] The other three studies were categorized as high risk of bias, one met 7 criteria[24] and two studies met 6 criteria[25,29] (Table 2). Reviewers showed a substancial agreement (kappa = 0.66).

Overall survival

OS for rs16 754 polymorphism was analyzed in 14 studies (Table 1) with 3618 total patients,[17,24,25,27–37] 10 of them included enough data to evaluate the recessive model (2875 patients).[17,24,27,30–33,35–37] We evaluated OS at 3 and 5 years and calculated OR and 95% CI (Table 3). Two studies only reported OS at 3 years[30,31] and we inferred these data as OS at 5 years. Percentage of OS at 5 years was estimated through Kaplan–Meier graphs in 8 studies that estimated OS at >5 years[24,25,29–31,34,36,37] and 8 studies for OS estimations at 3 years.[17,24,25,29,33,34,36,37]

We found evidence indicating that the variant allele G is associated with higher OS (Table 3). Statistical significance was obtained with the dominant model (OR: 1.24, 95% CI: 1.06–1.45, $P = 0.007$, I^2: 67%; Figure 2). Significant heterogeneity was detected using the fixed-effect model and the meta-analyses were repeated using the random-effect model (OR: 1.19, 95% CI: 0.86–1.63, I^2: 67%, *P*: 0.30) in which statistical significance was lost. This association was not replicated with recessive model as well as the analysis of OS at 3 years (Table 3).

The analysis for publication bias showed a little asymmetry in funnel plot of dominant model, as the studies of Damm *et al.*[17]

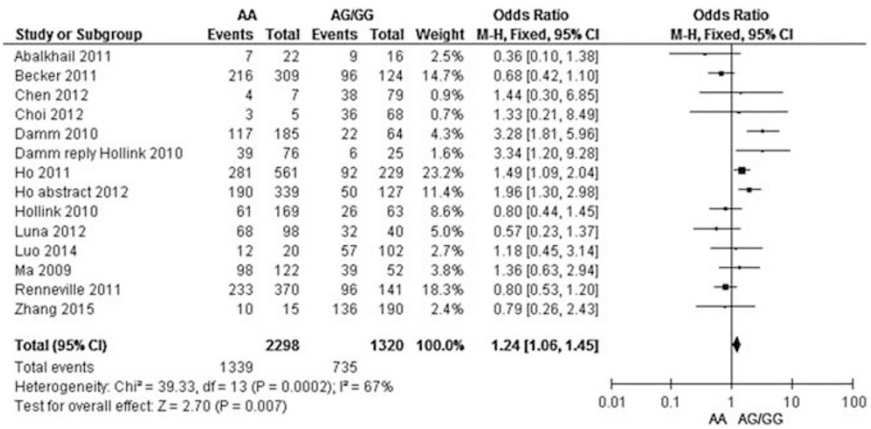

Figure 2. Overall survival at 5 years the dominant model (AA vs AG/GG).

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Table 4. Summary of ORs with 95% CIs for ordinary genetic contrasts of the association between the *WT1* rs16754 polymorphism and subgroup analyses with fixed effects

