

Altered expression of imprinted genes in patients with cytogenetically normal-acute myeloid leukemia: Implications for leukemogenesis and survival outcomes

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Abstract. Genomic imprinting, an epigenetic mechanism that regulates gene expression from parental chromosomes, holds substantial relevance in multiple cancers, including hematopoietic malignancies. In the present study, the expression of a panel of 16 human imprinted genes in bone marrow samples from 64 patients newly diagnosed with cytogenetically normal-acute myeloid leukemia (CN-AML) were examined alongside peripheral blood samples from 85 healthy subjects. The validated findings of the present study revealed significant upregulation of seven genes [COPI coat complex subunit

gamma 2 (*COPG2*), H19 imprinted maternally expressed transcript (*H19*), insulin like growth factor 2 (*IGF2*), PEG3 antisense RNA 1 (*PEG3-AS1*), DNA primase subunit 2 (*PRIM2*), solute carrier family 22 member 3 *SLC22A3* and Zinc finger protein 215 (*ZNF215*)] in patients with CN-AML ($P < 0.001$). Notably, the expression level of *H19* exhibited an inverse association with the survival duration of the patients ($P = 0.018$), establishing it as a predictive marker for two- and five-year survival in patients with CN-AML. Kaplan-Meier analysis demonstrated that patients with lower *H19* expression had superior two- and five-year survival rates compared with those with higher *H19* expression. The results of the present study highlighted the association between loss of imprinting and leukemogenesis in CN-AML, underscoring the significance of *H19* imprinting loss as a prognostic indicator for unfavorable two- and five-year survival in CN-AML patients.

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Abbreviations: AML, acute myeloid leukemia; AUC, area under the curve; BM, bone marrow; CA, cytogenetically abnormal; CN, cytogenetically normal; FAB, French-American-British; IRB, Institutional Review Board; MDS, myelodysplastic syndromes; PB, peripheral blood; qPCR, quantitative polymerase chain reaction; RBC, red blood cell; ROC, receiver operating characteristic; WHO, World Health Organization

Key words: imprinted genes, cytogenetically normal-acute myeloid leukemia, *H19* gene, two-year survival, five-year survival

Introduction

Acute myeloid leukemia (AML) is a hematopoietic stem cell disorder characterized by genetic alterations in precursor cells within the bone marrow (BM), leading to the proliferation of neoplastic clonal myeloid stem cells (1). AML presents a considerable global health burden, with ~500 new cases in adults annually in Taiwan alone (2). As the most prevalent form of acute leukemia in adults, AML accounts for the highest proportion of leukemia-related deaths (3). The dysregulation of transcription factors and signaling pathways in hematopoiesis contributes to the development of malignant phenotypes such as AML and myelodysplastic syndromes (MDS), ultimately leading to the formation of leukemic stem cells (4).

Prognostic stratification in AML is predominantly based on the World Health Organization (WHO) classification, which takes into account cytogenetic and molecular genetic

aberrations (5-7). These genetic alterations can serve as potent prognostic markers for AML (8). Additionally, epigenetic alterations have gained significance as contributors to AML pathogenesis (9). Consequently, AML is classified into favorable, intermediate and adverse risk groups based on newly recognized cytogenetic and molecular subsets, each displaying distinct responses to standard therapies (4). However, the translation of these novel biomarkers as tools for effective treatment decisions remains limited in clinical practice. Thus, the identification of molecular markers for risk-adapted therapies represents a critical goal in improving the clinical outcomes of AML (10).

Among the epigenetic mechanisms, genomic imprinting emerges as an interesting regulatory process that restricts gene expression to one of the two parental chromosomes. While the majority of the genes in the genome are expressed equally regardless of parental origin, a small subset of genes displays genomic imprinting (11). As of December 2018, ~165 imprinted genes in humans and 197 imprinted genes in mice have been reported (12). Although the exact mechanisms of imprinting remain elusive, the vital roles of imprinted genes in regulating cell proliferation and fetal growth are widely recognized (12,13). Consequently, dysregulated expression of imprinted genes has been implicated in tumorigenesis, exemplified by the upregulation of H19 imprinted maternally expressed transcript (*H19*) (10,14), insulin like growth factor 2 (*IGF2*) (14), maternally expressed 3 (*MEG3*) (15,16) and Zinc finger protein 215 (*ZNF215*) (14) genes in AML.

Significant progress has been achieved in the genomic profiling of AML, enabling the development of targeted therapies based on genomic characteristics. Nonetheless, the dysregulation of imprinted genes in AML remains relatively unexplored. A previous study conducted by the authors demonstrated that altered expression of imprinted genes and loss of *ZNF215* imprinting can function as prognostic indicators for poor five-year survival in patients with cytogenetically abnormal (CA)-AML (14). Building upon these findings, the present study investigated whether the expression of imprinted genes is similarly altered in patients with cytogenetically normal (CN)-AML. The primary aim of the authors was to establish a possible correlation between this disease subtype and loss of imprinting.

