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The incidence and prognostic effect of Fms-like tyrosine kinase 3 gene internal tandem and nucleolar phosphoprotein 1 genes in acute myeloid leukaemia

A PRISMA-compliant systematic review and meta-analysis

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Abstract

Background: Molecular genotyping is an important prognostic role in acute myeloid leukemia (AML) patients. We aimed to design this meta-analysis to discuss the incidence and prognostic effect of nucleolar phosphoprotein 1 (NPM1) and Fms-like tyrosine kinase 3 gene internal tandem (FLT3-ITD) gene in AML patients.

Methods: PubMed, Embase, Medline, and Cochrane library were systematically searched due to May 15, 2020. Four combinations of genotypes (FLT3-ITD^{neg}/NPM1^{mut}, FLT3-ITD^{pos}/NPM1^{mut}, FLT3-ITD^{neg}/NPM1^{wt}, FLT3-ITD^{pos}/NPM1^{wt}) were compared in association with the overall survival (OS) and leukemia-free survival (LFS) outcome, which expressed as pooled hazard ratio (HR) and 95% confidence intervals (CIs).

Results: Twenty-eight studies were included in our study. The incidence of FLT3-ITD^{neg}/NPM1^{mut}, FLT3-ITD^{pos}/NPM1^{mut}, FLT3-ITD^{neg}/NPM1^{wt}, and FLT3-ITD^{pos}/NPM1^{wt} was 16%, 13%, 50%, and 10%, respectively. The patients with FLT3-ITD^{neg}/NPM1^{mut} gene may have the best OS and LFS when comparing with FLT3-ITD^{pos}/NPM1^{mut} (HR = 1.94 and 1.70, $P < .01$), FLT3-ITD^{neg}/NPM1^{wt} (HR = 1.57 and 2.09, $P < .01$), and FLT3-ITD^{pos}/NPM1^{wt} (HR = 2.25 and 2.84, $P < .001$).

Conclusion: AML patients with FLT3-ITD^{neg}/NPM1^{mut} gene type have the best survival outcome than the other 3 gene types, which should be an independent genotyping in AML classification.

Abbreviations: AML = acute myeloid leukaemia, CEBPA = CCAAT/enhancer binding protein α gene mutation, CIs = confidence intervals, FLT3-ITD = Fms-like tyrosine kinase 3 gene internal tandem, HR = hazard ratio, LFS = leukemia-free survival, MeSH = the medical sub-headings terms, NOS = Newcastle-Ottawa Quality Assessment Scale, NK-AML = normal karyotype acute myeloid leukaemia, NPM1 = nucleolar phosphoprotein 1, OS = overall survival, PRISMA = the preferred reporting items for systematic review and meta-analysis guidelines.

Keywords: acute myeloid leukaemia, Fms-like tyrosine kinase 3, nucleolar phosphoprotein 1, survival analysis

1. Introduction

Acute myeloid leukemia (AML) is a highly heterogeneous malignant clonal disease caused by acquired myeloid progenitor/stem cell mutations.[1,2] Its diagnosis mainly relies on morphology, immunology, cytogenetics, and molecular biology detection, which referred to as "MICM" diagnostic typing.[3] With the continuous progress of cytogenetics research, it has been shown through research that changes in the chromosomal structure of patients with AML are not only clinical diagnostic markers for specific AML subtypes, but also the important prognostic factor of disease remission, risk of relapse, and overall survival (OS) in patients with AML.[4]

However, currently, about 40% to 49% of AML patients are not detected for abnormal karyotypes during routine chromosome testing, which is usually called normal karyotype acute myeloid leukemia (NK-AML). With continuous development, it has been found through testing that NK-AML patients have greater heterogeneity at the molecular biological level with different genetic variations. The heterogeneities are some genetic changes that have not been detected by conventional cytogenetic techniques and the molecular genetic changes are not only related to the pathogenesis of the disease but also affect its responsiveness

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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to treatment and its prognosis.[5] Studying genetic abnormalities related to the prognosis of NK-AML is one of the current research hotspots.[6,7]

Molecular biology techniques were used to detect molecular genetic changes including gene mutations and gene expression changes in NK-AML patients, for example, nucleolar phosphoprotein 1 (NPM1) gene mutation, Fms-like tyrosine kinase 3 gene internal tandem (FLT3-ITD) repeat, CCAAT/enhancer-binding protein α (CEBPA) gene mutation, etc. These molecular genetic changes have great clinical manifestations and prognosis of AML correlation and have been confirmed as molecular markers for further prognostic grading of AML patients.[8] NPM1 and FLT3 gene mutations are the most frequent forms of mutation in AML. These 2 genes have been recommended as necessary tests for AML in clinical practice guidelines, and are used as important reference indicators for treatment decisions.[9,10] However, it is still controversial whether the combination of the NPM1 and FLT3-ITD could be a prognostic factor for long-term outcomes, and therefore, could be available for classifying of the AML. Thus, we designed this systematic review and meta-analysis to evaluate the incidence of the combination of the 2 genes and the prognostic factor for AML based on the combination of NPM1 and FLT3-ITD genes.

2. Methods

This study was designed based on the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines.[11] The ethical approval was waived from the local institution due to the study design.

2.1. Search strategy

This study was aimed to analyze the incidence and prognostic effect of FLT3-ITD and NPM1 genes in AML patients and tried to classify the AML patients based on the 2 common gene types. PubMed, Embase, Medline, and Cochrane library were systematically searched due to May 15, 2020. The keywords and medical sub-headings (MeSH) terms were designed by an experienced librarian, and which was searched in the database above. The grey literature was searched in Google Scholar. The keywords included "FLT3," "NPM1," and "AML." All the studies were downloaded as cite into Endnote X7 (Thomson Reuters) for finding the duplication and for the further literature screening.

2.2. Selection criteria

The studies were included if met with the following criteria: the studies included both combination gene type, listed as FLT3-ITD^{neg}/NPM1^{mut}, FLT3-ITD^{pos}/NPM1^{mut}, FLT3-ITD^{neg}/NPM1^{wt} and FLT3-ITD^{pos}/NPM1^{wt}, in each study, which could be utilized to calculate the incidence of different categories; the studies mentioned the OS or leukemia-free survival (LFS) outcome, which were included for pooled hazard ratio (HR) in a meta-analysis for evaluating the prognostic effect of those 2 genes.

