Indian J Hematol Blood Transfus (Apr-June 2018) 34(2):240–252
https://doi.org/10.1007/s12288-017-0852-6

ORIGINAL ARTICLE

The Synonymous Isocitrate Dehydrogenase 1 315C>T SNP Confers an Adverse Prognosis in Egyptian Adult Patients with NPM1-/CEBPA-Negative Acute Myeloid Leukemia

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Received: 19 April 2017 / Accepted: 17 July 2017 / Published online: 24 July 2017

Abstract Although the clinical features of isocitrate dehydrogenase (*IDH*) genetic aberrations have been well-characterized in acute myeloid leukemia (AML), definitive information on their prognostic significance is lacking. We aimed to explore the prognostic significance of *IDH* gene alterations in an Egyptian cohort of adult patients with *de novo* AML. Diagnostic peripheral blood samples from 51 AML patients were analyzed for the presence of mutations/SNPs in exon 4 of *IDH1* and *IDH2* genes using polymerase chain reaction amplification followed by direct sequencing. *IDH* mutational status had no impact on event-free survival (EFS) and overall survival (OS), whereas the presence of *IDH1* 315C>T SNP was significantly associated with inferior EFS ($P = 0.037$) and OS ($P = 0.034$) as compared with wild-type *IDH1*. *IDH1* 315C>T SNP but not *IDH* mutations is associated with unfavorable outcomes, suggesting that AML patients with *IDH1* 315C>T SNP can represent a new subgroup of patients which allows refined risk stratification.

Keywords Acute myeloid leukemia · Isocitrate dehydrogenase 1 and 2 · Mutations · Single nucleotide polymorphisms · Prognostic markers

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Introduction

Acute myeloid leukemia (AML) is a clinically and pathogenetically heterogeneous group of hematopoietic malignancies characterized by maturation arrest and uncontrolled proliferation of early myeloid precursors accompanied by impaired normal hematopoiesis [1].

Conventional and molecular cytogenetics are essential components of risk stratification in the clinical management of AML patients. In addition to structural chromosomal alterations, molecular analysis of mutations in FMS-like tyrosine kinase 3-internal tandem duplication (*FLT3*-ITD), nucleophosmin 1 (*NPM1*) and CCAAT/enhancer binding protein alpha (*CEBPA*) are now broadly accepted in routine clinical practice. Particular, mutations in these three genes allow the stratification of patients with cytogenetically normal AML (CN-AML) into prognostic risk categories. Although the majority of CN-AML patients harbor one or more of the aforementioned mutations, in approximately 15% of the patients, no mutations have been detected, suggesting the existence of hitherto undiscovered genetic alterations that may contribute to further molecular risk stratification of this substantial percentage of patients [2].

A number of recurrently mutated genes have been identified in AML patients and many of the newly identified mutations are in genes that affect the epigenetic regulation of gene expression. Among a wide spectrum of genes, somatic mutations in the isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* have been identified as recurrent genetic aberration in AML [3].

IDH1 and *IDH2* are homodimeric enzymes that reversibly convert isocitrate to α-ketoglutarate (α-KG) in the cytosol/peroxisome and mitochondria, respectively, presumably for the purpose of the concomitant reduction of

nicotinamide adenine dinucleotide phosphate ($NADP^+$) to nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) [4]. Cancer-associated mutations in the *IDH1* and *IDH2* genes encoding isocitrate dehydrogenases have been shown to lead to a loss of the enzyme's ability to catalyze the oxidative decarboxylation of isocitrate to α-KG, and a neo-enzymatic gain of function, the NADPH-dependent reduction of α-KG to the oncometabolite 2-hydroxyglutarate (2-HG) [5, 6], which competitively inhabits α-KG-dependent enzymes that are important for normal DNA methylation [7]. Furthermore, it has been demonstrated that the expression of mutant IDH1/2 proteins leads to global DNA hypermethylation, which contributes to AML pathogenesis through the generation of increased 2-HG levels and impairment of hematopoietic differentiation [8, 9].

Several studies have investigated *IDH* mutations in different AML patient populations, with an incidence ranging from 15% to 20% [3]. Despite the recent insights into the distinct pathophysiology of *IDH* mutations, the prognostic significance of *IDH* mutations in AML is still controversial. While some studies have reported that *IDH* mutations were found to be associated with poor prognosis [10–13], others have suggested a lack of prognostic significance [14–16]. Previously, a synonymous single nucleotide polymorphism (SNP) located in codon 105 in exon 4 of the *IDH1* gene (315C>T: rs11554137) has been identified in AML patients, and has been reported to be associated with poor prognosis [15, 17].

To the best of our knowledge, the prevalence and prognostic value of *IDH* aberrations in Egyptian patients with AML has not been hitherto reported. The purpose of the present study was to investigate the frequency of genetic alterations in exon 4 of *IDH1/2* in an Egyptian cohort of AML patients. Furthermore, the association of *IDH* genetic aberrations with hematological, cytogenetic and known prognostic molecular markers as well as with clinical outcome was explored.

Patients and Methods

Study Cohort

Fifty-one Egyptian adult patients fulfilling the diagnostic criteria of *de novo* AML who were referred to the outpatient clinics of the Hematology unit, National Cancer Institute, Cairo University, Cairo, Egypt were enrolled in the present study. A written informed consent was obtained from all the recruited patients prior to inclusion into the study in accordance with the Declaration of Helsinki. The study protocol was approved by the review board of National Cancer Institute, Cairo University. Diagnosis and classification of the patients were based on the French-American-British classification (FAB) criteria [18, 19]. Data for immunophenotyping, cytogenetics and *FLT3*-ITD status were retrieved from the patients' medical records. All patients received standard first-line remission induction treatment with a DA-like regimen, which consisted of daunorubicin (DNR, 45 mg/m^2/day via intravenous (IV) infusion for 3 days, on days 1–3) and cytarabine (arabinofuranosyl cytidine, ara-C: 100 mg/m^2/day by IV infusion for 7 days, on days 1 through 7). All patients received two remission induction courses. After achieving complete remission (CR), patients were given three courses of postremission therapy (consolidation) with high-dose ara-C 3 g/m^2 every 12 h by continuous IV infusion over 3 h on days 1, 3 and 5. The three consolidation courses were administered at monthly intervals.

Analysis of *NPM1* and *CEBPA* Gene Aberrations

Prognostically-relevant genes were analyzed for frequently occurring aberrations as previously described. The most common mutation in exon 12 of *NPM1* (type A mutation) was tested using allele specific oligonucleotide-PCR [20]. *CEBPA* mutations were detected using PCR-single strand conformation polymorphism analysis [21].