Materials and methods

Patients and samples. Patients diagnosed with CN-AML were recruited from the Division of Hematology-Oncology, Department of Internal Medicine, Kaohsiung Medical University Hospital

(Kaohsiung, Taiwan) during the period spanning from August 1999 to October 2012. At the hospital, the standard protocol for diagnosis of AML included cytogenetic examination and mutational analysis. Immunophenotyping was conducted for the diagnosis of lymphocytic leukemia. The present study received ethical approval from the Institutional Review Board (IRB) of the Kaohsiung Medical University Hospital Ethical Committee (approval no. KMU-IRB-20130129; Kaohsiung, Taiwan). Written informed consent was provided by all participants.

BM samples were collected from 64 patients with CN-AML, while peripheral blood (PB) samples were obtained from 85 healthy adult volunteers. Within 1 h of collection, red blood cell (RBC) lysis buffer (MilliporeSigma) was added to the samples to deplete RBCs from both BM and PB samples. The isolated total leukocytes were then preserved in TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and stored in a -80°C deep freezer until RNA extraction.

Cytogenetic analysis. Cytogenetic analysis was conducted on BM aspirate samples during the initial diagnosis using conventional G-banding cytogenetic techniques. Short-term unstimulated cell cultures were established for one to three days in RPMI-1640 medium supplemented with 20% fetal bovine serum (both from Invitrogen; Thermo Fisher Scientific, Inc.). A total of 20 metaphase cells with G-banded patterns were karyotyped and described according to the ISCN 2013 guidelines (17,18).

Selection of imprinted genes. Candidate imprinted genes were identified based on the Catalogue of Imprinted Genes-University of Otago from 2001 (19), followed by a thorough evaluation using the Geneimprint Imprinted gene database (<https://www.geneimprint.com/site/genes-by-species>). Genes listed as either 'predicted' or 'not imprinted' were excluded from the study. Subsequently, a total of 16 human imprinted genes were selected, previously reported to be involved in various malignancies (14,20). Next, for primer design purposes, the sequences of these genes were acquired from the Ensembl human archive (Ensembl 54 NCBI 36; https://may2009.archive.ensembl.org/Homo_sapiens/Info/Index).

Expression analysis of imprinted genes. The expression analysis of imprinted genes adhered to the protocol is described in previous studies (14,20). Briefly, total RNA was isolated using TRIzol reagent (Invitrogen, Thermo Scientific, Inc.), followed by cDNA synthesis using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Thermo Scientific, Inc.) according to the manufacturer's protocols. Gene expression analysis was executed using SYBR® Green. Sequences of the forward and reverse primers of the 16 imprinted genes analyzed are listed in Table I. The primer sequences of reference gene *ACTB* (β -actin) were as follows: forward, 5'-GGCCAACCGCGAGAAGAT-3' and reverse, 5'-CGTCACCGGAGTCCATCAC-3'. In each reaction, 50 ng of cDNA, along with 200 nM of each primer, and 5 μ l of 2x Power SYBR® Green PCR Master Mix (Applied Biosystems; Thermo Scientific, Inc.) were combined to a final volume of 10 μ l. The PCR test was performed using a 7500 Fast Real-Time System (Applied Biosystems; Thermo Scientific, Inc.), employing the following thermal cycling parameters: An initial denaturation step at 95°C for 10 min, followed by 40 cycles of PCR reaction at 95°C for 20 sec and 60°C for 60 sec. The expression of the *ACTB* gene in the same sample was used as an endogenous control. The Δ Cq value for each imprinted gene was calculated using the equation Δ Cq=(Cq of imprinted gene-Cq of *ACTB* gene) (21). Higher- Δ Cq values indicate a higher expression level of the imprinted gene, while lower- Δ Cq values indicate a lower expression level.

Table I. List of imprinted genes and oligonucleotide primers for real-time quantitative polymerase chain reaction analysis.