The exclusion criteria were: no AML patients included; reviews, comments, or case reports; not containing both gene type; studies published other than English.

2.3. Literature screening and data extraction

Two researchers (HL and XZ) independently screened the titles and abstracts according to the inclusion and exclusion criteria. The full text was further assessed if the decision cannot be made by the titles and abstracts. The third investigator (CS) was adapted for discussion for any disagreement that existed when literature screening. Similarly, those 2 researchers independently extracted the data from the published articles and imported them into a standard form. The extracted information included: the study characteristics (author, publish year, recruitment period, country, institution, etc), the patient data (treatment, total sample, median age, sex, white blood cell count, karyotype, cytogenetics risks, etc), the incidence of the 4 types of genes and the patient characteristics for each type if possible, and the OS, LFS, relapse incidence, etc. The HR and 95% confidence intervals (CIs) associating with OS and LFS were extracted from Cox regression or Kaplan–Meier plots.

2.4. Quality assessment and definition

Two investigators independently assessed the quality of the including studies. The Newcastle-Ottawa Quality Assessment Scale (NOS) was used for evaluating the observation studies and case-control studies, with a high quality of 6 to 9, whereas low quality was scored as 0 to 5.[12]

The cytogenetic risk in AML was divided into 3 groups: favorable, intermediate, and adverse risk, which was useful for diagnosis, and guideline for treatment for AML patients.[13]

Based on the 4 types of combination of FLT3-ITD (positive or negative) and NPM1 (mutation or wild type), the patients were categorized into FLT3-ITD^{neg}/NPM1^{mut}, FLT3-ITD^{pos}/NPM1^{mut}, FLT3-ITD^{neg}/NPM1^{wt}, and FLT3-ITD^{pos}/NPM1^{wt} groups.

2.5. Statistical analysis

The survival analysis was combined using HR with 95% CIs. If the HR was not described in the univariate or multivariate analysis, we calculated the time-to-event data through the Kaplan–Meier survival curve based on Tierney method.[14] The likelihood Chi-squared test and I^2 statistics were used for detecting heterogeneity across studies ($I^2 \geq 50\%$ indicating the presence of heterogeneity). When the heterogeneity did not existed among studies, the fixed-effect model was used. On the opposite, the random-effect model was used for evaluating the pooled HRs if heterogeneity existed among studies. The P-value of $<.05$ was regarded as significant. All statistical analyses were performed by Stata 15.0 software (Stata Corporation, College Station, TX).

3. Results

There were 3977 studies were identified based on the search strategy in those 4 electrical databases. Other 5 studies were found in Google Scholar. After deleting the duplicated articles, 2980 studies were screened by titles and abstracts. Two thousand six hundred sixty seven studies were assessed as irrelevant studies, and rest 313 studies were further evaluated in full text. After excluding the articles based on the exclusion criteria, 28 studies were included for qualitative synthesis and 12 studies were identified for calculating the HRs among studies.[2,15–41] The flowchart was shown in Fig. 1.