Analysis of *IDH1* and *IDH2* Gene Alterations

Genomic DNA was extracted from mononuclear cells obtained from 51 AML patients using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) as per the manufacturer's instructions. DNA fragments spanning exons 4 of *IDH1* and *IDH2*, previously identified as hot spots for mutations in these genes [22], were amplified by PCR. Primer sequences for PCR were as follows: for *IDH1*, forward 5′- TGTGTTGAGATGGACGCCTATTTG-3′ and reverse 5′- TGCCACCAACGACCAAGTCA-3′; for *IDH2*, forward 5′-GGGGTTCAAATTCTGGTTGA-3′ and reverse 5′- CTAGGCGAGGAGCTCCAGT-3′ as previously described [23]. Both PCRs were performed in a 25 μL volume, containing 15 mM Tris–HCl + 50 mM KCl (Gene Amp 10X PCR Gold Buffer), 1.5 mM $MgCl_2$ (Gene Amp 25 mM $MgCl_2$), 200 μM of each dNTP (Gene Amp 10 mM dNTP Blend), 1 U Taq DNA polymerase (AmpliTaq Gold 5 U/μL DNA polymerase) (Applied Biosystems, Foster City, CA, USA), 0.5 μM each of forward and reverse primers (Sigma-Aldrich, St. Louis, MO, USA) and 100 ng of DNA. The thermal cycling conditions were as follows: initial denaturation at 94 °C for 3 min followed by 35 amplification cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min followed by a final extension step at 72 °C for 10 min. The PCR products were electrophoresed on a

2% agarose gel (Applied Biosystems/Ambion, Austin, USA) to verify the specificity of the products. The PCR products were purified using QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. The purified amplicons were directly sequenced in both directions with forward and reverse primers using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) in conjunction with GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Sequence data were analyzed using the Sequencing Analysis Software version 5.3.1 (Applied Biosystems, Foster City, CA, USA). The sequences were compared to the wild-type *IDH1* and *IDH2* cDNA. All aberrations were verified by sequencing non-amplified genomic DNA.

Statistical Analyses

Categorical variables were reported as the number of cases (percentage) and compared using the Pearson's Chi square (χ^2) test or Fisher's exact test as appropriate. Continuous variables were expressed as mean ± standard deviation (SD) if normally distributed and compared using the independent Student's *t* test or one-way analysis of variance (ANOVA) as appropriate. In contrast, continuous variables were expressed as median (range or interquartile range, IQR: 25th quartile to 75th quartile or minimum–maximum as appropriate) if non-normally distributed and compared using the non-parametric Mann–Whitney *U* test or Kruskal–Wallis test as appropriate. Univariate and multivariate logistic regression models were used to identify independent prognostic factors influencing CR, EFS and OS. The probabilities of EFS and OS were estimated using the Kaplan–Meier method and were compared among subsets of patients using the log-rank test. For all statistical analyses, the *P* values were two-sided, and a *P* value of <0.05 was deemed statistically significant. Data statistical analyses were performed using the statistical package for the social sciences (SPSS Statistics for Windows, Version 20.0; IBM Corp., Armonk, NY, USA). Genotypes and alleles frequency was estimated. Genotype frequencies were compared with the frequencies expected by the Hardy–Weinberg equilibrium (HWE) using a χ^2 goodness of fit test. All genetic analyses were performed using Haploview version 3.32 (Broad Institute, Cambridge, MA, USA) [24].

Results

Frequency of *IDH* Gene Alterations

In the overall cohort of 51 AML patients, IDH mutations were restricted to *IDH1* with 2 types of *IDH1* mutations have been identified in 4 patients. In contrast, no mutations affecting *IDH2* were detected in the entire cohort (Table 1). All patients with a mutated *IDH1* retained a wild-type allele, indicating heterozygosity of the mutant allele. Moreover, no patient concurrently had both c.C394T (p.R132C) and c.G395A (p.R132H) mutations, suggesting that these mutations are mutually exclusive.

The c.315C>T synonymous (p.G105G) *IDH1* SNP (rs11554137) was detected in 6 patients (11.8%) (Table 2). All patients with the c.315C>T SNP were heterozygous for the minor allele (T allele). The observed genotype frequencies of c.315C>T SNP did not deviate significantly from HWE expectations ($P = 0.655$). Two patients (3.9%) had *IDH2* SNPs, including one patient carried the intronic c.535-40G>A *IDH2* SNP (rs142033117) and another patient carried the synonymous c.429G>C (p.L143L) *IDH2* SNP (rs144712130) (Table 2). The patient with the c.535-40G>A SNP and the patient with the c.429G>C SNP were heterozygous for the minor allele (A allele and C allele, respectively). Similarly, the distribution of the observed genotypes of the two *IDH2* SNPs was not significantly different from the expected distribution according to HWE ($P = 0.944$).

We observed no association between the *IDH* SNP and the incidence of *IDH* mutations: Of the four patients with mutated *IDH1*, none harboring neither *IDH1* SNPs nor *IDH2* SNPs. Representative direct sequencing chromatograms of the identified *IDH* aberrations are shown in Fig. 1.

Association of *IDH* Gene Alterations with Pre-treatment Characteristics and Clinical Outcome

Patients with *IDH* mutations/SNPs showed no significant differences in demographic and clinical features as compared to those without *IDH* mutations (Table 1) or SNPs (Table 2).

The distribution of *IDH* mutations and SNPs didn't differ significantly among the different FAB subtypes, indicating that there was no preference of *IDH* mutations/SNPs among the different FAB subtypes. Interestingly, all patients carrying *IDH* mutations/SNPs had a normal karyotype. Furthermore, no significant association was observed between *IDH* mutations/SNPs and FLT3-ITD mutations.

There were no significant differences in CR rates between *IDH*-mutated and unmutated patients (Table 1) or between patients with and without *IDH* SNPs (Table 2). In addition, there were no significant differences in the distribution of *IDH* mutations/SNPs between patients with events and those without events. Likewise, the distribution of *IDH* mutations was not significantly different between surviving and non- surviving patients (Table 1). In

Table 1 Clinical characteristics and outcomes of AML patients with wild-type or mutant-type IDH1