Gene	Location	Expressed allele	GenBank Accession No.	Amplicon Size (bp)	Sequence (5→3')	Primer location
<i>C15orf2</i>	15q11-q13	Unknown	AB527113.1	61	F: GTG ACA GCA TTG CCT CAG C R: GGT CTC CTA TCT GCC TGT GC	3062-3080 3103-3122
<i>COPG2</i>	7q32 AS	Paternal	NM_012133.4	67	F: TTC CAG ATG AGG ATG GGT ATG R: TGG TCA GAC ACA GTC ACT TCG	2303-2323 2349-2369
<i>CPA4</i>	7q32	Maternal	NM_016352.3	85	F: GTC GGG CAC TGA GTA CCA A- R: GTT GTC ATA CGC CCA GTC G	1082-1100 1148-1166
<i>GABRB3</i>	15q11.2-q12 AS	Paternal	NM_000814.5	72	F: GGG TGT CCT TCT TGA TCA ATT A R: GTT GTC AGC ACA GTT GTG ATC C	899-920 950-970
<i>H19</i>	11p15.5 AS	Maternal	NR_002196.1	84	F: TTA CTT CCT CCA CGG AGT CG R: GCT GGG TAG CAC CAT TTC TT	3050-3069 4570-4589
<i>IGF2</i>	11p15.5 AS	Paternal	NM_000612.4	101	F: ACA CCC TCC AGT TCG TCT GT R: GAA ACA GCA CTC CTC AAC GA	868-887 949-968
<i>INPP5F</i>	10q26.11	Paternal	NM_014937.3	87	F: TTC TTG ATA TGA AGT GGT GTT GG R: GGC AGT CCA TAC AAT TAA CAC G	1514-1536 1579-1600
<i>L3MBTL</i>	20q13.12	Paternal	NM_015478.6	72	F: AGC GCA GGG AAT ACC AGA G R: TTC CTT CTTCTT GCT TCT CCA	564-582 615-635
<i>PEG3-AS1</i>	19q13.4 AS	Paternal	NR_023847.2	76	F: GGG TCA AGT CCT AGG TGA AGG R: CGC CAG ACA CCA GAA TAC C	4802-4822 4747-4765
<i>PPP1R9A</i>	7q21.3	Maternal	NM_001166160.1	76	F: GCC CAA AAC ATC ACT GGA G R: GGG ATG CTG TCA TTC CAA G	4141-4159
<i>PRIM2</i>	6p12-p11.1 AS	Biallelic	NM_000947.2	68	F: GCC TGC TGT GCA GTC TGA T R: GGC CAG TGT AGG AAT GAC TGA	798-816 845-865
<i>RTL1</i>	14q32.31	Paternal	NM_001134888.2	60	F: CGC AGA GAA TTC CAC GAG TT R: TCT TGG GTA GCT CTG TAA GGT CA	687-706 724-746
<i>SLC22A3</i>	6q26-q27	Maternal	NM_021977.3	71	F: CCA CCA TCG TCA GCG AGT R: CAG GAT GGC TTG GGT GAG	460-477 513-530
<i>SNURF</i>	15q12	Paternal	NM_022804.2	103	F: CTC ACT GAG CAA CCA AGA GTGT R: AGC TAA GAA TGC CTG CCT CA	219-240 302-321
<i>TCEB3C</i>	18q21.1 AS	Maternal	NM_145653.3	82	F: GGC CAA GAC GCC TTA TGA T R: TGG CTC CAT CTC TCC ATT TC	1601-1619 1663-1682
<i>ZNF215</i>	11p15.4	Maternal	NM_013250.2	75	F: TGT CCA AGA CAG CGA TTC C- R: TGC AAG TAG CTT AAG TGG CAA A	220-238 273-294

F, forward; R, reverse.

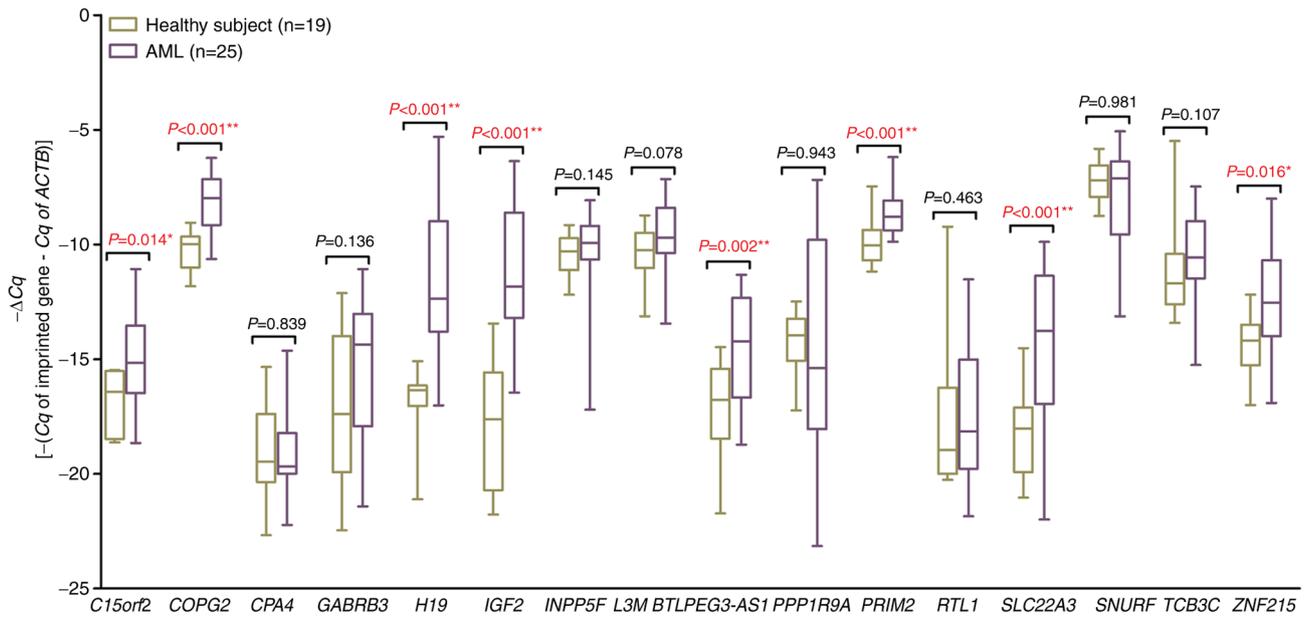


Figure 1. Screening the expression of imprinted genes in patients with AML. The expression of 16 imprinted genes was screened in 25 patients with AML and 19 healthy subjects using reverse transcription-quantitative PCR. Expression of 8 imprinted genes was significantly upregulated in patients with AML. The difference in expression of imprinted genes between healthy subjects and patients with AML was evaluated by the Mann-Whitney test using values of $-\Delta Cq$ [-(Cq of imprinted gene - Cq of *ACTB* gene)]. Data reported are median and range of $-\Delta Cq$. Higher $-\Delta Cq$ values represent higher expression levels. Statistical significance at * $P < 0.05$ and ** $P < 0.001$. AML, acute myeloid leukemia.