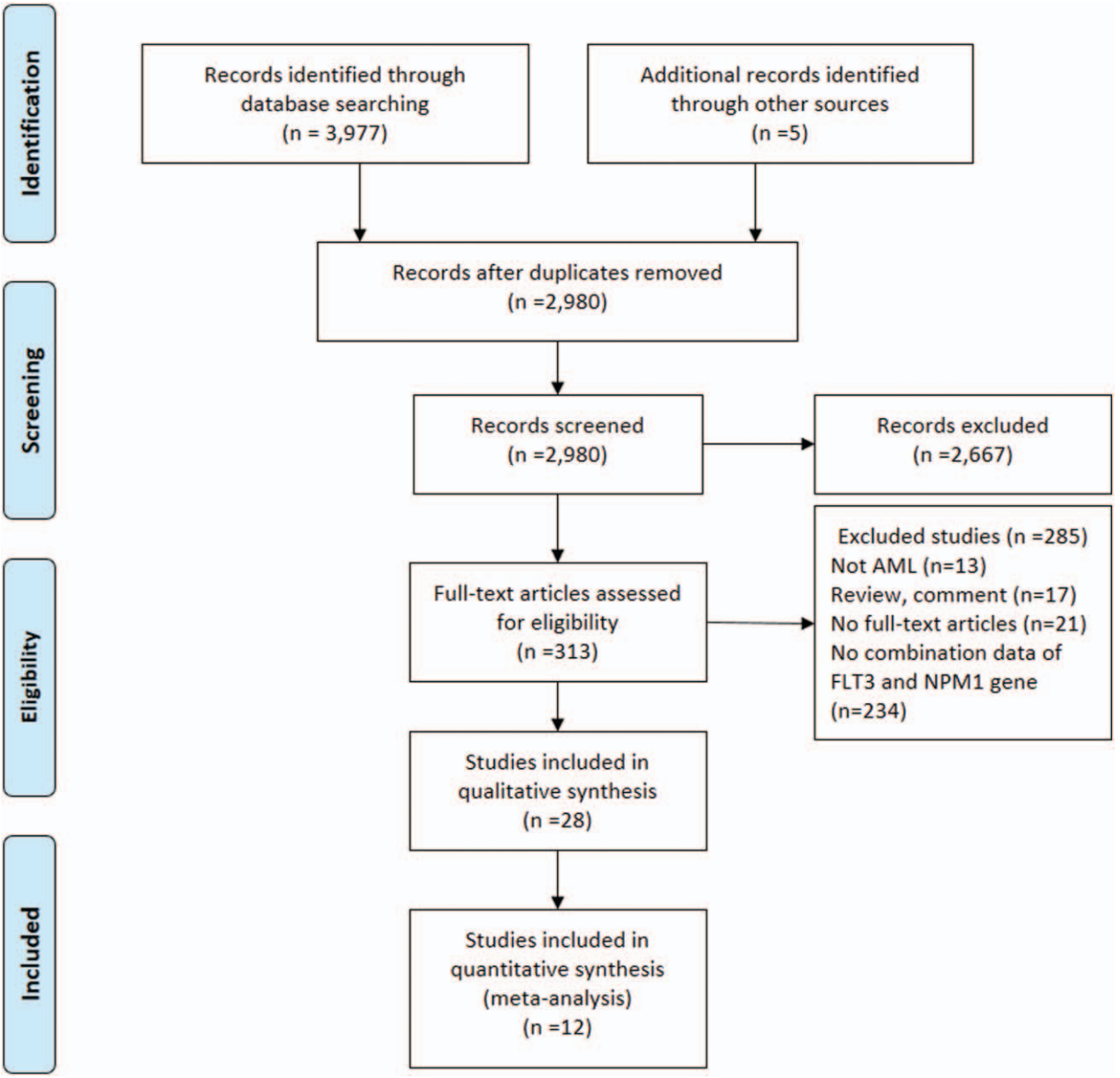

Figure 1. The flowchart of literature screening.

3.1. Characteristics of included studies

Twenty-eight studies were identified with 20,310 patients in our study (Table 1). The published year ranged from 2007 to 2020, with recruitment year ranging from 1995 to 2016. The patient data came from 13 countries, including Australia, France, Germany, India, Israel, Italy, Japan, Korea, Netherlands, South Africa, Spain, United Kingdom, and the United States of America. Two kinds of treatments were included (hematopoietic stem cell transplantation and chemotherapy). Half of the patients (51.2%, ranging from 43.0% to 63.3%) were men and the median occurrence age was 52 years.

The genetic classification based on FLT3-ITD and NPM1 was listed in Table 2. 16% of patients (ranging from 4% to 34%) were diagnosed as FLT3-ITD^{neg}/NPM1^{mut}, 13% of the AML patients (ranging from 3% to 38%) were diagnosed as FLT3-ITD^{pos}/NPM1^{mut}, 50% of patients (ranging from 9% to 75%) were diagnosed as FLT3-ITD^{neg}/NPM1^{wt}, and 10% of the patients (ranging from 3% to 23%) were diagnosed as FLT3-ITD^{pos}/NPM1^{wt}. The median incidences of favorable, intermediate, and adverse cytogenetics risk were 7%, 46%, and 17%, respectively.

The assessment of quality between studies was also shown in Table 2. Sixteen studies were regarded as median quality with scores of 5 to 6, and 12 studies were regarded as high quality with scores of >7.

3.2. The prognostic effect of FLT3-ITD and NPM1 in long-term outcome

The association between FLT3-ITD/NPM1 gene and OS was shown in Fig. 2 (fixed-effect model) and Fig. 3 (random-effect

Table 1

The characteristics of included studies.

Author	Year	Recruitment year	country	Treatment	Included patients	Male, %	Median age, year	Median WBC, 10^9/L
Shouval, R. et al	2020	2000-2014	Israel	HSCT	405	200 (49)	52.5 (42.9–60)	NG
Heiblig, M. et al	2019	2000-2016	France	Intensive chemotherapy	495	213 (43)	69 (64–73)	5.6 (1.9–32)
Pallarès, V. et al	2018	NG	Spain	Intensive chemotherapy	324	172 (53)	55 (17–70)	20 (0.03–325)
Kuwatsuka, Y. et al	2018	2001–2005	Japan	Intensive chemotherapy, HSCT	103	NG	NG	17.15 (0.23–203.3)
Craddock, C. et al	2018	2000–2015	Europe	HSCT	2028	1042 (51)	51 (18–77)	12.4 (0.1–780)
				Intensive chemotherapy	570	296 (52)	47 (16–77)	12 (0.3–510)
Sazawal, S. et al	2017	NG	India	NG	84	NG	NG	NG
Bradstock, K. F. et al	2017	2003–2010	Australia	Intensive chemotherapy, HSCT	176	NG	NG	NG
Alakel, N. et al.	2017	1996–2009	Germany	Intensive chemotherapy, HSCT	3240	1610 (50)	57 (15–87)	1.06 (0–2.67)
McGregor, A. K. et al	2016	2007–2011	UK	Intensive chemotherapy, HSCT	363	190 (52)	NG	NG
Ahn, J. S. et al.	2016	1998–2012	Korea	HSCT	115	57 (50)	42 (15–64)	31.3 (0.9–39.2)
Walter, R. B. et al	2015	1988–2010	USA	Intensive chemotherapy	4601	2442 (53)	52 (15–90)	15 (0–559)
Schmid, C. et al	2015	2006–2012	Italy	HSCT	702	357 (51)	51 (18–71)	NG
Lichtenegger, F. S. et al	2015	NG	Germany	NG	512	257 (50)	58 (18–85)	NG
Marshall, R. C. et al	2014	2004–2009	South Africa	NG	160	77 (48)	41 (17–81)	12.3 (0.69–582)
Lazenby, M. et al	2014	2006–2012	UK	Intensive chemotherapy	806	510 (63)	NG	NG
				Non-intensive chemotherapy	471	296 (63)	NG	NG
Pfeiffer, T. et al	2013	1999–2011	Germany	Intensive chemotherapy, HSCT	141	70 (50)	51 (18–69)	NG
Ribeiro, A. F. T et al	2012	NG	Netherlands	Intensive chemotherapy, HSCT	415	NG	NG	NG
Ibáñez, M. et al	2012	1998–2009	Spain	Intensive chemotherapy, HSCT	175	99 (57)	62 (16–88)	11.7 (1–396)
Haferlach, T. et al	2012	2005–2010	Germany	intensive chemotherapy, HSCT	805	410 (51)	66.6 (20.0–93.3)	37.7 (0.1–600.0)
Dufour, A. et al	2012	NG	Germany	Intensive chemotherapy, HSCT	663	NG	NG	NG
Becker, H. et al	2011	NG	USA	Intensive chemotherapy, HSCT	433	216 (50)	62 (18–83)	NG
Del Poeta, G. et al	2010	1996–2007	Italy	Intensive chemotherapy, HSCT	222	120 (54)	61	18.3
Abbas, S. et al	2010	NG	Netherlands	Intensive chemotherapy, HSCT	893	429 (48)	NG	NG
de Jonge, H. J. et al	2009	NG	Netherlands	Intensive chemotherapy, HSCT	525	NG	46.6 (15.2–77.2)	26 (0.3–510)
Scholl, S. et al	2008	1999–2005	Germany	Intensive chemotherapy, HSCT	99	48 (48)	71 (60–85)	14.8 (0.4–321)
Lo-Coco, F. et al	2008	1999–2003	Italy	intensive chemotherapy, HSCT	397	NG	NG	NG
Tamburini, J. et al	2007	NG	France	Intensive chemotherapy, HSCT	92	41 (45)	44 (12)	12 (0.4–252)
Brown, P. et al	2007	1995–1999	USA	Intensive chemotherapy, HSCT	295	144 (49)	9.5 (0–19.5)	47.7 (1.3–667)