Contrast	*Overall subgroup*	n	*OR (95% CI)*	I[a] *(%)*	P-*value*[b]
OS at 5 years AA vs AG/GG in the different ethnic subgroups (Figure 3)					
All subgroups	All studies[17,24,25,27–37]	3618	1.24 (1.06–1.45)	46	**0.008**
Caucasian	9 studies[17,24,25.27.28,32-35]	2619	1.21 (1.01–1.45)	75[a]	**0.04**
Asian	6 studies[27,28,30,31,36,37]	530	1.37 (0.78–2.40)	0	0.27
Hispanic	3 studies[27,28,35]	225	1.42 (0.82–2.46)	0	0.21
African	3 studies[27,28,35]	154	1.56 (0.70–3.51)	0	0.28
Arab	1 study[29]	38	0.36 (0.10–1.38)	Not applicable	0.14
Others	2 studies[27,35]	52	1.74 (0.56–5.36)	0	0.34
OS at 5 years AA/AG vs GG in the different ethnic subgroups					
All subgroups	10 studies[17,24,27,30–33,35–37]	2875	1.12 (0.85–1.49)	7	0.42
Caucasian	6 studies[17,24,27,32,33,35]	2058	1.41 (0.84–2.37)	0	0.20
Asian	5 studies[27,30,31,36,37]	515	0.97 (0.67–1.84)	55	0.85
Hispanic	2 studies[27,35]	143	1.05 (0.30–3.72)	0	0.94
African	1 study[27]	83	1.81 (0.16–20.77)	Not applicable	0.63
Others	2 studies[27,35]	76	2.43 (0.48–12.34)	0	0.28
CR at 5 years AA vs AG/GG in the different ethnic subgroups					
All subgroups	10 studies[17,27,30–37]	2793	1.12 (0.90–1.40)	0	0.30
Caucasian	6 studies[17,27,32–35]	2022	1.13 (0.88–1.45)	26	0.32
Asian	5 studies[27,30,31,36,37]	495	1.08 (0.55–2.14)	0	0.82
Hispanic	2 studies[27,35]	143	1.10 (0.47–2.57)	0	0.82
African	2 studies[27,35]	107	0.99 (0.29–3.35)	0	0.99
Others	2 studies[27,35]	26	1.29 (0.27–6.09)	0	0.75
CR at 5 years AA/AG vs GG in the different ethnic subgroups					
All subgroups	8 studies[17,27,30–33,35–37]	2655	0.82 (0.59–1.13)	0	0.22
Caucasian	5 studies[17,27,32,33,35]	1884	0.54 (0.30–0.98)	14	0.33
Asian	4 studies[27,30,31,36,37]	495	0.98 (0.65–1.48)	0	0.94
Hispanic	1 study[27]	130	0.42 (0.08–2.25)	Not applicable	0.31
Others	2 studies[27,35]	146	1.07 (0.24–4.81)	0	0.93
OS at 5 years AA vs AG/GG in the different age subgroups (Figure 4)					
All subgroups	All studies[17,24,25,27–37]	3618	1.24 (1.06–1.45)	67	**0.007**
Adult patients	8 studies[17,24,25,30,33–37]	2006	1.08 (0.87–1.35)	70	0.48
Pediatric patients	5 studies[27–29,31,32]	1612	1.42 (1.14–1.77)	60[c]	**0.002**
OS at 5 years AA/AG vs GG in the different age subgroups					
All subgroups	10 studies[17,24,27,30–33,35–37]	2875	1.12 (0.84–1.49)	41	0.44
Adult patients	7 studies[17,24,30,33,35–37]	1767	0.99 (0.70–1.40)	54	0.95
Pediatric patients	3 studies[27,31,32]	1108	1.47 (0.87–2.48)	0	0.15
CR at 5 years AA vs AG/GG in the different age subgroups					
All subgroups	9 studies[17,27,30–37]	2793	1.10 (0.89–1.37)	0	0.38
Adult patients	6 studies[17,30,33–37]	1731	1.06 (0.81–1.39)	6	0.68
Pediatric patients	3 studies[27,31,32]	1062	1.18 (0.82–1.69)	0	0.37
CR at 5 years AA/AG vs GG in the different age subgroups					
All subgroups	8 studies[17,27,30–33,35–37]	2655	0.80 (0.58–1.11)	7	0.18
Adult patients	5 studies[17,30,33,35–37]	1593	0.73 (0.50–1.06)	31	0.10
Pediatric patients	3 studies[27,31,32]	1062	1.08 (0.55–2.13)	0	0.82
OS at 5 years in CN-AML					
AA vs AG/GG	8 studies[17,27,30,32–36]	1483	1.03 (0.81–1.31)	68	0.83
AA/AG vs GG	7 studies[17,27,30,32,33,35,36]	1395	1.15 (0.71–1.87)	12	0.58
CR at 5 years in CN-AML					
AA vs AG/GG	5 studies[17,32,33,35,36]	1048	1.10 (0.78–1.56)	33	0.58
AA/AG vs GG	4 studies[17,33,35,36]	963	0.78 (0.37–1.65)	0	0.52
OS at 5 years AA vs AG/GG in the different chemotherapy scheme subgroups (Figure 5)					
With idarubicin	9 studies[17,25,27,28,30,32–34,36]	2682	1.38 (1.16–1.65)	71[d]	**0.0003**
With daunorubicin	9 studies[27,28,30–33,35–37]	2918	1.16 (0.97–1.38)	57	0.10
With etoposide	6 studies[17,25,27,28,31,34]	1830	1.74 (1.40–2.15)	63[e]	< **0.00001**
Without etoposide	6 studies[30,32,33,35–37]	1576	0.79 (0.61–1.02)	0	0.07
OS at 5 years AA/AG vs GG in the different chemotherapy scheme subgroups					
With idarubicin	6 studies[25,27,30,32,33,36]	1977	1.26 (0.87–1.84)	0	0.22
With daunorubicin	8 studies[27,30–33,35–37]	2452	1.03 (0.77–1.39)	0	0.20

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Table 4. (Continued)

Contrast	*Overall subgroup*	n	*OR (95% CI)*	I[a] *(%)*	P-*value*[b]
With etoposide	3 studies[17,27,31]	1125	1.63 (0.96–2.76)	0	0.07
Without etoposide	6 studies[30,32,33,35–37]	1576	0.87 (0.51–1.24)	25	0.44
CR at 5 years AA vs AG/GG in the different chemotherapy scheme subgroups					
With idarubicin	7 studies[17,27,30,32–34,36]	2089	1.21 (0.94–1.55)	0	0.15
With daunorubicin	8 studies[27,30–33,35–37]	2406	0.99 (0.78–1.26)	0	0.96
With etoposide	4 studies[17,27,31,34]	1217	1.32 (0.96–1.81)	0	0.09
Without etoposide	5 studies[30,32,33,35–37]	1576	0.94 (0.70–1.26)	0	0.68
CR at 5 years AA/AG vs GG in the different chemotherapy scheme subgroups					
With idarubicin	6 studies[25,27,30,32,33,36]	1951	0.78 (0.50–1.20)	34	0.25
With daunorubicin	8 studies[27,30–33,35–37]	2406	0.82 (0.59–1.13)	15	0.23
With etoposide	3 studies[17,27,31]	1079	1.01 (0.52–1.96)	0	0.97
Without etoposide	6 studies[30,32,33,35–37]	1576	0.74 (0.51–1.08)	28	0.12