Characteristic	All cases (n = 51) n(%)	IDH1 mutation status			IDH1 mutation type		
		$IDH1^{WT}$ (n = 47, 92.2%) n(%)	$IDH1^{MT}$ (n = 4, 7.8%) n(%)	*P* value	C394T (R132C) (n = 1, 25%) n(%)	G395A (R132H) (n = 3, 75%) n(%)	*P* value
Age	37.8 ± 12.4	37.5 ± 12.5	41.3 ± 11.2	0.563	50	38.3 ± 11.7	1.000
<40	27 (52.9)	25 (53.2)	2 (50)	0.649	0 (0)	2 (66.7)	0.500
≥40	24 (47.1)	22 (50.0)	2 (50)		1 (100)	1 (33.3)	
Sex, n(%)							
Male	23 (45.1)	23 (48.9)	0 (0)	0.082	0 (0)	0 (0)	1.000
Female	28 (54.9)	24 (51.1)	4 (100)		1 (100)	3 (100)	
Male/female ratio	0.82	0.96	0.0		0.0	0.0	
WBC ($\times 10^9$/L)	34 (7.4–84.7)	29.8 (7.2–93.8)	62.8 (29.2–72.8)	0.445	55	70.62 (20.5–73.5)	1.000
<25	24 (47.1)	23 (48.9)	1 (25)	0.351	0 (0)	1 (33.3)	0.750
≥25	27 (52.9)	24 (51.1)	3 (75)		1 (100)	2 (66.7)	
Hb (g/dL)	7.2 ± 1.8	7.2 ± 1.8	7.5 ± 1.0	0.696	6.7	7.8 ± 1.0	0.434
<8	35 (68.6)	32 (68.1)	3 (75)	0.629	1 (100)	2 (66.7)	0.750
≥8	16 (31.4)	15 (31.9)	1 (25)		0 (0)	1 (33.3)	
Plt ($\times 10^9$/L)	31 (20–56)	32 (20–57)	27.0 (18.5–31.7)	0.466	17.0	31 (23–32)	0.500
<30	25 (49)	23 (48.9)	2 (50)	0.680	1 (100)	1 (33.3)	0.500
≥30	26 (51)	24 (51.1)	2 (50)		0 (0)	2 (66.7)	
BM blasts (%)	62 (34–80)	62 (34–79)	67 (28–91)	0.800	91.0	43 (23–91)	0.500
<60	24 (47.1)	22 (46.8)	2 (50)	0.649	0 (0)	2 (66.7)	1.000
≥60	27 (52.9)	25 (53.2)	2 (50)		1 (100)	1 (33.3)	
FAB subtype, n(%)							
M0	2 (3.9)	2 (4.3)	0 (0.0)	0.229	0 (0.0)	0 (0.0)	0.500
M1	13 (25.5)	11 (23.4)	2 (50.0)		1 (100)	1 (33.3)	
M2	18 (35.3)	16 (34.0)	2 (50.0)		0 (0)	2 (66.7)	
M4	18 (35.3)	18 (38.3)	0.0 (0.0)		0 (0.0)	0 (0.0)	
Karyotype							
Normal	48 (94.1)	44 (93.6)	4 (100)	0.779	1 (100)	3 (100)	1.000
t(8;21)	3 (5.9)	3 (6.4)	0 (0)		0 (0)	0 (0)	
Cytogenetic risk group							
Favorable	3 (5.9)	3 (6.4)	0 (0)	0.779	0 (0)	0 (0)	1.000
Intermediate	48 (94.1)	44 (93.6)	4 (100)		1 (100)	3 (100)	
Immunophenotype							
CD4	18 (35.3)	18 (38.3)	0 (0)	0.164	1 (100)	3 (100)	1.000
CD7	2 (3.9)	2 (4.3)	0 (0)	0.848	1 (100)	3 (100)	1.000
CD13	51 (100.0)	47 (100.0)	4 (100)	1.000	1 (100)	3 (100)	1.000
CD14	18 (35.3)	18 (38.3)	0 (0)	0.164	1 (100)	3 (100)	1.000
CD15	36 (70.6)	34 (72.3)	2 (50)	0.336	0 (0.0)	2 (66.7)	0.500
CD33	49 (96.1)	45 (95.8)	4 (100)	0.848	1 (100)	3 (100)	1.000
CD34	20 (39.2)	18 (38.3)	2 (50)	0.514	1 (100)	1 (33.3)	0.500
CD117	40 (78.4)	36 (76.6)	4 (100)	0.366	1 (100)	3 (100)	1.000
HLA-DR	51 (100.0)	47 (100.0)	4 (100)	1.000	1 (100)	3 (100)	1.000
FLT3-ITD							
Present	11 (21.6)	10 (21.3)	1 (25)	0.634	0 (0.0)	1 (33.3)	0.750
Absent	40 (78.4)	37 (78.7)	3 (75)		1 (100)	2 (66.7)	
CR							
Yes	27 (52.9)	25 (53.2)	2 (50)	0.649	0 (0.0)	2 (66.7)	0.500
No	24 (47.1)	22 (46.8)	2 (50)		1 (100)	1 (33.3)	

Table 1 continued

Characteristic	All cases (n = 51) n(%)	IDH1 mutation status			IDH1 mutation type		
		$IDH1^{WT}$ (n = 47, 92.2%) n(%)	$IDH1^{MT}$ (n = 4, 7.8%) n(%)	*P* value	C394T (R132C) (n = 1, 25%) n(%)	G395A (R132H) (n = 3, 75%) n(%)	*P* value
Event-free survival (EFS, months)	0.69 (0.44–4.44)	0.68 (0.44–4.44)	0.76 (0.43–3.74)	0.933	0.72	43 (23–91)	0.500
Yes	7 (13.7)	7(14.9)	0 (0)	0534	0 (0.0)	0 (0.0)	1.000
No	44 (86.3)	40 (85.1)	4 (100)		1 (100)	3 (100)	
Overall survival (OS, months)	1.12 (0.79–5.39)	1.18 (0.72–5.39)	0.97 (0.87–4.9)	0.826	1.02	0.79 (0.33–4.73)	0.500
Alive	18 (35.3)	17 (36.2)	1 (25)	0.557	0 (0.0)	1 (33.3)	0.750
Dead	33 (64.7)	30 (63.8)	3 (75)		1 (100)	2 (66.7)	

Risk status: favorable-risk: t(8;21); Intermediate-risk: normal

Qualitative data are represented as the number of cases (%), whereas quantitative data are represented as mean ± SD (range, minimum–maximum) if normally distributed or as median (range or interquartile range, IQR: 25th quartile to 75th quartile) if non-normally distributed

The nucleotide sequence variations and amino acid changes are designated according to the recommendations of the Human Genome Variation Society (http://www.hgvs.org/mutnomen/). IDH1 nucleotide numbering uses the first base of the translation start codon as nucleotide _1 on the basis of National Center for Biotechnology Information sequence NM_005896.2

BM bone marrow, *C* cysteine, *CD* cluster of differentiation, *CR* complete remission, *EFS* event-free survival, *FAB* French-American-British, *FLT3* FMS-like tyrosine kinase-3, *H* histidine, *Hb* hemoglobin, *IDH* isocitrate dehydrogenase, *ITD* internal tandem duplication, *MT* mutant-type, *OS* overall survival, *Plt* platelets, *R* arginine, *WBC* white blood cells, *WT* wild-type

contrast, the distribution of *IDH* SNPs differed significantly between surviving and non- surviving patients (Table 2).

Prognostic Impact of *IDH* Gene Alterations

As shown in Table 3, univariate analysis showed that only the percentage of BM blasts had a significant impact on CR and OS. Furthermore, only WBC count had a significant impact on EFS. The significant variables were selected as prognostic variables, and multivariate analysis was performed stepwise. Consequently, high BM blasts percentage (≥60%) was identified as independent prognostic predictor for lower CR rate and shorter OS after adjusting for other variables. Interestingly, high WBC count ($\geq 25 \times 10^9$/L) tended to be independently associated with shorter EFS when considering other factors.