HSCT = hematopoietic stem cell transplantation, NG = not given, WBC = white blood cell.

Table 2

The incidence of FLT3 and NPM1 mutation in AML patients.

Author	Year	NOS	FLT3-ITD^{neg}/ NPM1^{mut}, %	FLT3-ITD^{pos}/ NPM1^{mut}, %	FLT3-ITD^{neg}/ NPM1^{wt}, %	FLT3-ITD^{pos}/ NPM1^{wt}, %	Cytogenetics risk		
							Favorable	Intermediate	Adverse
Shouval, R. et al	2020	8	120 (30)	46 (11)	201 (50)	38 (9)	NG	NG	NG
Heiblig, M. et al	2019	7	46 (9)	37 (7)	107 (22)	18 (4)	16 (3)	34 (7)	3 (1)
Pallarès, V. et al	2018	5	92 (28)	63 (19)	125 (39)	31 (10)	NG	NG	NG
Kuwatsuka, Y. et al	2018	7	9 (9)	5 (5)	69 (67)	17 (17)	NG	NG	NG
Craddock, C. et al	2018	5	278 (14)	536 (26)	1061 (52)	153 (8)	NG	NG	NG
Craddock, C. et al	2018	5	25 (4)	48 (8)	184 (32)	54 (9)	NG	NG	NG
Sazawal, S. et al	2017	7	12 (14)	3 (4)	63 (75)	6 (7)	NG	NG	NG
Bradstock, K. F. et al	2017	8	51 (29)	40 (23)	70 (40)	26 (15)	NG	NG	NG
Alakel, N. et al	2017	6	265 (8)	335 (10)	1668 (51)	494 (15)	214 (7)	2331 (72)	695 (21)
McGregor, A. K. et al	2016	8	25 (7)	35 (10)	54 (15)	11 (3)	23 (6)	192 (53)	72 (20)
Ahn, J. S. et al	2016	8	25 (22)	23 (20)	27 (23)	12 (10)	NG	NG	NG
Walter, R. B. et al	2015	9	773 (17)	594 (13)	2792 (61)	442 (10)	259 (6)	1447 (31)	275 (6)
Schmid, C. et al	2015	8	68 (10)	269 (38)	290 (41)	75 (11)	NG	NG	NG
Lichtenegger, F. S. et al	2015	7	54 (11)	54 (11)	377 (74)	27 (5)	8 (2)	115 (22)	36 (7)
Marshall, R. C. et al	2014	5	12 (8)	9 (6)	120 (75)	19 (12)	NG	NG	NG
Lazenby, M. et al	2014	7	96 (12)	65 (8)	504 (63)	54 (7)	24 (3)	408 (51)	113 (14)
Lazenby, M. et al	2014	7	49 (10)	38 (8)	344 (73)	26 (6)	5 (1)	245 (52)	73 (15)
Pfeiffer, T. et al	2013	7	13 (9)	18 (13)	77 (55)	33 (23)	NG	NG	NG
Ribeiro, A. F. T et al	2012	6	64 (15)	69 (17)	235 (57)	47 (11)	57 (14)	191 (46)	64 (15)
Ibáñez, M. et al	2012	6	28 (16)	14 (8)	85 (49)	14 (8)	12 (7)	102 (59)	37 (21)
Haferlach, T. et al.	2012	5	240 (30)	151 (19)	346 (43)	68 (8)	NG	NG	NG
Dufour, A. et al	2012	5	206 (31)	136 (21)	235 (35)	55 (8)	NG	NG	NG
Becker, H. et al	2011	5	148 (34)	115 (27)	136 (31)	34 (8)	NG	NG	NG
Del Poeta, G. et al	2010	6	37 (17)	17 (8)	133 (60)	35 (16)	13 (6)	92 (41)	73 (33)
Abbas, S. et al	2010	5	140 (16)	126 (14)	544 (61)	85 (10)	NG	NG	NG
de Jonge, H. J. et al	2009	6	77 (15)	82 (16)	305 (58)	61 (12)	89 (17)	331 (63)	85 (16)
Scholl, S. et al	2008	6	16 (16)	7 (7)	67 (68)	9 (9)	3 (3)	48 (48)	29 (29)
Lo-Coco, F. et al	2008	5	46 (12)	21 (5)	37 (9)	7 (2)	NG	NG	NG
Tamburini, J. et al	2007	6	17 (18)	8 (9)	45 (49)	8 (9)	14 (15)	54 (59)	20 (22)
Brown, P. et al	2007	5	14 (5)	8 (3)	204 (69)	44 (15)	NG	NG	NG

NG = not given.

