Spectrum of mitochondrial genome instability and implication of mitochondrial haplogroups in Korean patients with acute myeloid leukemia

Hye Ran Kim[1#], Min-Gu Kang[2#], Young Eun Lee[3,4], Bo Ram Na[3,4], Min Seo Noh[3,4], Seung Hyun Yang[3,4], Jong-Hee Shin[3], Myun-Geun Shin[3,4,5]

[1]College of Korean Medicine, Dongshin University, Naju, [2]Department of Laboratory Medicine, Gwangyang Sarang General Hospital, Gwangyang, [3]Department of Laboratory Medicine, Chonnam National University Medical School and Chonnam National University Hwasun Hospital, Hwasun, [4]Brain Korea 21 Plus Project, Chonnam National University Medical School, Gwangju, [5]Environmental Health Center for Childhood Leukemia and Cancer, Chonnam National University Medical School and Chonnam National University Hwasun Hospital, Hwasun, Korea

Background
Mitochondrial DNA (mtDNA) mutations may regulate the progression and chemosensitivity of leukemia. Few studies regarding mitochondrial aberrations and haplogroups in acute myeloid leukemia (AML) and their clinical impacts have been reported. Therefore, we focused on the mtDNA length heteroplasmies minisatellite instability (MSI), copy number alterations, and distribution of mitochondrial haplogroups in Korean patients with AML.

Methods
This study investigated 74 adult patients with AML and 70 controls to evaluate mtDNA sequence alterations, MSI, mtDNA copy number, haplogroups, and their clinical implications. The hypervariable (HV) control regions (*HV1* and *HV2*), *tRNA*^{leu1} gene, and *cytochrome b* gene of mtDNA were analyzed. Two mtDNA minisatellite markers, 16189 poly-C (^{16184}CCCCCTCCCC^{16193}, 5CT4C) and 303 poly-C (^{303}CCCCCCCTCCCCC^{315}, 7CT5C), were used to examine the mtDNA MSI.

Results
In AML, most mtDNA sequence variants were single nucleotide substitutions, but there were no significant differences compared to those in controls. The number of mtMSI patterns increased in AML. The mean mtDNA copy number of AML patients increased approximately 9-fold compared to that of controls ($P < 0.0001$). Haplogroup D4 was found in AML with a higher frequency compared to that in controls (31.0% vs. 15.7%, $P = 0.046$). None of the aforementioned factors showed significant impacts on the outcomes.

Conclusion
AML cells disclosed more heterogeneous patterns with the mtMSI markers and had increased mtDNA copy numbers. These findings implicate mitochondrial genome instability in primary AML cells. Therefore, mtDNA haplogroup D4 might be associated with AML risk among Koreans.

Key Words   AML, Mitochondrial genome, Instability, Haplogroup, Outcome

INTRODUCTION

Human mitochondrial DNA (mtDNA) is a circular molecule of 16,569 bp that contains 37 genes coding for 13 polypeptides of the mitochondrial electron respiratory chain, two ribosomal RNAs, and 22 transfer RNAs (tRNA) required for polypeptide synthesis [1, 2]. In comparison to the nuclear genome, mtDNA has a modified genetic code, paucity of introns, and lack of histone protection [3, 4]. Moreover,

the turnover of mtDNA is high, as degradation and replication is a continuous process in the mitochondria during one cell cycle, and mtDNA polymerase does not have proof-reading capabilities. Therefore, the accumulation of somatic mutations is greater in mtDNA compared to that in nuclear DNA [1]. The mtDNA molecule has a noncoding region, which includes a unique displacement loop (D-loop) responsible for replication and transcription control. This control region includes a mtDNA production regulation fraction and a hypervariable (HV) region known as a gene mutation "hot spot," resulting in mtDNA sequence and length heteroplasmies [5]. The sequence heteroplasmies consist of base substitutions and small deletions and insertions. The length heteroplasmies are usually detected by varying numbers of a particular repeated nucleotide, usually poly-C, known as the mtDNA minisatellite instability (mtMSI) [6]. In conjunction with the control region, the *tRNA leucine 1* (*tRNA^leu1*) and *cytochrome b* (*CYTB*) genes in the coding region represent approximately 13% of the total mtDNA sequence, and pathological heteroplasmic mtDNA mutations were observed in these regions of various cancer tissues [6-8].

The mitochondrion is an important micro-organelle that produces energy for cell development, differentiation, and growth. It also controls cell growth by inducing apoptosis. The mtDNA is comprised of 0.1% to 1.0% of the total DNA in most mammalian cells, and 2 to 10 copies come packaged in each nucleated cell per mitochondrion up to 1,000 mitochondria. The copy number per cell is controlled within a constant range to fulfill the energy requirement of the cell and to maintain normal physiological functions [9, 10]. Variations in the mitochondria copy number have been reported to reflect the net results of gene-environmental interactions, and the copy number has been implicated as a potential biomarker for various cancers [11, 12].