Abbreviations: CN-AML, cytogenetically normal acute myeloid leukemia; CR, complete remission; DFS, disease-free survival; OR, odds ratio; OS, overall survival; RFS, relapse-free survival; RR, rate of relapse; *WT1*, Wilms tumor 1. [a]Significant heterogeneity using the fixed-effect model. We calculated with random effects (OR: 1.27, 95% CI: 0.86–1.87, I^2: 75%, *P*: 0.24). [b]*P*-value of test of overall effect. [c]Significant heterogeneity using the fixed-effect model. We calculated with random effects (OR: 1.24, 95% CI: 0.80–1.92, I^2: 60%, *P*: 0.33). [d]Significant heterogeneity using the fixed-effect model. We calculated with random effects (OR: 1.38, 95% CI: 0.94–2.02, I^2: 71%, *P*: 0.10). [e]Significant heterogeneity using the fixed-effect model. We calculated with random effects (OR: 1.78, 95% CI: 1.17–2.72, I^2: 63%, *P*: 0.007). Results with statistical significance are in bold.

and Becker *et al.*[35] were slightly out the funnel in the dominant model, but this was not significant in the Egger's test ($P = 0.65$ for OS at 5 years and $P = 0.76$ at 3 years).

Complete remission

Ten studies evaluated CR for *WT1* polymorphism[17,27,30–37] with 2793 total patients. The pooled effect estimate did not show association between CR and *WT1* genotypes in dominant (OR: 1.10, 95% CI: 0.89–1.37, $P = 0.38$, I^2: 0%; Table 3) or recessive model (OR: 0.80, 95% CI: 0.58–1.11, $P = 0.18$, I^2: 7%; Table 3).

The publication bias analysis yielded nonsignificant asymmetry in funnel plot (though Renneville *et al.*[33] was a bit out the funnel in recessive model) as well as in the Egger's test ($P = 0.74$).

Other effectiveness variables

The included studies analyzed other effectiveness variables (Table 3), as relapse-free survival (RFS) (5 studies, 703 patients),[17,31,34,36,37] disease-free survival (DFS; 4 studies, 1303 patients)[27,29,34,35] and rate of relapse (RR; 5 studies, 1387 patients).[27,30,31,33,36] Two studies evaluated event-free survival[32,36] and other two studies analyzed treatment-related mortality,[27,28] but the limited data published did not allow to measure these variables.

We evaluated separately these variables at 5 years with dominant and recessive models (Table 3). We estimated RFS at 5 years in 4 studies[31,34,36,37] and DFS at 5 years in 2 studies,[30,34] using the Kaplan–Meier graphs.

We did not find any difference in RFS and RR for this SNP with both models. The pooled effect estimate showed a higher DFS in G allele carriers, statistically significant in the recessive model (OR: 1.77, 95% CI: 1.09–2.86, $P = 0.02$, I^2: 5%).

Subgroup analysis

Ethnics: An interaction between OS and ethnic origen for *WT1* was found with the dominant model (Table 4). Caucasian participants carrying G allele showed statistically significant higher OS (OR: 1.21, 95% CI: 1.01–1.45, $P = 0.04$, I^2: 75%; Figure 3), but with significant heterogeneity. We recalculated with random effects (OR: 1.27, 95% CI: 0.86–1.87, $P = 0.24$, I^2: 75%). Regarding CR, no significant associations were found.

Age: Pediatric patients carrying G allele showed statistically significant higher OS with the recessive model (OR: 1.42, 95% CI: 1.14–1.77, $P = 0.002$, I^2: 60%; Figure 4). It was reanalyzed with random effects model (OR: 1.24, 95% CI: 0.80–1.92, I^2: 60%, *P*: 0.33). We found no significant correlations regarding CR (Table 4). Evaluation of publication bias was nonsignificant in all comparisions.

CN-AML: Our results did not demonstrate an effect of *WT1* genotypes in OS and CR with dominant or recessive model in AML patients with normal cytogenetics (Table 4).

Chemotherapy scheme: All the studies included in this systematic review employed cytarabine plus anthracyclines with or without etoposide (Table 1). Patients with G allele treated with idarubicin (OR: 1.38, 95% CI: 1.16–1.65, $P = 0.0003$, I^2: 71%; Figure 5) showed higher OS with the dominant model, but this effect was not reproducible with daunorubicin schemes and was lost with random effects model (OR: 1.38, 95% CI: 0.94–2.02, I^2: 71%, *P*: 0.10). Regarding etoposide schemes, the variant allele was associated with higher OS in patients treated with etoposide (OR: 1.74, 95% CI: 1.40–2.15, $P < 0.00001$, I^2: 63%; Figure 5) and the opposite effect in treatments without etoposide (OR: 0.79, 95% CI: 0.61–1.02, $P = 0.07$, I^2: 0%; Figure 5). In this association a significant heterogeneity was detected and random effects analysis was performed (OR: 1.78, 95% CI: 1.17–2.72, I^2: 63%, *P*: 0.007; Table 4). No significant correlations were found with CR (Table 4).

Sensitivity analyses

After excluding outlier studies[17,35] significant differences were not found in OS analysis (OR: 1.23, 95% CI: 1.04–1.47, $P = 0.02$, I^2: 52%), denoting that our results are robust and reliable. The pooled effect estimated with fixed or random-effect models did not show significant discrepancies (Table 3).

The studies of Chen *et al.*[31] and Luo *et al.*[30] are the only two studies that evaluated OS at 3 years. No differences were obtained after their exclusion in OS with both models.