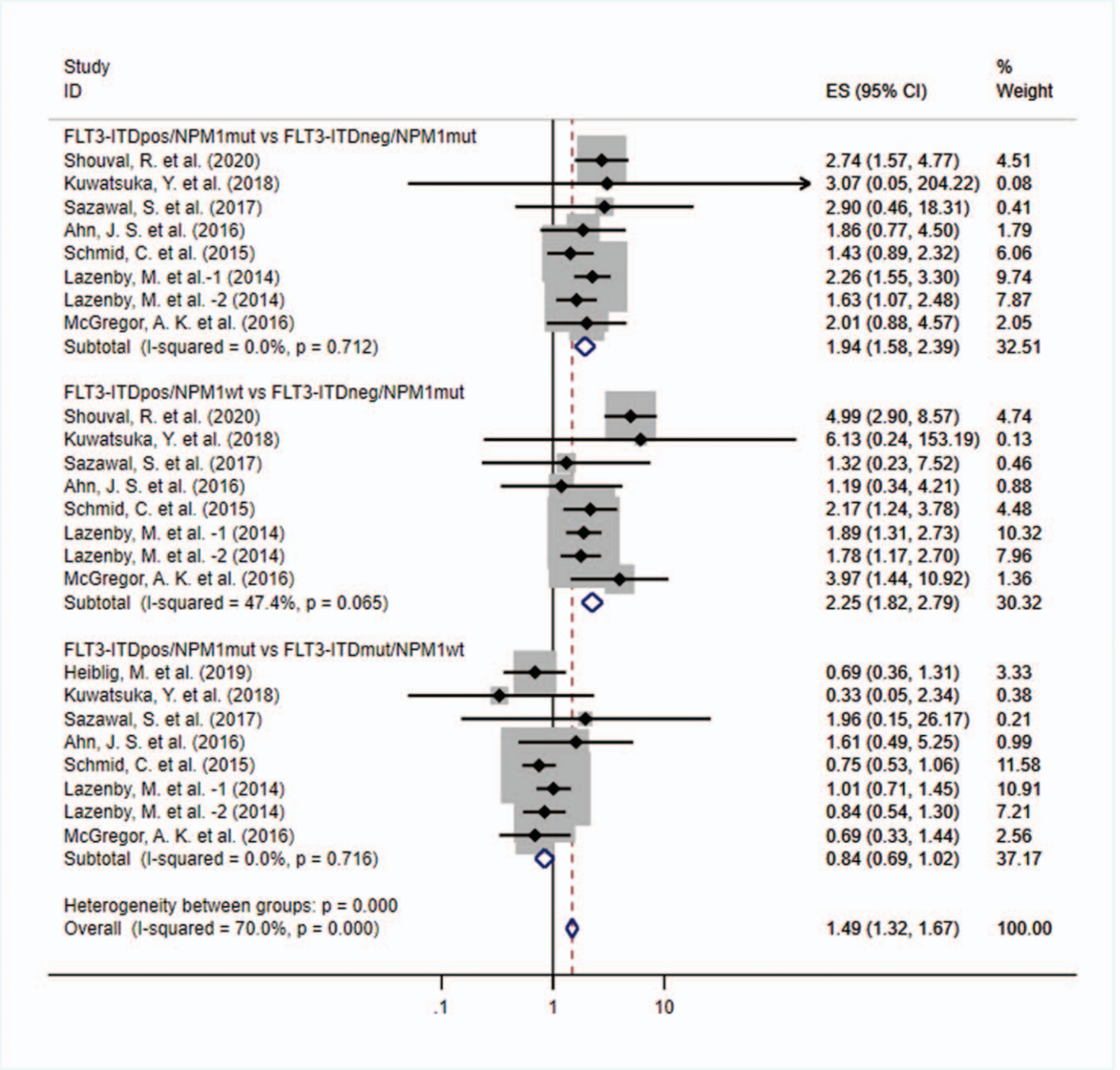

Figure 2. The pooled HRs of OS in comparison among 4 combination genotypes ($I^2 < 50\%$, fixed-effect model). HR = hazard ratio; OS = overall survival.

model). The FLT3-ITD^{neg}/NPM1^{mut} gene patients may have the best OS when comparing with FLT3-ITD^{pos}/NPM1^{mut} (HR = 1.94, 95%CI = 1.58–2.39, $P < .001$), FLT3-ITD^{neg}/NPM1^{wt} (HR = 1.57, 95%CI = 1.11–2.21, $P = .011$), and FLT3-ITD^{pos}/NPM1^{wt} (HR = 2.25, 95%CI = 1.82–2.79, $P < .001$). Besides, patients with FLT3-ITD^{neg}/NPM1^{wt} have a better OS than patients with FLT3-ITD^{pos}/NPM1^{mut} (HR = 1.36, 95%CI = 1.03–1.81, $P = .033$, Table 3) and FLT3-ITD^{pos}/NPM1^{wt} (HR = 1.86, 95%CI = 1.30–2.68, $P = .001$). There was no significant difference between FLT3-ITD^{pos}/NPM1^{wt} and FLT3-ITD^{pos}/NPM1^{mut} in terms of OS (HR = 0.84, 95%CI = 0.69–1.02, $P = .716$).

The association between FLT3-ITD/NPM1 gene and LFS was shown in Fig. 4 (fixed-effect model) and Fig. 5 (random effect model). Similarly, the FLT3-ITD^{neg}/NPM1^{mut} gene patients may have the best LFS when comparing with FLT3-ITD^{pos}/NPM1^{mut} (HR = 1.70, 95%CI = 1.25–2.31, $P = .001$), FLT3-ITD^{neg}/NPM1^{wt} (HR = 2.09, 95%CI = 1.66–2.64, $P < .001$), and FLT3-ITD^{pos}/NPM1^{wt} (HR = 2.84, 95%CI = 1.53–5.18, $P < .001$). However, there were no significantly differences between FLT3-ITD^{neg}/NPM1^{wt} and FLT3-ITD^{pos}/NPM1^{mut} (HR = 1.16, 95%CI = 0.77–1.73, $P = .479$ Table 3) and FLT3-ITD^{pos}/NPM1^{wt} (HR = 1.64, 95%CI = 0.86–3.15, $P = .136$) in terms of LFS. Interestingly, patients with FLT3-ITD^{pos}/NPM1^{mut} had a