Statistical analysis. Statistical analyses were performed using SPSS 22.0 software (IBM Corp.) and GraphPad Prism 7.04 (Dotmatics). Results were presented as the mean \pm SEM (standard error of mean). Variations in the expression of each imprinted gene between healthy subjects and CN-AML patients were assessed using the unpaired Student's t-test or Mann-Whitney test, depending on data distribution. Pearson correlation analysis was used to evaluate the correlation between two variables. To ascertain whether the expression of a specific imprinted gene could function as a predictor of survival in CN-AML, multivariate analysis using the Cox proportional hazards regression model was employed. The predictive capacity of the gene for the survival of the patients was evaluated by constructing a receiver operating characteristic (ROC) curve, with the calculation of the area under the curve (AUC). For comparison of patient survival across groups, Kaplan-Meier survival analysis and log-rank test were performed. Statistical analyses for expression analysis of imprinted genes were based on the $-\Delta Cq$ values, with $P < 0.05$ signifying statistical significance in all assessments.

Results

Patient characteristics. The present study included a total of 64 patients with CN-AML, consisting of 38 males and 26 females. Additionally, 85 healthy subjects, consisting of 47 males and 38 females, were enrolled in the present study. The age of the participants ranged from 23 to 57 years, with a median age of 36.3 years. Detailed information on the clinical characteristics of the patients, such as sex, age, laboratory data, and French-American-British (FAB) subtypes are presented in Table II. The diagnosis and subtyping of AML were determined based on the classification systems of FAB

and WHO (15). To examine the karyotypes of patients at the time of initial diagnosis, conventional G-banding cytogenetic analysis was performed on BM aspirate samples.

Expression of imprinted genes in patients with CN-AML. In a previous study conducted by the authors, altered expressions of imprinted genes in patients with CA-AML were observed (14). In the present study, the objective was to determine whether these genes were also altered in patients with CN-AML. For analysis, the same panel of 16 human imprinted genes was selected, namely *C15orf2*, *COPG2*, *CPA4*, *GABRB3*, *H19*, *IGF2*, *INPP5F*, *L3MBTL*, *PEG3-AS1*, *PPP1R9A*, *PRIM2*, *RTL1*, *SLC22A3*, *SNURF*, *TCEB3C* and *ZNF215*. To initially screen the expression of these 16 genes, a cohort of 25 patients with AML, were randomly chosen regardless of their karyotypes, alongside 19 healthy subjects. The findings revealed that 8 of the genes exhibited upregulation in patients compared with the healthy subjects (Fig. 1). After the initial screening of 25 patients regardless of their karyotypes, the results were analyzed by segregating the cohort into CN-AML and CA-AML groups. The results from the broader 25-patient group aligned consistently with those exclusively from CN-AML patients. Therefore, the apprehension about discarding genes uniquely regulated in patients with CN-AML rather than patients with CA-AML during the initial screening was effectively addressed. Subsequently, for further validation, the focus shifted to a larger group comprising 64 patients with CN-AML and 85 healthy subjects. From the 8 genes previously identified (excluding *C15orf2*), the remaining 7 imprinted genes (*COPG2*, *H19*, *IGF2*, *PEG3-AS1*, *PRIM2*, *SLC22A3* and *ZNF215*) that were upregulated in patients with CA-AML were similarly observed to be significantly upregulated in patients with CN-AML ($P < 0.001$) when compared with healthy subjects (Fig. 2).

Table II. Characteristics of patients newly diagnosed with cytogenetically normal-acute myeloid leukemia (n=64).

Characteristic	Values	Mutational analysis												
		<i>ACEBP</i> (%)	<i>DNMT3A</i> (%)	<i>FLT3-ITD</i> (%)	<i>FLT3-TKD</i> (%)	<i>IDH1</i> (%)	<i>IDH2</i> (%)	<i>MLL</i> (%)	<i>NPM1</i> (%)					
Sex, n (%)														
Male	38 (59.4%)													
Female	26 (40.6%)													
Age, year, median (range)	53.5 (22-86)													
Laboratory data, median (range)														
WBCs, per μ l	39.37 (0.10-328.30)													
Hemoglobin, g/dl	8.45 (4.10-15.60)													
Platelets, x1,000/ μ l	43.00 (8.00-369.00)													
Blasts in PB (%)	66.25 (2.00-97.50)													
Blasts in BM (%)	74.90 (3.40-96.60)													
LDH, U/l	444 (52-4,023)													
FAB subtype, n														
M0	2	2 (100) ^a	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)
M1	20	9 (45)	0 (0)	5 (25)	1 (5)	0 (0)	0 (0)	0 (0)	1 (5)	1 (5)	1 (5)	1 (5)	9 (45)	0 (0)
M2	23	8 (35)	3 (13)	8 (35)	1 (4)	1 (4)	1 (4)	1 (4)	1 (4)	4 (17)	4 (17)	4 (17)	7 (30)	0 (0)
M3	1	0 (0%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
M4	16	3 (19)	5 (31)	6 (38)	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	10 (63)	0 (0)
M5	2	1 (50)	2 (100)	0 (0)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)
M6	0													
M7	0													

^aNumbers presented are the number of cases with mutation (%). BM, bone marrow; FAB, French-American-British classification; LDH, lactic dehydrogenase; PB, peripheral blood; WBC, white blood cell.