DISCUSSION

The findings of this meta-analysis suggest that the *WT1* rs16754 polymorphism in AML could influence the standard chemotherapy effectiveness, specifically the variant allele (G) could be associated with increased OS. Our results suggest that the variant genotype of *WT1* SNP increased OS, with statistical significance using the dominant model, although the slight increase in OR reveals that

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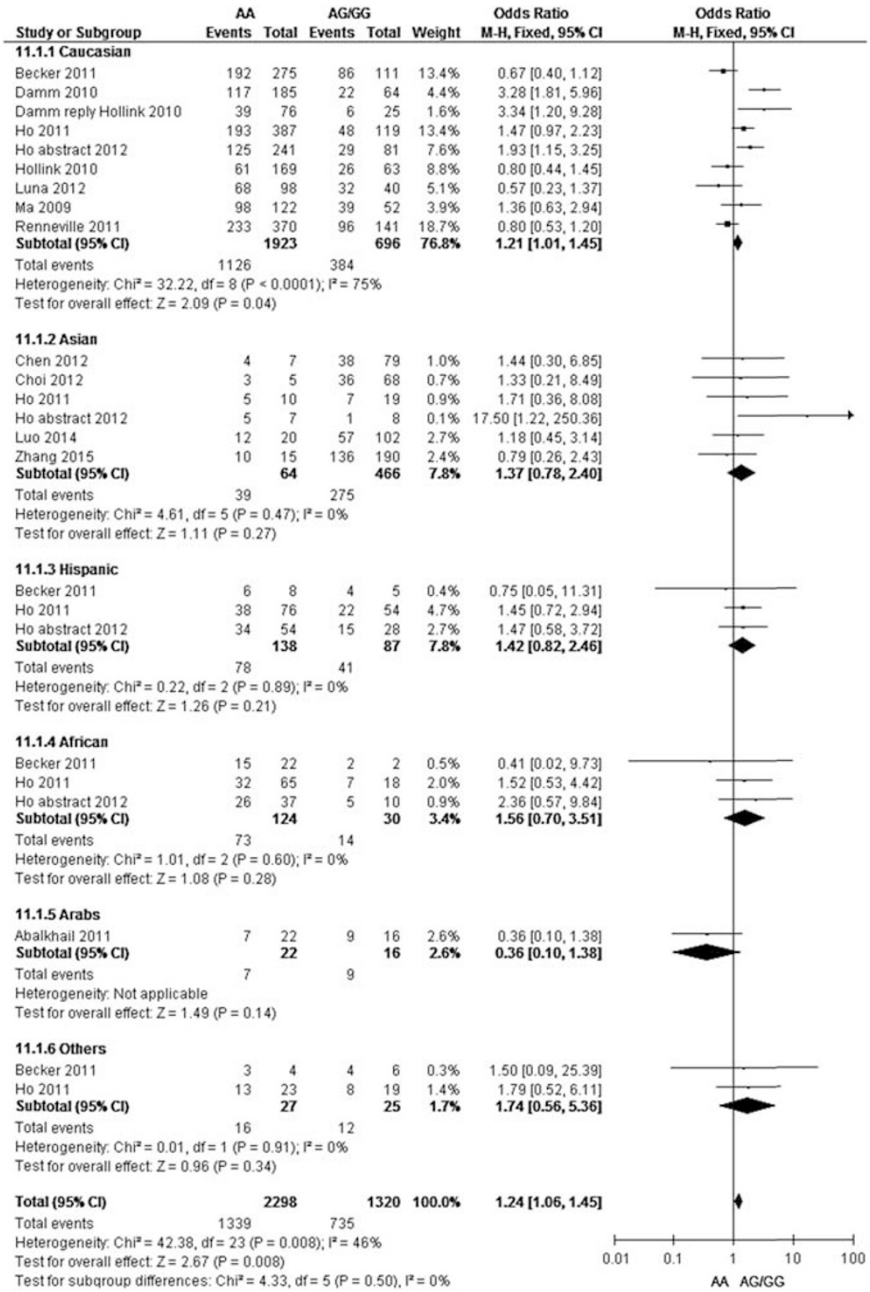

Figure 3. Overall survival at 5 years AA vs AG/GG in the different ethnic subgroups.

the clinical impact of the variant genotype could be limited. In Caucasian and pediatric patients this effect was more manifest. We found significant heterogeneity in OS analysis with the dominant model, and we recalculated this analysis with random effects and statistical significance was lost. This heterogeneity was introduced by two studies,[17,35] which were performed only in CN-AML patients, thus providing a reason for this heterogeneity. The exclusion of these studies in the sensitivity analysis produced a decrease in heterogeneity, whereas significant results in OS were maintained. Similar results with variant G allele were obtained with DFS.

We did not observe any significant effect for CR, possibly as a consequence of the small number of studies that evaluated it. These results were consistent with the CR obtained in the individual studies, all of them without significant differences in this outcome. Other variables related with CR, such as RFS and RR, showed similar values after meta-analysis.