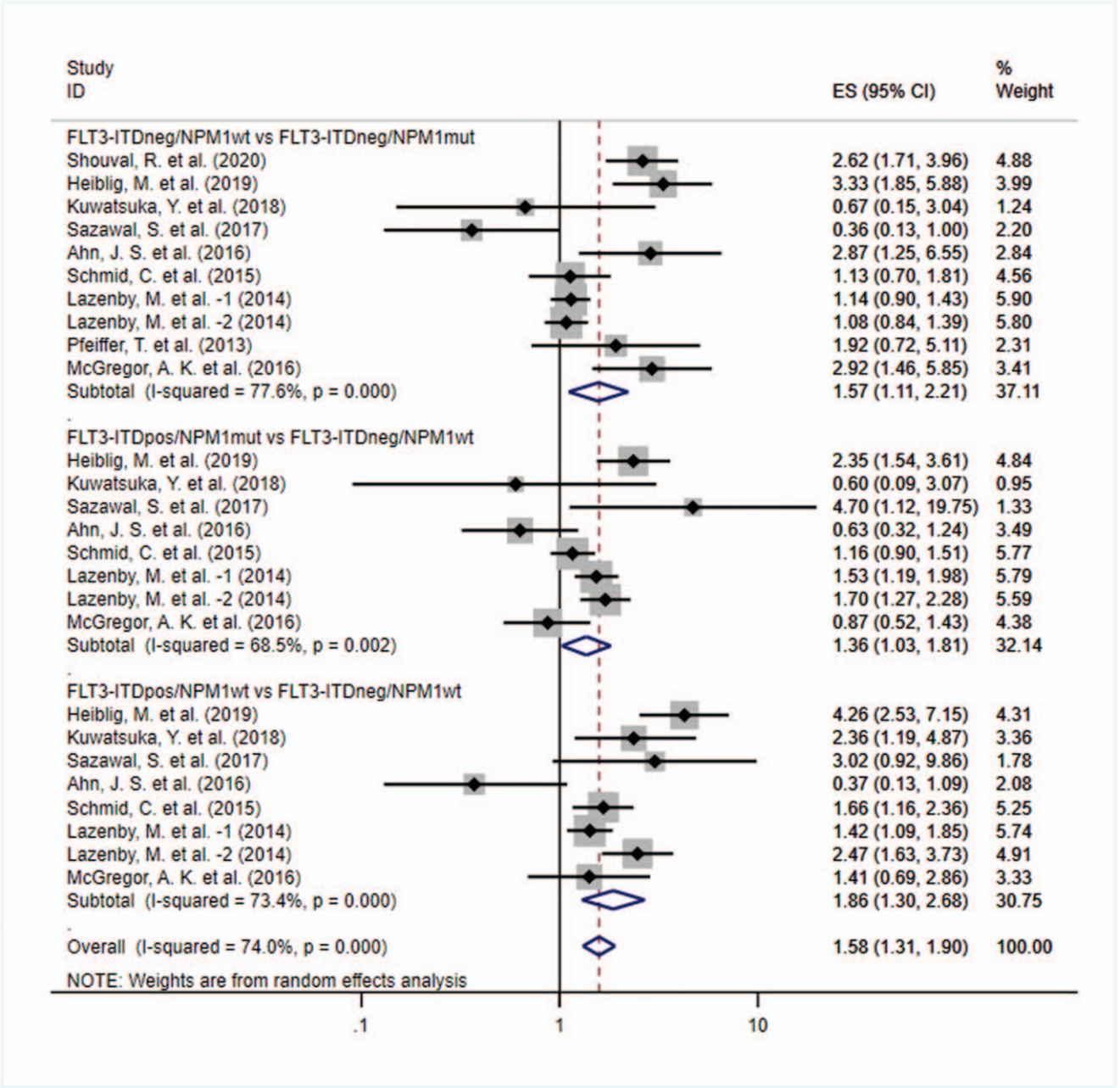

Figure 3. The pooled HRs of OS in comparison among 4 combination genotypes ($I^2 > 50\%$, randomized-effect model). HR = hazard ratio; OS = overall survival.

Table 3

Summary of the effect of FLT3 and NPM1 gene in assessing the outcome of AML patients.

	OS				LFS			
	HR	95%CI	I^2	*P*	HR	95%CI	I^2	*P*
FLT3-ITD^{pos}/NPM1^{mut} vs FLT3-ITD^{neg}/NPM1^{mut}	1.94	1.58–2.39	0	<.001	1.70	1.25–2.31	12.1	.001
FLT3-ITD^{neg}/NPM1^{wt} versus FLT3-ITD^{neg}/NPM1^{mut}	1.57	1.11–2.21	77.6	.011	2.09	1.66–2.64	46.9	<.001
FLT3-ITD^{pos}/NPM1^{wt} versus FLT3-ITD^{neg}/NPM1^{mut}	2.25	1.82–2.79	47.4	<.001	2.84	1.53–5.18	61.6	<.001
FLT3-ITD^{pos}/NPM1^{mut} versus FLT3-ITD^{neg}/NPM1^{wt}	1.36	1.03–1.81	68.5	.033	1.16	0.77–1.73	57.3	.479
FLT3-ITD^{pos}/NPM1^{wt} versus FLT3-ITD^{neg}/NPM1^{wt}	1.86	1.30–2.68	73.4	.001	1.64	0.86–3.15	78.3	.136
FLT3-ITD^{pos}/NPM1^{mut} versus FLT3-ITD^{pos}/NPM1^{wt}	0.84	0.69–1.02	0	.716	0.63	0.48–0.83	0	.001

AML = acute myeloid leukaemia; NPM1 = nucleolar phosphoprotein 1.