Kaplan–Meier survival analyses were carried out to estimate the distribution of EFS (Fig. 2) and OS (Fig. 3). There was no significant difference in terms of EFS between patients with and without *IDH* mutations (Fig. 2a). Similarly, no significant difference regarding EFS between patients with and without *IDH* SNPs, neither in the entire group (Fig. 2b) nor in the subgroup of *IDH2* SNPs (Fig. 2d). In contrast, patients with the c.315C>T *IDH1* SNP had poorer EFS as compared to those with wild-type *IDH1* (Fig. 2c). OS didn't differ significantly between patients with and without *IDH* mutations (Fig. 3a). In contrast, patients with *IDH* SNPs showed inferior OS as compared to those with wild-type *IDH* (Fig. 3b). Furthermore, patients with the c.315C>T *IDH1* SNP were significantly associated with inferior OS as compared to those with wild-type *IDH1* (Fig. 3c). On the other hand, OS was not significantly different between patients with and without *IDH2* SNPs (Fig. 3D). However, the power of this analysis is limited by the relatively small number of patients carrying *IDH2* SNPs.

Discussion

To date, analysis of cytogenetic aberrations at diagnosis provides the most important prognostic information of AML. In addition, the identification of *FLT3*- ITD, *NPM1* and *CEBPA* mutations and their incorporation into prognostic models was shown to improve risk stratification, especially in the large group of patients with CN-AML [2].

Although these genes have become clinically established prognostic markers in CN-AML, there is still a large group of intermediate risk patients without *FLT3*-ITD, *NPM1* and *CEBPA* mutations or other reliable prognostic markers, highlighting the need for additional markers that could explain the differential outcome in this heterogeneous patient group [25].

Mutations in the genes encoding, *IDH1* and *IDH2*, are among the most commonly occurring mutations found in

Table 2 Clinical characteristics and outcomes of AML patients with or without IDH1/2 SNPs

Characteristic	All cases (n = 51) n(%)	IDH SNP status			IDH1 SNP status			IDH2 SNP status		
		IDH SNP− (n = 43, 84.3%) n(%)	IDH SNP+ (n = 8, 15.7%) n(%)	*P* value	IDH1 SNP− (n = 45, 88.2%) n(%)	IDH1 SNP+ (n = 6, 11.8%) n(%)	*P* value	IDH2 SNP − (n = 49, 96.1%) n(%)	IDH2 SNP + (n = 2, 3.9%) n(%)	*P* value
Age	37.8 ± 12.4	38.5 ± 12.5	33.9 ± 10.9	0.333	38.5 ± 12.2	32.2 ± 12.3	0.575	37.7 ± 12.5	39.0 ± 2.8	0.888
<40	27 (52.9)	22 (51.2)	5 (62.5)	0.422	23 (51.1)	4 (66.7)		26 (53.1)	1 (50)	
≥40	24 (47.1)	21 (48.8)	5 (37.5)		22 (49.8)	2 (33.3)	0.393	23 (46.9)	1 (50)	0.725
Sex, n(%)										
Male	23 (45.1)	19 (44.2)	4 (50)	0.529	21 (46.7)	2 (33.3)	0.434	21 (42.9)	2 (100)	0.198
Female	28 (54.9)	24 (55.8)	4 (50)		24 (53.3)	4 (66.7)		28 (57.1)	0 (0)	
Male/female ratio	0.82	0.79	1.0					0.75	–	
WBC ($\times 10^9$/L)	34 (7.4–84.7)	39.0 (5.0–93.8)	29.0 (17.2–58.5)	0.829	39.0 (6.1–89.3)	61.8 (14.3–103.2)	0.853	34 (7.3–89.3)	39.2 (24.6–53.8)	0.941
<25	24 (47.1)	20 (46.5)	4 (50)	0.578	21 (46.7)	3 (50)	0.607	23 (46.9)	1 (50)	0.725
≥25	27 (52.9)	23 (53.5)	4 (50)		24 (53.3)	3 (50)		26 (53.1)	1 (50)	
Hb (g/dL)	7.2 ± 1.8	7.2 ± 1.7	7.5 ± 2.4	0.685	7.2 ± 1.8	7.2 ± 2.0	0.850	7.2 ± 1.7	8.4 ± 4.2	0.359
<8	35 (68.6)	30 (69.8)	5 (62.5)	0.488	31 (68.9)	4 (66.7)	0.621	34 (69.4)	1 (50)	0.533
≥8	16 (31.4)	13 (30.2)	3 (37.5)		14 (31.1)	2 (33.3)		15 (30.6)	1 (50)	
Plt ($\times 10^9$/L)	31 (20–56)	32 (20–60)	20.5 (17.2–34.5)	0.112	32.0 (20.0–58.5)	20.0 (10.8–35.5)	0.082	31 (19–56.5)	29.5 (21–38)	0.941
<30	25 (49)	20 (48.8)	5 (62.5)	0.329	21 (46.7)	4 (66.7)	0.315	24 (49)	1 (50)	0.745
≥30	26 (51)	23 (51.2)	3 (37.5)		24 (53.3)	2 (33.3)		25 (51)	1 (50)	
BM blasts (%)	62 (34–80)	62 (30–76)	71.5 (52–89)	0.254	62 (32–77)	72 (45.7–93.3)	0.294	62 (32–80)	65.5 (52–79)	0.692
<60	24 (47.1)	21 (48.7)	3 (37.5)	0.422	22 (49.8)	2 (33.3)	0.393	23 (46.9)	1 (50)	0.725
≥60	27 (52.9)	22 (51.3)	5 (37.5)		23 (51.1)	4 (66.7)		26 (53.1)	1 (50)	
FAB subtype, n(%)										
M0	2 (3.9)	2 (4.7)	0 (0.0)	0.677	2 (4.4)	0 (0.0)	0.607	2 (4.1)	0 (0.0)	0.699
M1	13 (25.5)	11 (25.6)	2 (25.0)		11 (24.4)	2 (33.3)		13 (26.5)	0 (0.0)	
M2	18 (35.3)	14 (32.6)	4 (50.0)		15 (33.4)	3 (50.0)		17 (34.7)	1 (50.0)	
M4	18 (35.3)	16 (37.2)	2 (25.0)		17 (37.8)	1 (16.7)		17 (34.7)	1 (50.0)	
Karyotype, n(%)										
Normal	48 (94.1)	40 (93.0)	8 (100)	0.593	42 (93.3)	6 (100)	0.681	46 (93.9)	2 (100)	0.885
t(8;21)	3 (5.9)	3 (7.0)	0 (0)		3 (6.7)	0 (0)		3 (6.1)	0 (0)	
Cytogenetic risk group, n(%)										
Favorable	3 (5.9)	3 (7.0)	0 (0)	0.593	3 (6.7)	0 (0)	0.681	3 (6.1)	0 (0)	0.885
Intermediate	48 (94.1)	40 (93)	8 (100)		38 (93.3)	6 (100)		46 (93.9)	2 (100)	