The reason for the increase in survival rates associated with the variant allele of rs16754 remains undetermined. Several action mechanisms have been proposed to explain how this synonymous SNP may alter protein amount, structure and/or activity of *WT1*. This polymorphism consists of the replacement of CGA by CGG codon, and the latter is used two times more often than the former to encode arginine.[39] Therefore, the presence of G allele is predicted to increase the rate of translation, which could potentially affect protein folding. A second possibility is that SNP rs16754 is in linkage disequilibrium with another genetic variant that affects drug metabolism, although only few studies[40–42] reported tag SNPs of WT1 gene (any of them in AML patients), and only one of them[40] found other WT1 polymorphisms in linkage

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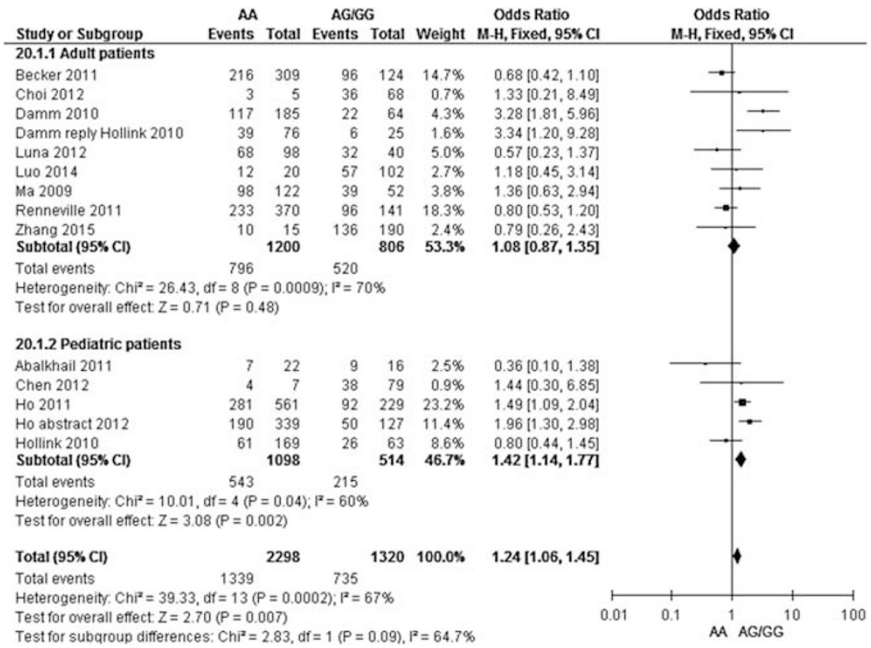

Figure 4. Overall survival at 5 years AA vs AG/GG in the different age subgroups.

disequilibrium with rs16754. A third possibility is that this SNP can affect the sensitivity or timing of co-translational folding and binding of non-coding RNAs. In other synonymous polymorphism, such as C3435T SNP of the multidrug resistance 1 *(ABCB1)* gene, the variant has been postulated to change P-glycoprotein substrate and inhibitor interaction sites.[43] Other explanation could be that GG genotype was more sensitive to cytarabine, and therefore this genotype conferred a favorable outcome in treatment with high-dose cytarabine.[17]

Although some studies associated the presence of G allele with an increase in *WT1* expression,[27,30,37] this relation is not completely understood. One of these studies[30] also demonstrated that GG genotype did not predict improved outcome in stratified groups according to the median expression of *WT1*. This fact and the contradictory data exposed in other studies[17,32] suggest that the influence on *WT1* expression of *WT1* SNP rs16754 is not as significant as that of other genetic or nongenetic factors. The subgroup analysis by ethnic origin showed similar results in different races in OS and CR. The opposite genotype frequencies in Asian and Caucasian populations are well known. Specifically, G is a minor allele in Western populations, whereas it is a major allele in Asian populations. Nevertheless, the effect of this SNP in AML outcomes seems to be equivalent in both ethnic groups. Caucasian patients were the unique ethnic origin that obtained statistical significant increase of OS related with variant allele. The impact of this polymorphism in other races remains poorly studied.

The age subgroup analysis found a significant relation between variant allele and higher OS in pediatric patients, not detected in adults. This association was not reproduced with the recessive model, possibly because of the limited number of studies and their contradictory results. Only one study[29] compared *WT1* SNPs in both age subgroups directly, in which AG genotype was associated with shorter OS and EFS in pediatric patients, but not in adults. These results are contradictory with ours, but it should be noted that this study included a small cohort of Arabic patients undergoing allogeneic stem cell transplant.

Another factor that could explain result variability among studies could be the variability in AML induction therapy. Chemotherapy schemes used in eligible studies were based on cytarabine and anthracyclines. Previous studies did not find differences in the type of anthracycline used and the effect of *WT1* polymorphisms. We obtained a significant higher OS with G allele in patients treated with idarubicin, not observed in daunorubicin treatments. Similar results were found with etoposide schemes. These studies mixed different chemotherapy agents, even both anthracyclines, therefore it is difficult to clearly differentiate the drug effect of the schemes employed.

Heterogeneity could difficult the interpretation of the pooled estimations of meta-analyses. We repeated the meta-analysis using the random effects model when heterogeneity was present, and significant results were lost in most of these analyses, therefore limiting their clinical implications. This model involves an assumption that the effects being estimated in the different studies are not identical, but follow some kind of distribution, and it is the appropriate model when heterogeneity is present. In our results, heterogeneity was identified in one of the statistical significant results, which was introduced by two studies[17,35] that only included CN-AML patients in their analysis. After excluding these studies in a *post hoc* analysis, the heterogeneity decreased while maintaining the OS significant results. Our subgroup analysis of CN-AML patients reveals that *WT1* polymorphism does not influence OS or CR. Unfortunately we could analyze the impact of this SNP in other cytogenetic risk groups, such as high-risk or core binding factor leukemia. The ethnic and chemotherapy scheme subgroup analyses showed similar heterogeneity, probably related with the two previously cited studies.[17,35]

Some limitations of this meta-analysis should be addressed. First, the role of *WT1* in the development of AML and its effect in treatment outcomes are not completely understood. Second, our results are based on unadjusted estimates, whereas a more precise analysis should be conducted if more detailed individual data were available. Third, we found that genotype distributions were not in agreement with Hardy–Weinberg equilibrium in three studies[27,30,31] and unknown in other two studies.[24,25] Fourth, regarding the subgroup analysis, we find novel associations with G allele of rs16754 and OS that generate new hypothesis but that we cannot explain properly. Finally, subgroup analyses regarding other variables (sex, AML and mutation status, and so on) were not performed due to insufficient data available in the original studies.