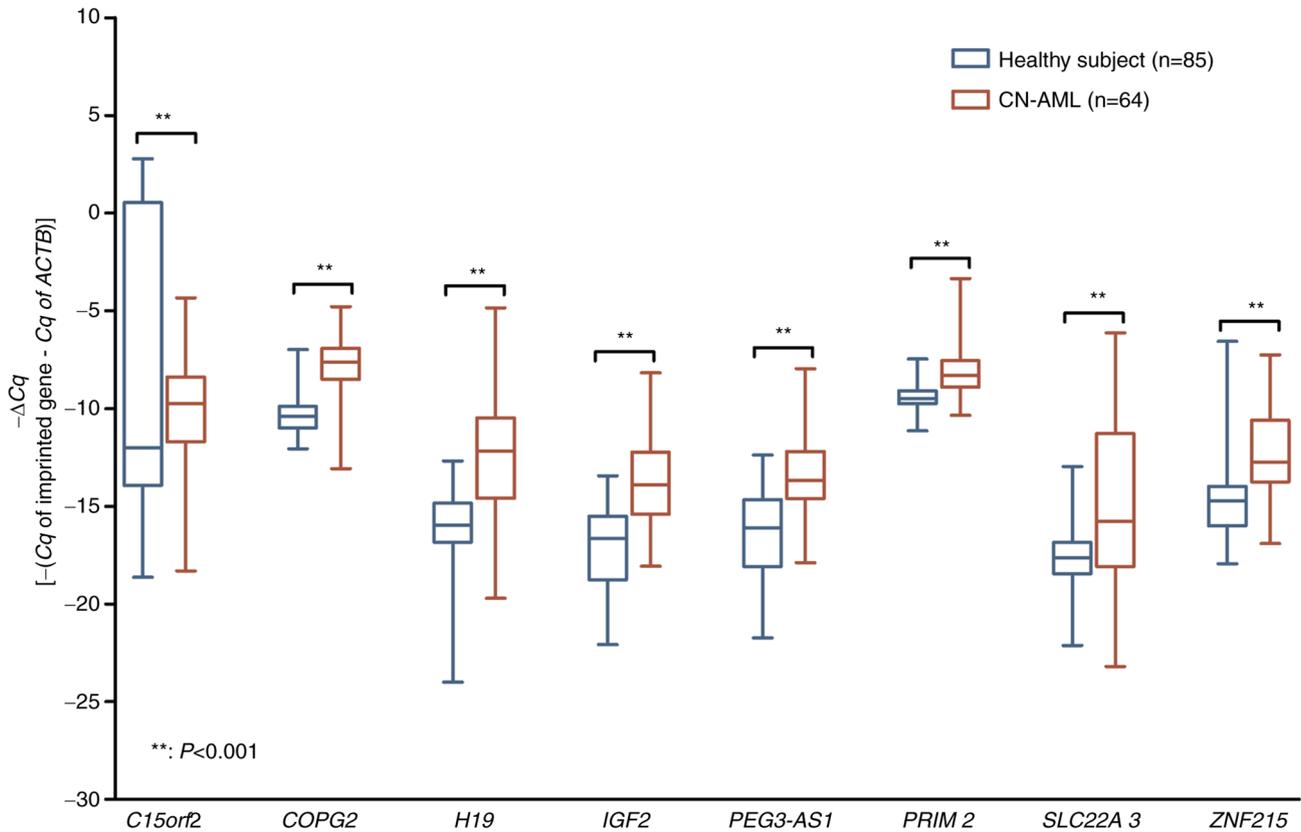


Figure 2. Validation of altered expression of imprinted genes in patients with CN-AML. The altered expression of imprinted genes was validated in patients with CN-AML using reverse transcription-quantitative PCR. Expression of the 7 imprinted genes was significantly upregulated in patients with CN-AML (n=64) compared with healthy subjects (n=85). The difference in expression of imprinted genes between healthy subjects and patients with CN-AML was evaluated by the Mann-Whitney test using values of $-\Delta Cq$ [-(Cq of imprinted gene - Cq of ACTB gene)]. Data reported are median and range of $-\Delta Cq$. Higher $-\Delta Cq$ values represent higher expression levels. **P<0.001. CN-AML, cytogenetically-normal acute myeloid leukemia.

Correlation between expression of imprinted genes and clinical parameters of patients. To determine a possible association, Pearson correlation analysis was performed on the altered expression levels of seven imprinted genes in patients with CN-AML. The results, presented in Table III, revealed several significant correlations. Specifically, the expression levels of *COPG2*, *PEG3AS*, *PRIM2* and *ZNF215* were positively correlated with the percentage of blasts in PB (P<0.05). Finally, the expression level of *H19* was positively correlated with the level of lactic dehydrogenase (P=0.040) in patients with CN-AML.