Table 2 continued

Characteristic	All cases (n = 51) n(%)	IDH SNP status			IDH1 SNP status			IDH2 SNP status		
		IDH SNP− (n = 43, 84.3%) n(%)	IDH SNP+ (n = 8, 15.7%) n(%)	*P* value	IDH1 SNP− (n = 45, 88.2%) n(%)	IDH1 SNP+ (n = 6, 11.8%) n(%)	*P* value	IDH2 SNP − (n = 49, 96.1%) n(%)	IDH2 SNP + (n = 2, 3.9%) n(%)	*P* value
Immunophenotype, n(%)										
CD4	18 (35.3)	16 (37.2)	2 (25.0)	0.409	17 (37.8)	1 (16.7)	0.299	17 (34.7)	1 (50)	0.586
CD7	2 (3.9)	2 (4.7)	0 (0.0)	0.708	2 (4.4)	0 (0)	0.776	2 (4.1)	0 (0)	0.922
CD13	51 (100.0)	43 (100.0)	8 (100)	1.000	45 (100)	6 (100)	1.000	49 (100)	2 (100)	1.000
CD14	18 (35.3)	16 (37.2)	2 (25.0)	0.113	17 (37.8)	1 (16.7)	0.229	17 (41)	1 (10)	0.062
CD15	36 (70.6)	30 (69.8)	6 (75.0)	0.565	32 (71.1)	4 (66.7)	0.776	34 (69.4)	2 (100)	0.494
CD33	49 (96.1)	41 (95.3)	8 (10.00)	0.708	43 (95.6)	6 (100)	0.562	47 (95.9)	2 (100)	0.922
CD34	20 (39.2)	18 (41.9)	2 (25.0)	0.315	18 (40.0)	2 (33.3)	0.613	20 (40.8)	0 (0)	0.365
CD117	40 (78.4)	34 (79.0)	6 (75.0)	0.557	35 (77.8)	5 (83.3)	0.615	39 (79.6)	1 (50)	0.388
HLA-DR	51 (100.0)	43 (100.0)	8 (100.0)	1.000	45 (100)	6 (100)	1.000	49 (100)	2 (100)	1.000
FLT3-ITD, n(%)										
Present	11 (21.6)	9 (20.9)	2 (25.0)	0.557	9 (20.0)	2 (33.3)	0.385	11 (22.4)	0 (0)	0.612
Absent	40 (78.4)	34 (79.1)	6 (75.0)		36 (80.0)	4 (66.7)		38 (77.6)	2 (100)	
CR, n(%)										
Yes	27 (52.9)	24 (55.8)	3 (37.5)	0.285	25 (55.6)	4 (33.3)	0.278	26 (53.1)	1 (50)	0.725
No	24 (47.1)	19 (44.2)	5 (62.5)		20 (44.4)	6 (66.7)		23 (46.9)	1 (50)	
Event-free survival (EFS, months)	0.69 (0.44–4.44)	0.79 (0.49–4.44)	0.49 (0.38–3.46)	0.186	0.79 (0.49–4.4)	0.44 (0.34–2.37)	0.120	0.69 (0.43–4.37)	2.47 (0.49–4.44)	0.904
Yes	7 (13.7)	5 (11.6)	2 (25.0)	0.300	5 (11.1)	2 (33.3)	0.186	7 (14.3)	0 (0)	0.742
No	44 (86.3)	38 (88.4)	6 (75.0)		40 (88.9)	4 (66.7)		42 (85.7)	2 (100)	
Overall survival (OS, months)	1.12 (0.79–5.39)	1.22 (0.86–6.2)	0.81 (0.61–4.33)	0.213	1.2 (0.84–5.8)	0.77 (0.57–3.5)	0.179	1.12 (0.81–5.8)	3.06 (0.72–5.39)	0.941
Alive	18 (35.3)	18 (41.9)	0 (0.0)	0.022*	18 (40.0)	0 (0.0)	0.061	18 (36.7)	0 (0)	0.414
Dead	33 (64.7)	25 (58.1)	8 (100.0)		27 (60.0)	6 (100.0)		31 (63.3)	2 (100)	

Risk status: favorable-risk: t(8;21); Intermediate-risk: normal

Qualitative data are represented as the number of cases (%), whereas quantitative data are represented as mean ± SD (range, minimum–maximum) if normally distributed or as median (range or interquartile range, IQR: 25th quartile to 75th quartile) if non-normally distributed

The nucleotide sequence variations and amino acid changes are designated according to the recommendations of the Human Genome Variation Society (http://www.hgvs.org/mutnomen/). IDH1 nucleotide numbering uses the first base of the translation start codon as nucleotide _1 on the basis of National Center for Biotechnology Information sequence NM_005896.2

BM bone marrow, *CD* cluster of differentiation, *CR* complete remission, *EFS* event-free survival, *FAB* French-American-British, *FLT3* FMS-like tyrosine kinase-3, *Hb* hemoglobin, *IDH* isocitrate dehydrogenase, *ITD* internal tandem duplication, *OS* Overall survival, *Plt* platelets, *SNP* single nucleotide polymorphism, *WBC* white blood cells