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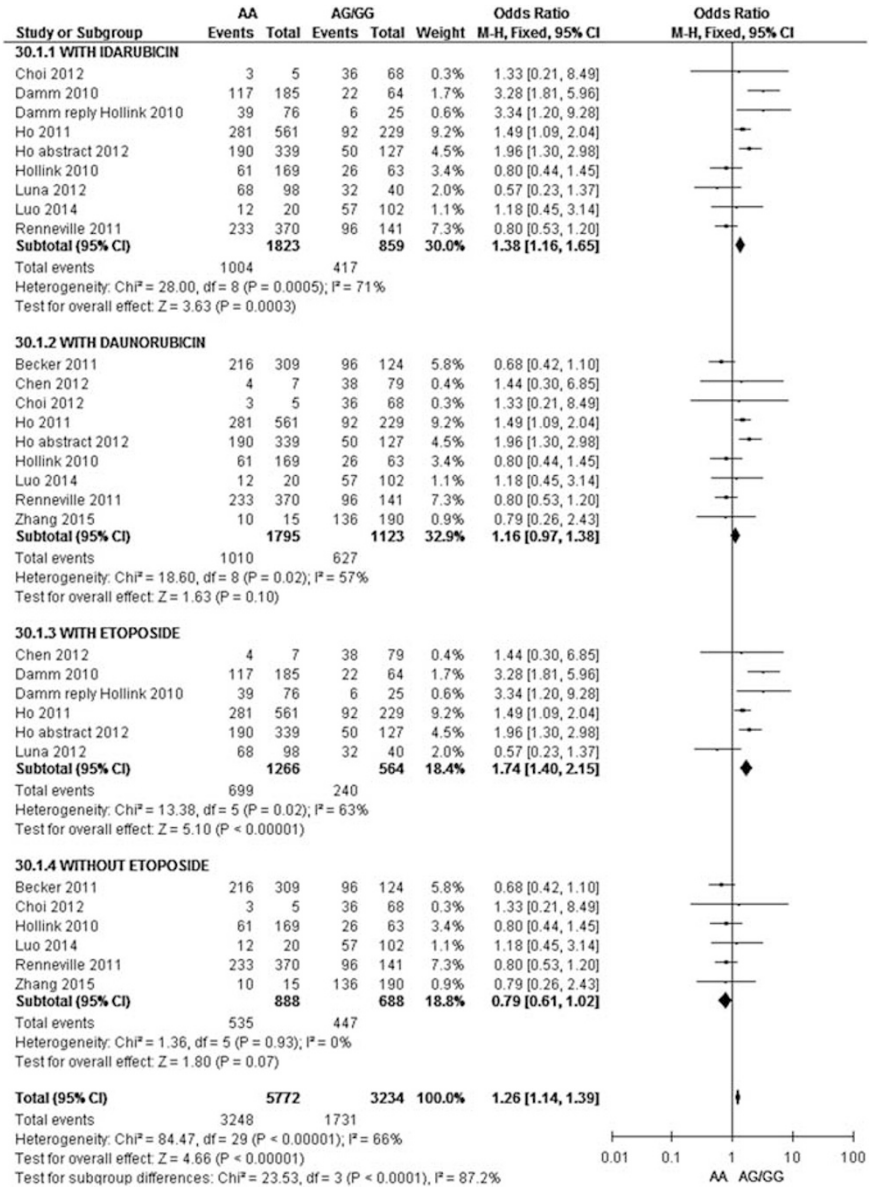

Figure 5. Overall survival at 5 years AA vs AG/GG in the different chemotherapy scheme subgroups.

WT1 rs16754 polymorphism has never been formerly meta-analyzed and individual studies have shown inconclusive results. Our meta-analysis consisted in the use of extensive search strategies of the literature together with selection criteria. Effectiveness variables were extracted and their pooled effects were estimated with appropriate statistical analyses. Likewise, results obtained were demonstrated to be robust in posterior sensitivity analyses.