Correlation, regression and survival analysis of expression of imprinted genes in patients with CN-AML. The correlation between the expression levels of the seven altered imprinted genes and the survival rate of patients with CN-AML was additionally explored. Pearson correlation analysis was conducted, revealing that among these seven genes, solely the expression level of *H19* exhibited a negative correlation with the survival rate of the patients (P=0.018) (Table IV). Furthermore, the binary logistic analysis indicated that *H19* could function as a predictive factor for the survival of patients with CN-AML [$\beta=142.90$, 95% Confidence interval (CI): 30.23-255.28, P=0.014].

To assess the impact of *H19* on survival, a Cox proportional hazard model was utilized. The hazard ratio of *H19* for two-year survival was computed as 0.852 (95.0% CI:

0.774-0.938, P=0.001), and for five-year survival, it stood at 0.882 (95.0% CI: 0.808-0.963, P=0.005).

Additionally, ROC analysis was performed to evaluate the area under the ROC curve (AUC) for two-year survival (AUC: 0.712, 95% CI: 0.582-0.843, P=0.006) (Fig. 3A). Using the cut-off point of *H19* expression (-12.165), Kaplan-Meier survival analysis revealed that patients with lower *H19* expression had a higher two-year survival rate (log-rank P=0.006; Fig. 3B) and improved five-year survival rate (log-rank P=0.029; Fig. 3C) compared with those with higher *H19* expression.

Discussion

CN-AML constitutes a significant proportion (40-50%) of newly diagnosed AML cases and the identification of somatically acquired genetic alterations in both CA-AML and CN-AML groups is crucial for effective risk stratification. Despite presenting with a normal karyotype, patients with CN-AML exhibit various mutations, rendering them a heterogeneous group characterized by distinctive clinical outcomes (22). In the present study, by investigating the expression of imprinted genes, the authors aimed to identify possible prognostic biomarkers that could enhance treatment decisions for CN-AML.

Genomic imprinting plays a vital role in regulating growth and development, with its disruption frequently observed in the early stages of tumorigenesis (23,24). Consistent with the

Table III. Correlation between expression of the 7 imprinted genes and clinical parameters in patients with cytogenetically normal-acute myeloid leukemia.

Gene	Blasts in BM (%)	Blasts in PB (%)	WBC (/μl)	Hb (g/dl)	Platelets (x1,000/μl)	LDH (U/l)
<i>COPG2</i>	0.173 (0.187)	0.344 (0.011a)	0.198 (0.130)	-0.026 (0.845)	0.061 (0.642)	-0.098 (0.475)
<i>H19</i>	0.238 (0.067)	0.141 (0.310)	0.184 (0.160)	-0.235 (0.071)	0.059 (0.655)	0.277 (0.040 ^a)
<i>IGF2</i>	-0.146 (0.267)	-0.112 (0.418)	-0.113 (0.390)	0.129 (0.326)	0.159 (0.224)	0.050 (0.715)
<i>PEG3-AS</i>	-0.077 (0.567)	0.337 (0.017 ^a)	0.067 (0.625)	0.077 (0.574)	0.277 (0.039 ^a)	0.043 (0.759)
<i>PRIM2</i>	-0.072 (0.597)	0.525 (<0.001 ^b)	0.038 (0.781)	0.175 (0.196)	0.358 (0.007 ^b)	-0.038 (0.788)
<i>SLC22A3</i>	0.081 (0.541)	0.187 (0.181)	0.107 (0.419)	-0.054 (0.687)	0.165 (0.211)	-0.009 (0.949)
<i>ZNF215</i>	-0.031 (0.820)	0.281 (0.048 ^a)	-0.017 (0.900)	0.277 (0.039 ^a)	0.311 (0.020 ^a)	0.064 (0.646)

Numbers presented are Pearson correlation coefficient (P-value). Statistically significant at ^aP<0.05 and ^bP<0.01. BM, bone marrow; FAB, French-American-British classification; LDH, lactic dehydrogenase; PB, peripheral blood; WBC, white blood cell.

Table IV. Correlation between expression of the 7 imprinted genes and survival days in patients with cytogenetically normal-acute myeloid leukemia.

Imprinted gene	Survival days
<i>COPG2</i>	-0.013 (0.920)
<i>H19</i>	-0.295 (0.018 ^a)
<i>IGF2</i>	0.070 (0.581)
<i>PEG-3AS</i>	-0.171 (0.192)
<i>PRIM2</i>	-0.062 (0.637)
<i>SLC22A3</i>	-0.074 (0.566)
<i>ZNF215</i>	-0.126 (0.338)

Numbers presented are Pearson correlation coefficient (P-value). ^aStatistically significant at P<0.05.

previous findings in CA-AML (14), significantly higher expression was observed in 7 out of the 16 imprinted genes analyzed in CN-AML. These results underscore the significance of imprinting and its dysregulation in AML, manifesting in cases with both normal and abnormal karyotypes.