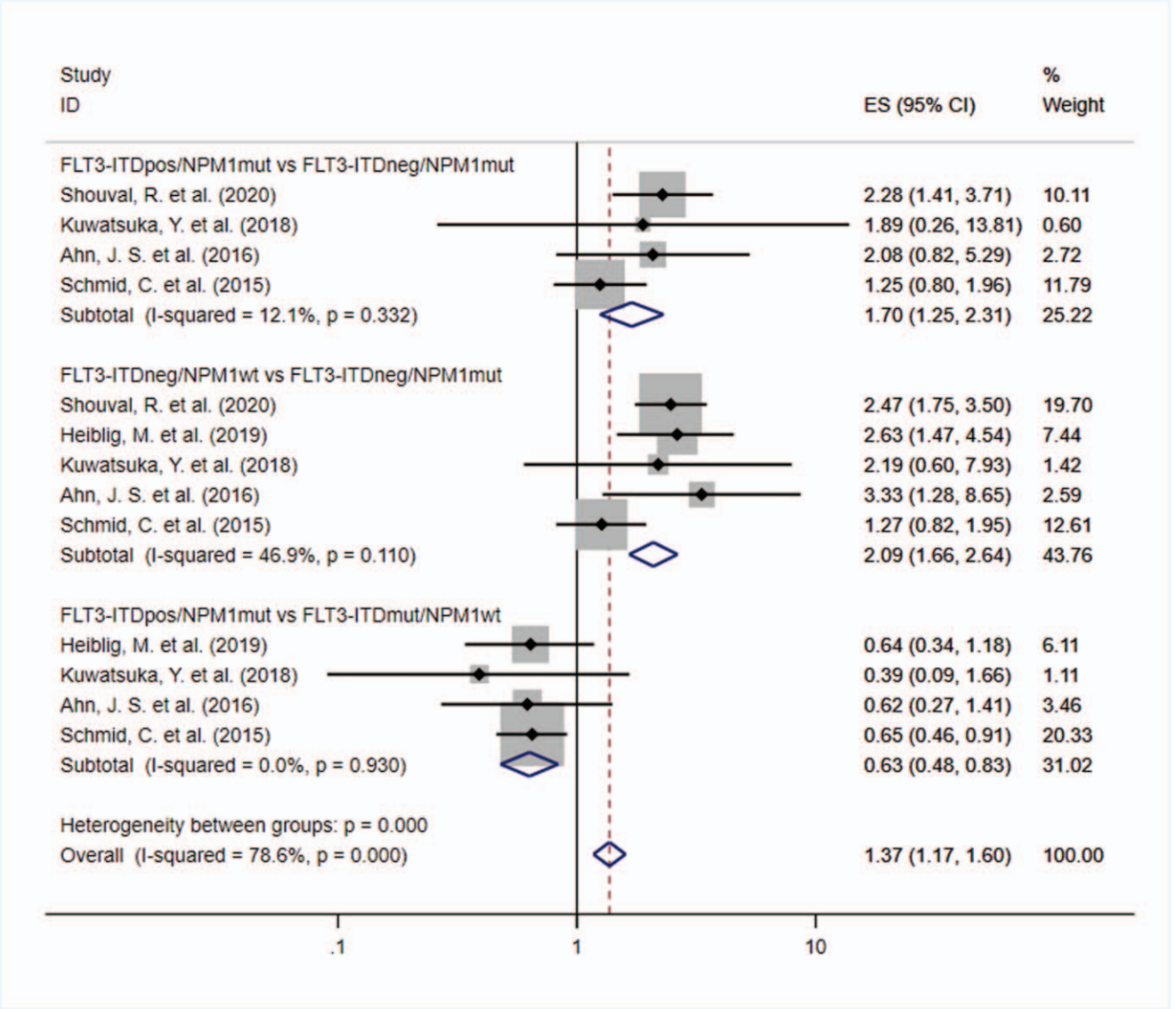

Figure 4. The pooled HRs of LFS in comparison among 4 combination genotypes ($I^2 < 50\%$, fixed-effect model). HR = hazard ratio; LFS = leukemia-free survival.

better LFS than patients with FLT3-ITD^{pos}/NPM1^{wt} (HR = 1.64, 95%CI = 0.86–3.15, $P = .136$).

4. Discussion

As far as we concern, this is the first and the largest meta-analysis to compare 4 different categories based on the FLT3-ITD and NPM1 genes in assessing the prognosis of AML. In our meta-analysis, we demonstrated that FLT3-ITD^{neg}/NPM1^{mut} AML patients may have the best OS and LFS, which demonstrated it should be a favorable prognosis group compared with the other 3 gene types. However, it is still controversial if there were significant differences among the rest 3 gene categories, even FLT3-ITD^{neg}/NPM1^{wt} patients have a better OS than FLT3-ITD^{pos}/NPM1^{mut} and FLT3-ITD^{pos}/NPM1^{wt}, while FLT3-ITD^{pos}/NPM1^{mut} patients had a better LFS than patients with FLT3-ITD^{pos}/NPM1^{wt}.

NPM1 protein is a multifunctional shuttle protein, which has a molecular chaperone role, regulates cell cycle progression and proliferation development through various signaling pathways, and is involved in the occurrence of various tumors.[6] Recent studies have shown that mutation of exon 12 of the NPM1 gene is a common form of mutation in AML patients, and its mutation rate is 25% to 35%. While in NK-AML patients, the mutation rate of it is higher, reaching 47% to 60%.[42] Schnittger et al[43,44] found that positive cases of NPM1 mutations are highly sensitive to chemotherapy-induced remission, complete remission (CR), LFS, and event-free survival. Therefore, the NPM1 gene mutation is considered to be an independent factor that predicts a good prognosis of AML.[45] Similarly, in our meta-analysis, we demonstrated that the FLT3-ITD^{neg}/NPM1^{mut} AML patients have a superior survival outcome than the other 3 gene types. But we cannot demonstrate that FLT3-ITD^{pos}/NPM1^{mut} patients have a better OS than patients with FLT3-ITD^{pos}/NPM1^{wt}, even they have a better LFS. At the same time, it was found that the NPM1 gene disappeared during the remission period of AML and appeared during the relapse period of AML. Its gene