In summary, this meta-analysis showed an association between rs16754 polymorphism and OS with fixed effects, but the statistical significance was lost with random effects model. This effect was especially shown in subgroups of Caucasian patients, pediatric patients, and those receiving combined idarubicin-etoposide treatments. The polymorphism showed no association with CR. Future studies based on larger populations and using different age, ethnic and cytogenetic groups should clarify the effect of *WT1* in AML outcomes, as well as its influence in different chemotherapy schemes. Moreover, further studies that investigate gene–gene and gene–environment interactions may help understand the role of *WT1* in the chemotherapy effectiveness.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Bloomfield CD, Lawrence D, Byrd JC, Carroll A, Pettenati MJ, Tantravahi R *et al.* Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. *Cancer Res* 1998; **58**: 4173–4179.
2 Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A *et al.* Karyotypic analysis predicts outcome of preremission and postremission therapy

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in adult acute myeloid leukemia: A Southwest Oncology Group/ Eastern Cooperative Oncology Group Study. *Blood* 2000; **96**: 4075–4083.
3 Rombouts WJ, Blokland I, Lowenberg B, Ploemacher RE. Biological characteristics and prognosis of adult acute myeloid leukemia with internal tandem duplications in the *Flt3 gene*. *Leukemia* 2000; **14**: 675–683.
4 Schnittger S, Schoch C, Kern W, Mecucci C, Tschulik C, Martelli MF *et al. Nucleophosmin gene* mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. *Blood* 2005; **106**: 3733–3739.
5 Preudhomme C, Sagot C, Boissel N, Cayuela JM, Tigaud I, de Botton S *et al.* Favorable prognostic significance of *CEBPA* mutations in patients with de novo acute myeloid leukemia: A study from the Acute Leukemia French Association (ALFA). *Blood* 2002; **100**: 2717–2723.
6 Whitman SP, Liu S, Vukosavljevic T, Rush LJ, Yu L, Liu C *et al.* The *MLL* partial tandem duplication: Evidence for recessive gain-of-function in acute myeloid leukemia identifies a novel patient subgroup for molecular targeted therapy. *Blood* 2005; **106**: 345–352.
7 Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A *et al.* The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 2009; **114**: 937–951.
8 King-Underwood L, Renshaw J, Pritchard-Jones K. Mutations in the *Wilms' tumor gene WT1* in leukemias. *Blood* 1996; **87**: 2171–2179.
9 Summers K, Stevens J, Kakkas I, Smith M, Smith LL, Macdougall F *et al. Wilms' tumour 1* mutations are associated with *FLT3-ITD* and failure of standard induction chemotherapy in patients with normal karyotype AML. *Leukemia* 2007; **21**: 550–551.
10 Paschka P, Marcucci G, Ruppert AS, Whitman SP, Mrózek K, Maharry K *et al. Wilms' tumor 1 gene* mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol* 2008; **26**: 4595–4602.
11 Virappane P, Gale RA, Hills R, Kakkas I, Summers K, Stevens J *et al.* Mutations of the *Wilms' tumor 1 gene* is a poor prognostic factor associated with chemoresistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical Research Council Adult Leukaemia Working Party. *J Clin Oncol* 2008; **26**: 5429–5435.
12 Renneville A, Boissel N, Zurawski V, Llopis L, Biggio V, Nibourel O *et al. Wilms tumor 1 gene* mutations are associated with a higher risk of recurrence in young adults with acute myeloid leukemia: a study from the Acute Leukemia French Association. *Cancer* 2009; **115**: 3719–3727.
13 Hollink IH, van den Heuvel-Eibrink MM, Zimmermann M, Balgobind BV, Arentsen-Peters ST, Alders M *et al.* Clinical relevance of *Wilms tumor 1 gene* mutations in childhood acute myeloid leukemia. *Blood* 2009; **113**: 5951–5960.
14 Becker H, Marcucci G, Maharry K, Radmacher MD, Mrózek K, Margeson D *et al.* Mutations of the *Wilms tumor 1 gene* (*WT1*) in older patients with primary cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *Blood* 2010; **116**: 788–792.
15 Hou H-A, Huang T-C, Lin L-I, Liu C-Y, Chen C-Y, Chou W-C *et al. WT1* mutation in 470 adult patients with acute myeloid leukemia: stability during disease evolution and implication of its incorporation into a survival scoring system. *Blood* 2010; **115**: 5222–5231.
16 Gaidzik VI, Schlenk RF, Moschny S, Becker A, Bullinger L, Corbacioglu A *et al.* Prognostic impact of *WT1* mutations in cytogenetically normal acute myeloid leukemia: a study of the German-Austrian AML Study Group. *Blood* 2009; **113**: 4505–4511.
17 Damm F, Heuser M, Morgan M, Yun H, Grosshennig A, Göhring G *et al.* Single nucleotide polymorphism in the mutational hotspot of *WT1* predicts a favorable outcome in patients with cytogenetically normal acute myeloid leukemia. *J Clin Oncol* 2010; **28**: 578–585.
18 Ho PA, Zeng R, Alonzo TA, Gerbing RB, Miller KL, Pollard JA *et al.* Prevalence and prognostic implications of *WT1* mutations in pediatric acute myeloid leukemia: A report from the Children's Oncology Group. *Blood* 2010; **116**: 702–710.
19 Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA *et al.* Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 *Wilms tumor locus*. *Cell* 1990; **60**: 509–520.