* indicates a statistical significant difference

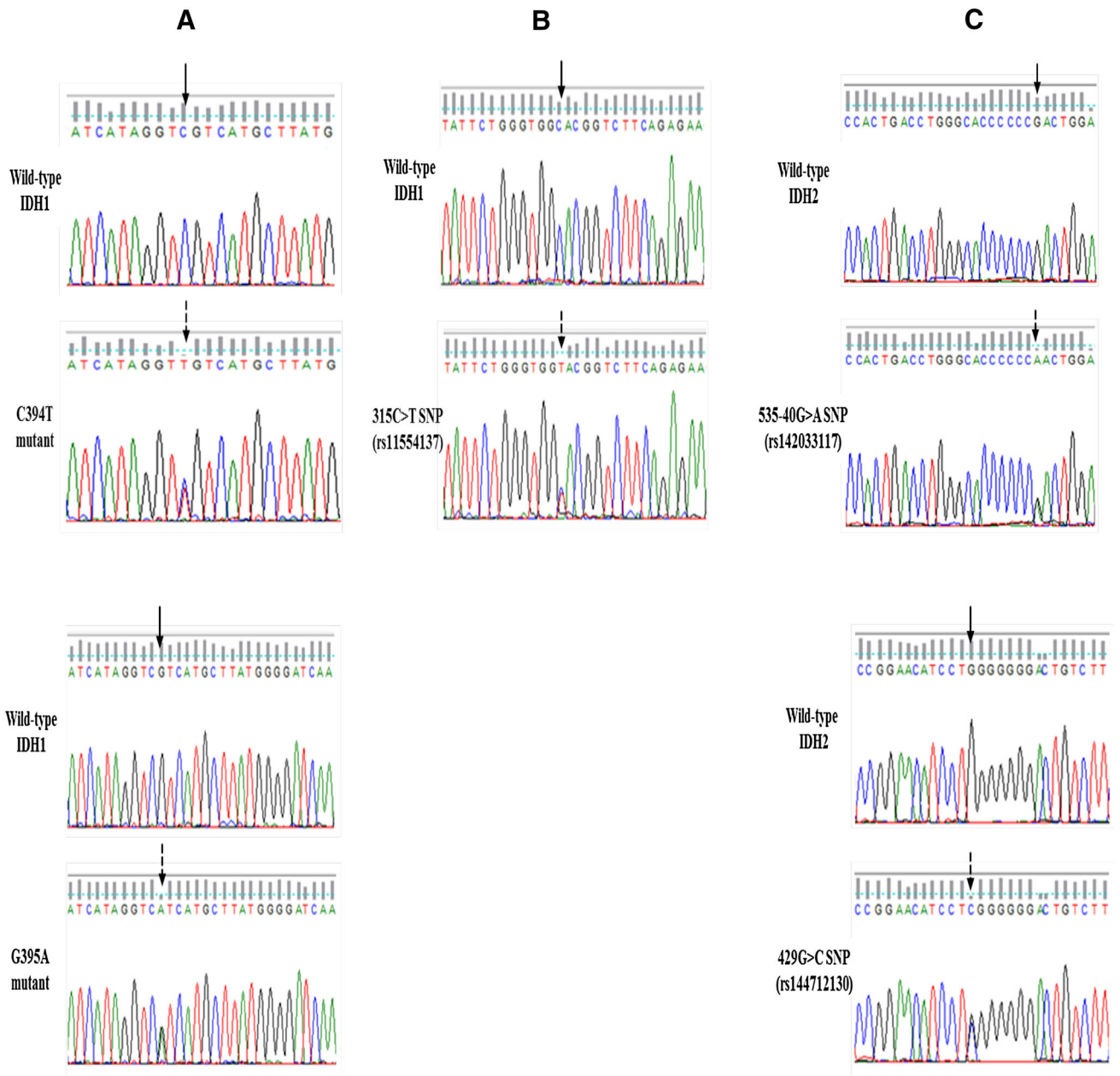

Fig. 1 Direct sequencing chromatograms of IDH1 mutations and IDH1/2 SNPs. **a** Representative chromatograms of the C394T and G395A IDH1 mutations. **b** Representative chromatograms of the 315C>T (rs11554137) IDH1 SNP. **c** Representative chromatograms of the 535-40G > A (rs142033117) and 429G > C (rs144712130) IDH2 SNPs. The *solid arrows* indicate the position of the wild-type nucleotide, whereas the *dotted arrows* indicate the position of the mutated nucleotide

AML. Although several studies have reported on the incidence and prognosis of *IDH* mutations in patients with AML, available results are complicated by the fact that a number of studies (1) examined the clinical relevance of only 1 or 2 mutations in *IDH1* and *IDH2*, (2) have included a limited number of additional genetic alterations beyond *IDH1/2* in the analysis, and/or (3) have studied patients receiving different therapeutic modalities [26]. In the present study, we investigated the incidence and prognostic impact of *IDH* aberrations in an Egyptian cohort of AML patients.

Multiple studies have evaluated *IDH* mutations in different patient populations, with an incidence of 6–16% for *IDH1* and 8–19% for *IDH2* [3]. In our study cohort, similar to the frequency of *IDH1* mutations in previously published reports, 7.8% of patients had *IDH1* mutations. Interestingly, we did not find any mutations affecting *IDH2* gene. This finding appears to be in line with a previous study by

Table 3 Univariate and multivariate analyses of prognostic factors for CR, EFS and OS in 51 patients with de novo AML

Variable	CR			EFS			OS		
	OR	95% CI (Lower–Upper)	*P* value	OR	95% CI (Lower–Upper)	*P* value	OR	95% CI (Lower–Upper)	*P* value
Univariate logistic regression analysis									
Gender (Male vs. Female)	0.297	(0.06–1.40)	0.125	0.13	0.01–1.31	0.084	0.987	0.23–4.26	0.986
Age (years) <40 versus ≥40	0.280	(0.07–1.14)	0.076	1.71	0.24–11.96	0.589	2.16	0.53–8.82	0.284
WBC ($\times 10^9$/L) <25 versus ≥25	0.706	(0.20–2.50)	0.588	0.08	0.01–0.86	0.037*	1.71	0.48–6.15	0.411
Hb (g/dL) <8 versus ≥8	0.760	(0.19–3.11)	0.703	1.30	0.18–9.21	0.796	0.50	0.12–2.09	0.341
Plt ($\times 10^9$/L) <30 versus ≥30	0.534	(0.15–1.92)	0.336	1.12	0.16–7.94	0.910	1.48	0.41–5.41	0.553
BM blasts (%) <60 versus ≥60	0.108	(0.02–0.53)	0.006*	0.35	0.04–2.89	0.331	6.48	1.46–28.75	0.014*
Constant	80.916		0.019	7.52		0.338	0.44		0.606
Multivariate logistic regression analysis									
WBC ($\times 10^9$/L) <25 versus ≥25				0.12	0.01–1.04	0.054			
BM blasts (%) <60 versus ≥60	0.24	(0.08–0.79)	0.018*				5.2	1.48–18.33	0.01*
Constant	2.43		0.048	0.33		0.020	0.85		0.683

Risk status: favorable-risk: t(8;21); Intermediate-risk: normal

CR is defined as bone marrow cellularity of at least 20% with maturation in all cell lineages, fewer than 5% bone marrow blast cells, no Auer rods, recovery of neutrophils ≥1.5 × 10^9/L and platelets >100 × 10^9/L in peripheral blood, as well as no evidence for circulating blasts and/or extramedullary leukemia, all of which had persisted for at least 1 month. Relapse is defined as reoccurrence of more than 5% of leukemic blasts in bone marrow, reappearance of circulating blasts or the development of extramedullary leukemia. OS is defined as the time elapsed from the date of diagnosis to the date of last follow-up or death from any cause. EFS is defined as the time interval between the date of diagnosis and the date of CR induction failure, relapse, secondary malignancy or death from any cause, whichever occurred first

BM bone marrow, *CR* complete remission, *EFS* event-free survival, *Hb* hemoglobin, *ITD* internal tandem duplication, *OR* odds ratio, *OS* overall survival, *95% CI* 95% confidence interval, *Plt* platelets, *WBC* white blood cells

* indicates a statistical significant difference

Mardis et al., who did not detect *IDH2* mutations in 188 patients with AML [3].