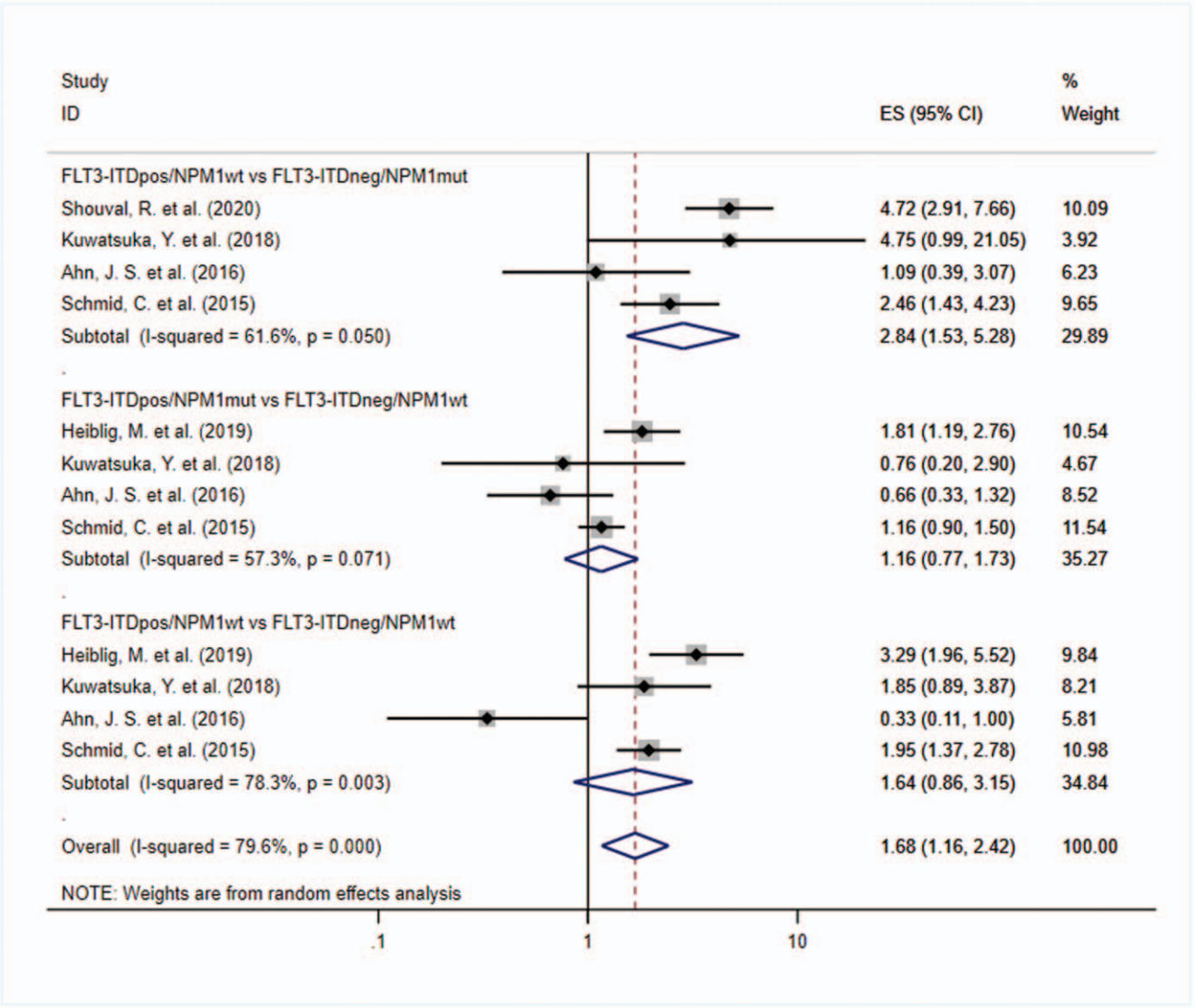

Figure 5. The pooled HRs of LFS in comparison among four combination genotypes ($I^2 > 50\%$, randomized-effect model). HR = hazard ratio; LFS = leukemia-free survival.

expression is related to the disease process. Therefore, quantitative detection of NPM1 gene expression can be used to monitor a minimal residual disease of leukemia.[44]

The FLT3 gene is an early hematopoietic growth factor receptor gene discovered in 1991. The encoded membrane-bound protein binds with the corresponding ligand to form a dimer and transmits activation signals through various cytoplasmic related proteins to regulate the growth and differentiation of hematopoietic cells.[7] It had 2 common manifestations in AML. One was first reported by Nakao et al in 1996 FLT3 internal tandem duplication (FLT3-ITD). The other is FLT3 tyrosine kinase domain point mutation (FLT3-TKD).[46] Of the 2 common mutations, FLT3-ITD is the most common type of mutation in the FLT3 gene mutation.[47] FLT3-ITD has a relatively high detection rate in NK-AML cases, approximately 28% to 38%.[47] Several studies have shown that both the LFS and OS of FLT3-ITD mutation-positive cases were significantly lower than those of FLT3-ITD negative patients.[48] In our study, we demonstrated that FLT3-ITD^{neg}/NPM1^{mut} patients have a better prognosis than FLT3-ITD^{neg}/NPM1^{wt} AML patients. And also, FLT3-ITD^{neg}/NPM1^{wt} patients have a better OS than FLT3-ITD^{pos}/NPM1^{wt} patients, which were similar to the previous report.

NPM1 and FLT3 gene mutations are the most common types of gene mutations in NK-AML cases. A previous study found that patients with only a simple mutation of the NPM1 gene are sensitive to chemotherapeutic drugs, with a high complete remission rate and a good prognosis.[45] The patients with a simple FLT3-ITD gene mutation have high white blood cells in the peripheral blood and high primitive bone marrow cells. Their induction remission rate is low, and the prognosis is poor.[15,48] In recent years, studies have shown that NPM1 and FLT3 gene mutations have a higher probability of co-occurrence,[49] but their prognosis reports are different. More large-scale prospective randomized controlled studies are needed to confirm this finding.

There were some limitations in our study. Firstly, most included studies were observational studies, the selection bias

could not be abandoned among 4 gene types. Secondly, due to the lack of data of the individual patients and the other important variables associated with survival outcome, the heterogeneity among studies could not be controlled. Further individual patient meta-analysis and meta-regression were needed for analyzing the independent prognostic effect of the 4 genes.

5. Conclusion

AML patients with FLT3-ITD^{neg}/NPM1^{mut} gene type have the best survival outcome than the other three gene types, which should be an independent genotyping in AML classification.

Author contributions

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Statistics analysis: Guangrong Jiang.
Supervision: Chunping Song.
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