20 Yang L, Han Y, Saurez Saiz F, Minden MD. A tumor suppressor and oncogene: the *WT1* story. *Leukemia* 2007; **21**: 868–876.
21 Ariyaratana S, Loeb DM. The role of the *Wilms tumour gene* (*WT1*) in normal and malignant haematopoiesis. *Expert Rev Mol Med* 2007; **9**: 1–17.
22 Altshuler DM, Gibbs RA, Peltonen L, Altshuler DM, Gibbs RA, Peltonen L *et al.* Integrating common and rare genetic variation in diverse human populations. *Nature* 2010; **467**: 52–58.
23 Komar AA. Silent SNPs: impact on gene function and phenotype. *Pharmacogenomics* 2007; **8**: 1075–1080.
24 Ma W, Kantarjian H, Zhang X, Wang X, Zhang Z, Yeh C-H *et al.* Mutation and single- nucleotide polymorphism (rs16754) in Wilms tumor-1 gene are independent prognostic factors in acute myeloid leukemia. *Blood* 2009; **114**: 413.
25 Damm F, Heuser M, Ganser A, Krauter J. Reply to I.H.I.M Hollink et al. *J Clin Oncol* 2010; **28**: e527–e528.
26 Renneville A, Boissel N, Helevaut N, Nibourel O, Terré C, Pautas C *et al.* Prognostic impact of *Wilms tumor 1* single nucleotide polymorphism rs16754 in older patients with acute myeloid leukemia. *Blood* 2010; **161**: 1113.
27 Ho PA, Kuhn J, Gerbing RB, Pollard JA, Zeng R, Miller KL *et al. WT1* synonymous single nucleotide polymorphism rs16754 correlates with higher mRNA expression and predicts significantly improved outcome in favorable-risk pediatric acute myeloid leukemia: a report from the children's oncology group. *J Clin Oncol* 2011; **29**: 704–711.
28 Ho PA, Alonzo TA, Gerbing RB, Sung L, Aplenc R, Pollard JA *et al. WT1* snp rs16754 Genotype Predicts Treatment Related Mortality (TRM) in African-American and Asian Pediatric AML Patients: A Report From the Children's Oncology Group. *Blood* 2012; **120**: 1385.
29 Abalkhail H, El-Solh H, Alseraihy A, Ayas M, Al-Ahmari A, Belgaumi AF *et al.* The AG Genotype of the *Wilms Tumor-1* rs16754 SNP is associated with poor outcome in pediatric AML patients treated with stem cell transplantation but not in adults. *Blood* 2011; **118**: 5327.
30 Luo S, Yu K, Yan QX, Shen ZJ, Wu JB, Chen HM *et al.* Analysis of *WT1* mutations, expression levels and single nucleotide polymorphism rs16754 in de novo non-M3 acute myeloid leukemia. *Leuk Lymphoma* 2014; **55**: 349–357.
31 Chen X, Yang Y, Huang Y, Tan J, Chen Y, Yang J *et al. WT1* mutations and single nucleotide polymorphism rs16754 analysis of patients with pediatric acute myeloid leukemia in a Chinese population. *Leuk Lymphoma* 2012; **53**: 2195–2204.
32 Hollink IH, van den Heuvel-Eibrink MM, Zimmermann M, Balgobind BV, Arentsen-Peters ST, Alders M *et al.* No prognostic impact of the *WT1* gene single nucleotide polymorphism rs16754 in pediatric acute myeloid leukemia. *J Clin Oncol* 2010; **28**: e523–e526.
33 Renneville A, Boissel N, Helevaut N, Nibourel O, Terré C, Pautas C *et al. Wilms' tumor 1* single-nucleotide polymorphism rs16754 does not predict clinical outcome in adult acute myeloid leukemia. *Leukemia* 2011; **25**: 1918–1921.
34 Luna I, Such E, Cervera J, Barragán E, Jiménez-Velasco A, Dolz S *et al.* Analysis of SNP rs16754 of *WT1* gene in a series of de novo acute myeloid leukemia patients. *Ann Hematol* 2012; **91**: 1845–1853.
35 Becker H, Maharry K, Radmacher MD, Mrózek K, Metzeler KH, Whitman SP *et al.* Clinical outcome and gene- and microRNA-expression profiling according to the *Wilms tumor 1* (*WT1*) single nucleotide polymorphism rs16754 in adult de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *Haematologica* 2011; **96**: 1488–1495.
36 Choi Y, Lee JH, Hur EH, Kang MJ, Kim SD, Lee JH *et al.* Single nucleotide polymorphism of *Wilms' tumor 1 gene* rs16754 in Korean patients with cytogenetically normal acute myeloid leukemia. *Ann Hematol* 2012; **91**: 671–677.
37 Zhang DY, Yan H, Cao S, Zhang W, Li XL, Zeng H *et al. Wilms Tumor 1* rs16754 predicts favorable clinical outcomes for acute myeloid leukemia patients in South Chinese population. *Leuk Res* 2015; **39**: 568–574.
38 Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP *et al.* The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009; **339**: b2700.
39 Komar AA, Lesnick T, Reiss C. Synonymous codon substitutions affect ribosome traffic and protein folding during in vitro translation. *FEBS Lett* 1999; **462**: 387–391.
40 Qi XW, Zheng XD, Zong BG, Chen QQ, Zhang F, Yang XH *et al.* Association between WT1 polymorphisms and susceptibility to breast cancer: results from a case-control study in a southwestern Chinese population. *Am J Cancer Res* 2015; **5**: 1234–1250.
41 Kim DH, Xu W, Ma C, Liu X, Siminovitch K, Messner HA *et al.* Genetic variants in the candidate genes of the apoptosis pathway and susceptibility to chronic myeloid leukemia. *Blood* 2009; **113**: 2517–2525.
42 Carmichael SL, Ma C, Choudhry S, Lammer EJ, Witte JS, Shaw GM. Hypospadias and genes related to genital tubercle and early urethral development. *J Urol* 2013; **190**: 1884–1892.
43 Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV *et al.* A "silent" polymorphism in the *MDR1 gene* changes substrate specificity. *Science* 2007; **315**: 525–528.

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