The frequency discrepancies among various reports may be explained by the variable inclusion criteria of the study samples, the variable sensitivity of the detection assays, the selective inclusion or exclusion of certain *IDH* aberrations, or the ethnic variability. In addition, most of the previous studies have focused on patients with the normal karyotype of AML, so data from patients belonging to other cytogenetic risk groups are scarce [27].

In the present study, all detected *IDH1* mutations were heterozygous, consistent with the retention of the wild-type allele, a finding which is in agreement with previous reports [5, 6].

In the current study, we identified the 315C>T *IDH1* SNP in 11.8% of patients, a frequency which was similar to that previously reported by Wagner et al. (12%) in a cohort of CN-AML patients [15]. In contrast to the 315C>T *IDH1* SNP, *IDH2* SNPs were exceedingly rare, with only 3.9% of the patients harboring *IDH2* SNPs. To the best of our knowledge, there were no previous reports documenting the frequencies of the *IDH2* SNPs in patients with AML worldwide and our study is the first to report *IDH2* SNPs in a cohort of AML patients. Notably, although the 315C>T *IDH1* SNP is located very close to *IDH1* codon 132, we observed no correlation between this SNP and the overall occurrence of *IDH1* codon 132 mutations.

It has been previously reported that AML patients with *IDH* mutations shared several common clinical characteristics, such as manifestation at older age or higher Plt count at diagnosis [11]. However, in the present study, *IDH* mutations were nearly evenly distributed between the two age groups (<40 vs. ≥40 years) and were not significantly associated with higher Plt count. Consistent with a previous observation [28], in our cohort, *IDH* mutated cases were found to be frequently females rather than males. Contrary

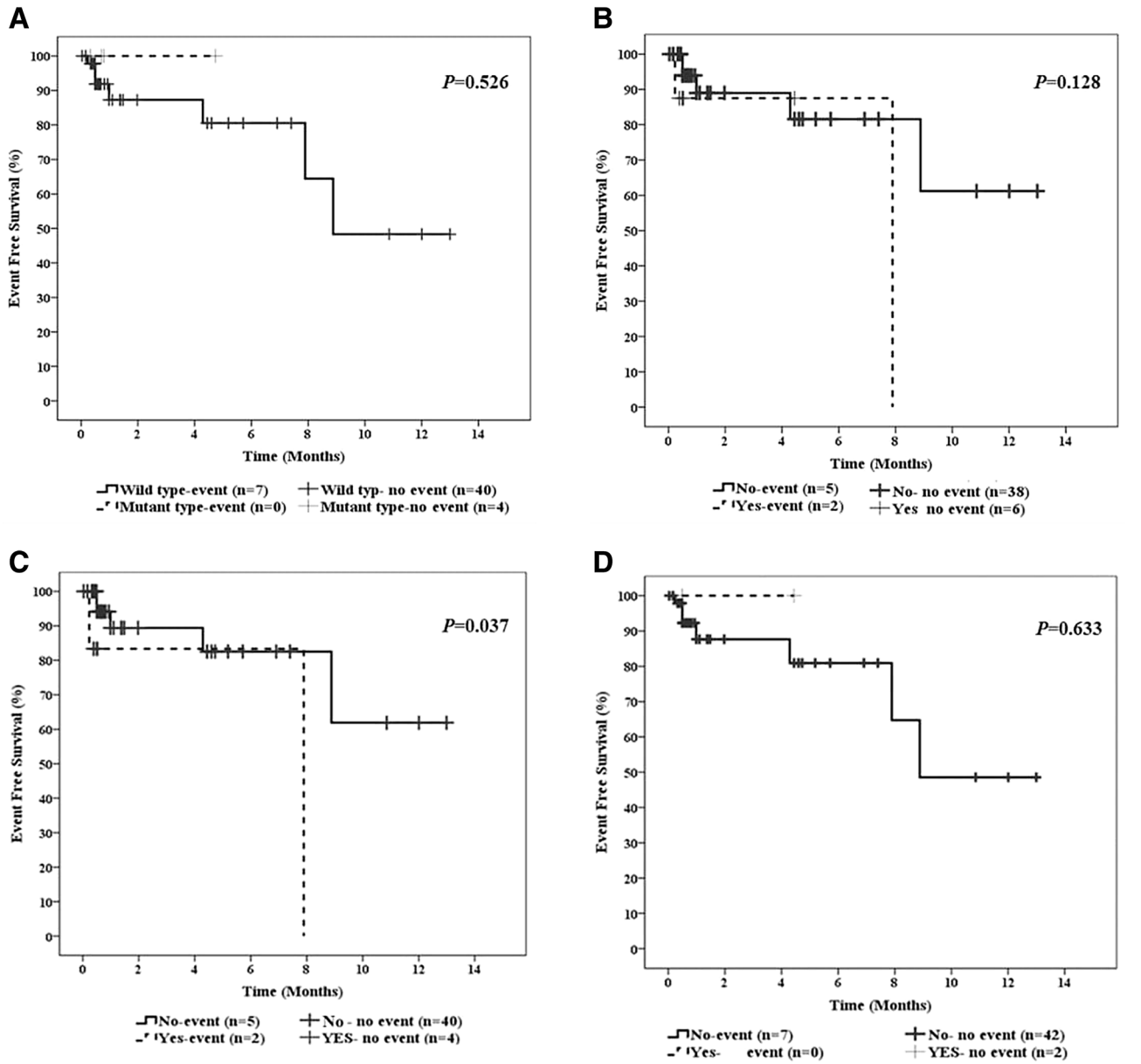

Fig. 2 Impact of IDH1 mutations and IDH1/2 SNPs on event-free survival (EFS) in the entire series of AML patients. **a** EFS of AML patients harboring wild-type or mutated IDH. **b** EFS of AML patients stratified by IDH SNPs status. **c** EFS of AML patients with or without IDH1 SNPs. **d** EFS of AML patients with or without IDH2 SNPs. The log-rank test *P* value is indicated per Kaplan–Meier survival curve

to previous studies [3, 28], which indicated that *IDH1* mutations were significantly associated with FAB AML-M1, our study suggested that the distribution of *IDH1* mutations didn't differ significantly among the different FAB subtypes.

The prognostic impact of *IDH* mutations in AML has been intensively studied but remains debatable, and it varies considerably among the different mutation types detected, and also by the presence of concurrent mutations in other clinically relevant genes. Although some studies have suggested a worse prognostic effect in CN-AML patients [10–13], others have not found any prognostic effect [14–16], which is in accordance with our findings. In our cohort of patients, we did not demonstrate any significant association of *IDH* mutations with EFS or OS, although it should be stressed that this finding is certainly limited by the small sample size as well as the low number of *IDH*-mutated patients. Although it seems counter-intuitive to see differences between the different studies, it has been suggested that differences in sizes of patient cohorts analyzed, varying inclusion criteria, age, and treatment administered might contribute to these discrepancies among studies.