The novel finding of the present study is the significant correlation between the *H19* expression and the survival of patients with CN-AML. *H19*, the first reported human imprinted gene (25), has been demonstrated to promote leukemogenesis across diverse types of leukemia. In AML, increased *H19* expression has been associated with poor prognosis (10,14,25,26) by inhibiting apoptosis through the targeting of miR-29a-3p (26) and miR-19a/b (27). Furthermore, loss of imprinting in the *IGF2-H19* cluster has also been observed in a high percentage of patients with MDS and AML (28). Elevated *H19* expression has been documented in acute lymphoblastic leukemia (29,30) and chronic myeloid leukemia (31,32), correlating with adverse outcomes in both cases. The oncogenic properties of *H19*, including inhibition of apoptosis, promotion of tumor cell proliferation, invasion, metastasis and chemoresistance, have been demonstrated across multiple solid tumors (33). Downstream targets of *H19* in cancer include *Akt2*, *β-catenin*, *FOXMI*, *RUNXI*, *STAT3*

and miRNAs. Additionally, *H19* can be transmitted to cancer cells via exosomes to promote tumor progression (34). Thus, *H19* emerges as a potential biomarker and therapeutic target in both leukemia and solid tumors.

In addition to *H19* and *IGF2*, the loss of imprinting of *COPG2*, *PEG3-AS*, *PRIM2*, *SLC22A3* and *ZNF215* genes in patients with CN-AML was observed. These genes have been associated with various malignancies and disorders, as discussed in the previous study conducted by the authors (14). The present study detected correlations between the expression of *COPG2*, *H19*, *PEG3-AS*, *PRIM2*, *ZNF215* and clinical parameters in patients with CN-AML. However, only *H19* expression was negatively correlated with patient survival. These results differ from the observations made in patients with CA-AML, further supporting the critical role of the loss of imprinting in the leukemogenesis of AML. The findings of the present study also indicated that specific imprinted genes are influenced under CA or CN conditions, leading to different clinical outcomes.

Over the past decade, numerous gene mutations in AML have been identified through genomics technologies (for example, *RUNXI*, *IDH1*, *IDH2*, *DNMT3A*, *WT1*, *TET2*, *MLL*, *ASXL1*, *CBL*, *NRAS*, *KRAS* and *TP53* genes) (5,35). Patients with CN-AML, being the largest subgroup of AML, can be further classified into molecular subgroups based on these mutations. Targeted therapies are being developed to address these genetic and epigenetic changes, including demethylating agents and tyrosine kinase inhibitors (36). Although mutations in several genes (*ACEBP*, *DNMT3A*, *FLT3-ITD*, *FLT3-TKD*, *IDH1*, *IDH2*, *MLL* and *NPM1*) in the patients of the present study with CN-AML were analyzed, the presence or absence of these mutations did not significantly contribute to the prediction of patient survival (data not shown). Therefore, despite the inclusion of *H19* as a prognostic marker, the application of novel biomarkers for treatment decisions remains limited. Future goals include improving the specificity of diagnostic classification to facilitate more efficacious targeted therapies and the development of personalized treatment strategies.

Similar to the previous study of the authors on CA-AML, the present study on CN-AML included certain limitations. Firstly, the samples obtained from healthy subjects were PB rather than BM. Secondly, the patient subgroups were