Several lines of evidence suggested that the 315C>T *IDH1* SNP was associated with poor prognosis in AML

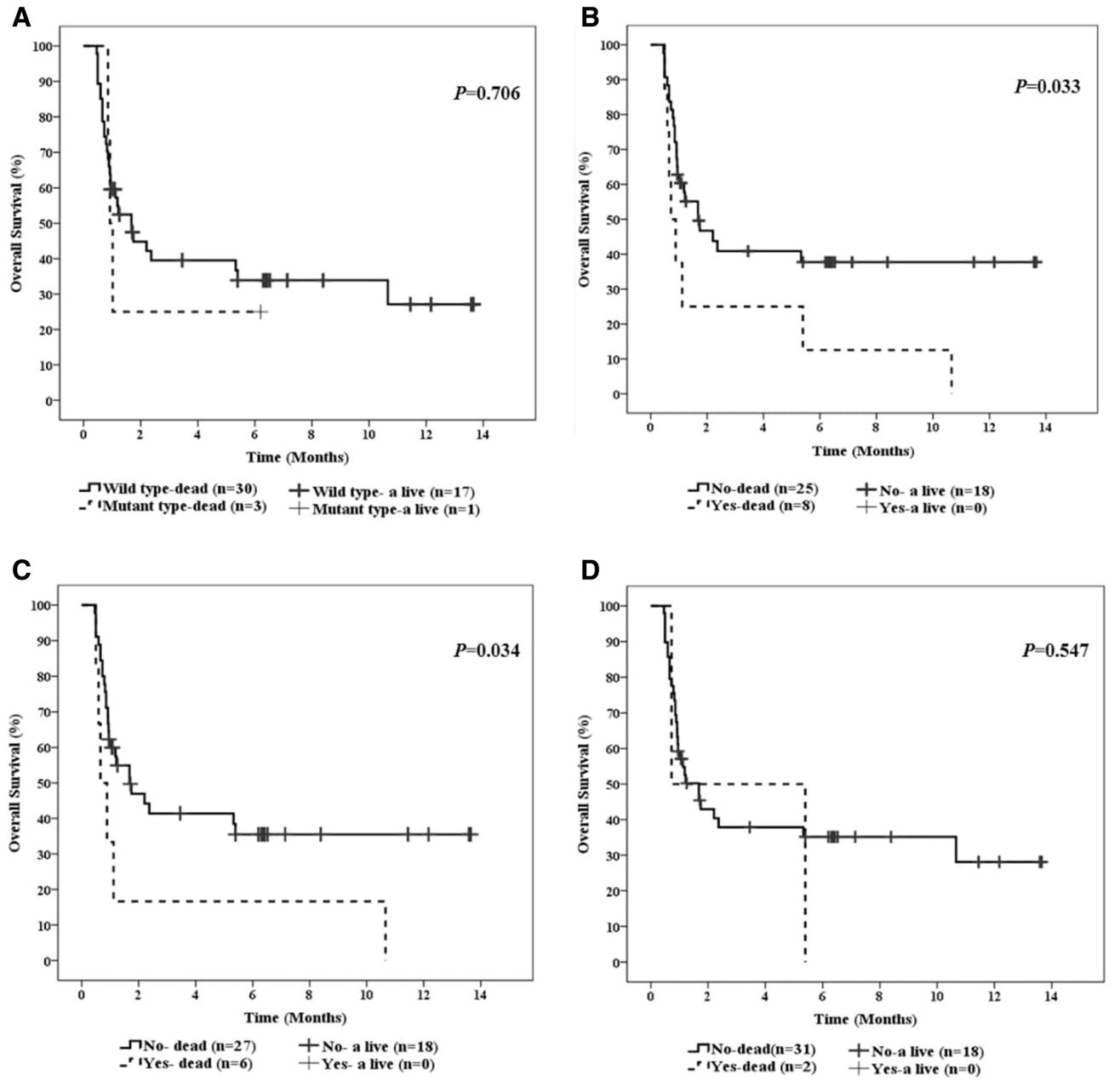

Fig. 3 Impact of IDH1 mutations and IDH1/2 SNPs on overall survival (OS) in the entire series of AML patients. **a** OS of AML patients harboring wild-type or mutated IDH. **b** OS of AML patients stratified by IDH SNPs status. **c** OS of AML patients with or without IDH1 SNPs. **d** OS of AML patients with or without IDH2 SNPs. The log-rank test *P* value is indicated per Kaplan–Meier survival curve

[15, 17]. In line with this finding, we have demonstrated in our cohort of patients that the presence of the 315C>T *IDH1* SNP could identify a subset of patients with particularly poor prognosis in terms of both EFS and OS.

Potential mechanisms by which a "silent" SNP may alter gene function include alterations in mRNA stability, folding, and splicing, differences in tRNA selection, or binding of non-coding RNAs [29]. The biological consequences of the silent 315C>T *IDH1* SNP remains to be investigated in AML. One speculative explanation is the alteration of the kinetics of protein translation, which may be affected by the frequency of usage of a particular codon. The 315C>T *IDH1* SNP represents a GGC>GGT transversion, resulting in the replacement of a common (GGC) codon by a rare (GGT) codon encoding glycine. The replacement of a commonly used codon with a rarely used one via a synonymous SNP may slow down the rate of protein translation, resulting in altered protein folding and, ultimately, decreased protein function. In support of this possibility, the C3435T SNP in the multidrug resistance 1 (*MDR1*) gene, which also encodes a GGC>GGT transversion, generates a protein with altered structure and function [30].

In conclusion, our findings further strengthen previous data suggesting that *IDH* mutations is a recurrent event in AML patients. Although *IDH* mutations did not confer an adverse outcome in our cohort of AML patients, further studies in larger cohorts with a less heterogeneous genetic background may be warranted to provide further insight into their association with the clinical outcome of AML patients. Our preliminary results suggest that the synonymous 315C>T *IDH1* SNP is associated with a negative prognostic impact in patients with CN-AML. Our findings clearly provide a solid rationale to include the molecular testing of 315C>T *IDH1* SNP in future prospective studies with larger patient numbers from various ethnic backgrounds to confirm the prognostic significance of this SNP.

Author Contributions Study conception and design: M.A.M.A. and R.H.; Patients recruitment and clinical monitoring: M.M.A.A.; Conducting the mutations analysis: M.A.M.A., E.K.A. and R.H.; acquisition of data: M.M.A.A. and R.H.; Analysis and interpretation of data: M.A.M.A.; Conceptualization and drafting of the manuscript: M.A.M.A.; Critical revision of the manuscript: M.A.M.A., E.K.A., M.M.A.A. and R.H.; All authors have approved the final version of the submitted manuscript. All authors have contributed significantly to this study, and all of them are in agreement with the content of the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the National Cancer Institute research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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