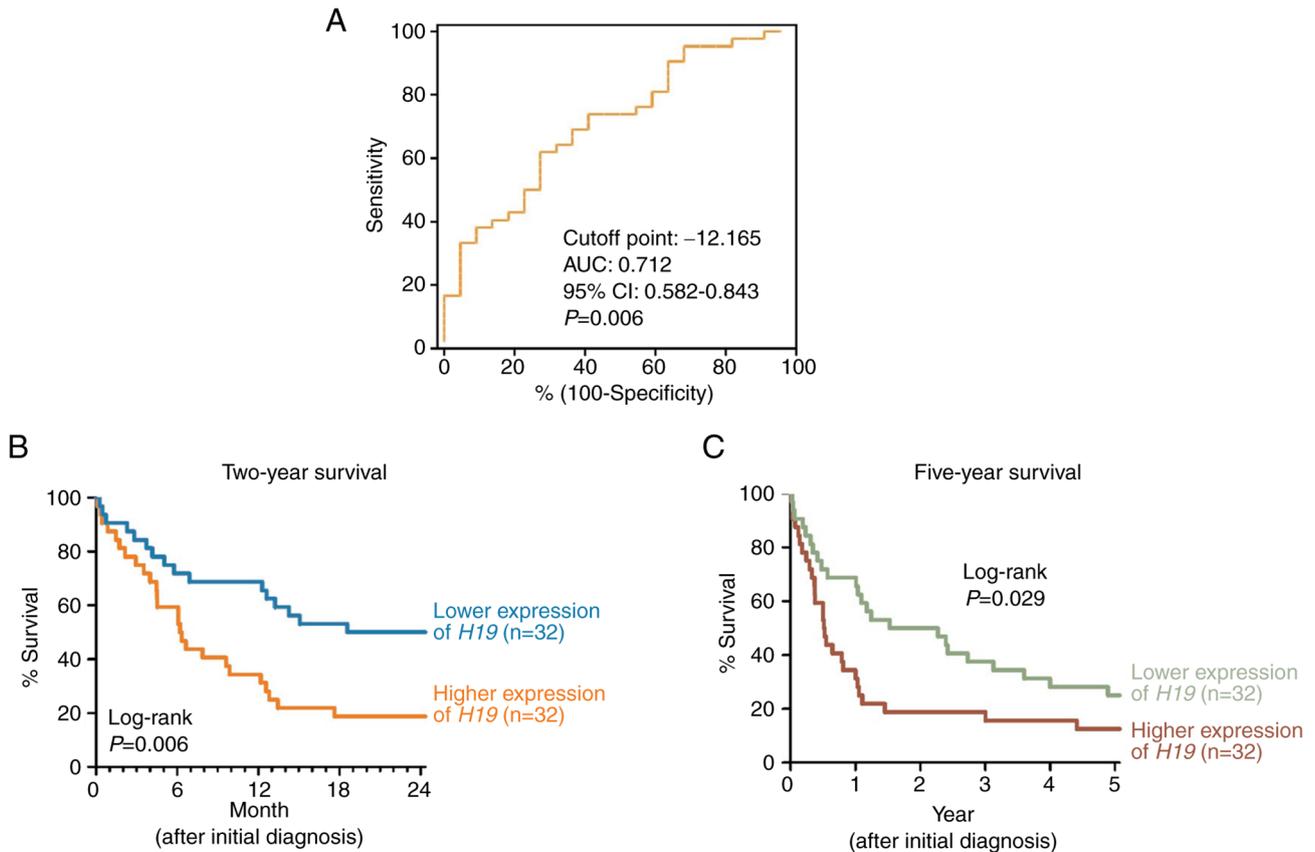


Figure 3. *H19* expression as a potential predictor for survival in patients with CN-AML. (A) ROC curve demonstrating the predictive ability of *H19* expression for two-year survival in patients with CN-AML. Patients with lower expression of *H19* exhibited improved two-year survival. (B) Kaplan-Meier survival curve showing the two-year survival rates in patients with CN-AML with lower expression of *H19* compared with those with higher *H19* expression (log-rank $P=0.006$). (C) Kaplan-Meier survival curve displaying the five-year survival rates in patients with CN-AML with lower *H19* expression compared with those with higher *H19* expression (log-rank $P=0.029$). CN-AML, cytogenetically normal acute myeloid leukemia; AUC, area under the curve; CI, Confidence interval; ROC, receiver operating characteristic.

relatively modest in size, posing constraints on more rigorous correlation analysis. Lastly, an inadequacy of post-treatment cases hindered the validation of the relationship between imprinted gene expression and disease status. Additionally, although novel and clinically relevant findings were observed, the functions of the altered imprinted genes in CN-AML were not examined. Studying *H19* comes with its share of limitations and challenges. While it is established that *H19* plays crucial roles in both normal development and disease, its functional complexity adds a layer of difficulty to comprehending its mechanisms. Moreover, the functions of *H19* may vary among different cell types and tissues, highlighting the need to consider cell type specificity. Consequently, the effects observed in a specific context may not universally apply to all cell types or biological circumstances. *H19* has been implicated in cancers. However, disease progression can involve numerous factors. Isolating the specific contribution of *H19* among these factors can be intricate. Furthermore, it shows promise as a potential biomarker or therapeutic target in various diseases. Translating these findings into clinical applications can be a complex process involving validation, safety assessments and regulatory approvals.

Despite the extensive identification of cytogenetic and molecular biomarkers for AML, their application for diagnosis, prediction and treatment decisions remains limited. A

recent study designed diagnostic models for diverse cancer types using measurements of elevated expression of imprinted genes (*GNAS*, *GRB10* and *SNRPN* genes) (37). In combination with other molecular biomarkers such as gene mutations and expression of histone modifying genes, the integration of a panel of biomarkers could significantly enhance prediction sensitivity and specificity. In pursuit of this goal, forthcoming research should focus on elucidating the functions of altered imprinted genes in CA-AML and CN-AML, establishing histone modifications and exploring their clinical applications.

In conclusion, the present study revealed upregulated expression of 7 out of the 16 examined imprinted genes in BM samples from patients with CN-AML, indicating the involvement of loss of imprinting in the leukemogenesis of CN-AML. Notably, a significant negative correlation was observed between the *H19* expression and the survival rate of CN-AML patients, highlighting its potential as a prognostic predictor for two- and five-year survival. The upregulated *H19* expression observed in CN-AML implies its potential involvement in modulating cell proliferation, apoptosis and differentiation. Additionally, its interactions with other genes and regulatory factors may contribute to the intricate molecular pathways underlying CN-AML development and progression. By aligning the findings of the present study in CN-AML with the previous study on CA-AML, a substantiated connection

between imprinted genes and AML has been established. Furthermore, the results suggest that expression of different imprinted genes are influenced by distinct cytogenetic conditions, leading to divergent clinical outcomes. To gain a deeper understanding of the roles these imprinted genes play in the leukemogenesis of both CA-AML and CN-AML, further functional studies are needed.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MYY conceptualized and supervised the study, acquired funding, wrote the original draft, wrote, reviewed and edited the manuscript. CMH conceptualized the study, acquired funding, wrote, reviewed and edited the manuscript. PML conducted investigation and developed methodology. CHY and MLH conducted investigation and project administration. IYC curated and validated data, performed formal analysis, and conducted software analysis. SFL conceptualized the study, acquired funding, wrote, reviewed and edited the manuscript. MY and SFL confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of the Kaohsiung Medical University Hospital Ethical Committee (approval no. KMU-IRB-20130129; Kaohsiung, Taiwan).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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