Since mtDNA is haploid and lacks recombination, specific mutations in the mtDNA genome leading to human diseases arise in particular genetic backgrounds referred to as haplogroups [13]. Human populations usually carry several mtDNA haplogroups defined by unique sets of mtDNA polymorphisms, reflecting mutations accumulated by a discrete maternal lineage [14, 15], but the sets and their frequencies differ between populations. Thus, haplogroup association studies have been used to assess the role of mtDNA variants in various diseases and cancers [16].

Acute myeloid leukemia (AML) results from the accumulation of abnormal blasts in the bone marrow. It is likely that many different mutations, epigenetic aberrations, or abnormalities in microRNA expression can produce the same morphological disease; however, these differences may be responsible for the variable responses to therapy, which is a principal feature of AML [17]. In addition to these nuclear genetic changes, non-chromosomal mitochondrial mutations may play a role in the progression and chemosensitivity of leukemias [18-20]. In recent years, mtDNA variants within the D-loop or the entire mitochondrial genome in patients with AML and their prognostic impacts have been reported [21, 22]. However, few studies have focused on the mtMSI, copy number, or haplogroups in association with AML.

In this study, we analyzed the control region and two coding regions in bone marrow cells of patients with AML to demonstrate the sequence and length heteroplasmies of mtDNA, alterations in mtDNA copy number, haplogroup distribution, and their impacts on patient outcomes.

MATERIALS AND METHODS

Patients and samples

Seventy-four patients with AML and 70 control subjects were enrolled in this study after obtaining Institutional Review Board approval and informed consent. The control subjects were selected from normal adults visiting the health examination center. The total DNA of bone marrow samples at the diagnostic stage from patients with AML was extracted

Table 1. Primers for the mtDNA control region PCR, direct sequencing, and gene scan.

mtDNA segment	Primer sequence (5' to 3') for PCR		Sequencing primers (5' to 3')	
Control region 1.12 kb (np 16024–16569; np 1–576)	F15971	TTAACTCCACCATTAGCACC	F15971	TTAACTCCACCATTAGCACC
			R48	GCATGGAGAGCTCCCGTGAGTGG
	R611	CAGTGTATTGCTTTGAGGAGG	F15	CACCCTATTAACCACTCACG
			R611	CAGTGTATTGCTTTGAGGAGG
tRNA^leu1, *ND1* 585 bp (np 2972–3557)	F2972	ATAGGGTTTACGACCTCGATG	F2972	ATAGGGTTTACGACCTCGATG
	R3557	AGAAGAGCGATGGTGAGAGC	R3557	AGAAGAGCGATGGTGAGAGC
CYTB 487 bp (np 14909–15396)	F14909	TACTCACCAGACGCCTCAACCG	F14909	TACTCACCAGACGCCTCAACCG
	R15396	TTATCGGAATGGGAGGTGATTC	R15396	TTATCGGAATGGGAGGTGATTC

Minisatellite markers	Location (bp)	Size (bp)	Primer sequences (5' to 3')
303 poly C	D-loop (303–315)	109–118	F: Hex-CTTTCCACACAGACATCATAAC
			R: ATCTGGTTAGGCTGGTGTTAG
16189 poly C	D-loop (16184–16193)	102–105	F: Hex-CTTGACCACCTGTAGTACATA
			R: GGAGTTGCAGTTGATGTGTGA

Abbreviations: bp, base pair; *CYTB*, *cytochrome b*; F, Forward; Kb, kilo base; np, nucleotide position; R, Reverse; *tRNA^leu1*, tRNA leucine1.

with the AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). The BM specimens from the patients with AML were frozen in liquid nitrogen immediately after acquisition for further evaluation. The total DNA from the peripheral blood of control subjects was processed using the same protocol. The DNA from control subjects was used for detecting mtDNA sequence variation, measuring mtDNA copy number, and classifying haplogroups.

Direct sequencing of the mtDNA control and coding region

Using samples from AML patients and control subjects, we directly sequenced the control regions (*HV1* and *HV2*), the *tRNA^leu1*gene including a small portion of NADH dehydrogenase (*ND1*), and the *cytochrome b* (CYTB) gene of mtDNA, using a set of primer pairs according to our protocols (Table 1) [6]. The sequencing analysis was performed using the ABI Prism 3130XL Genetic Analyzer with the BigDye Terminator v3.1 Ready Reaction Kit (Applied Biosystems, Foster City, CA). To exclude potential artifacts, PCR amplifications from the original cell lysates were replicated once or twice and sequenced. The mtDNA sequences obtained were analyzed using the MitoAnalyzer (http://www.cstl.nist.gov/biotech/strbase/mitoanalyzer.html) with the Revised Cambridge Reference Sequence (http://mitomap.

org/MITOMAP) and the Blast2 program (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi) to determine mtDNA aberrations.

Detection of mtDNA length heteroplasmy using minisatellite markers

Our previous protocols were used in order to examine the length of heteroplasmies using two mtDNA minisatellite markers 16189 poly-C (^{16184}CCCCCTCCCC^{16193}, 5CT4C) and 303 poly-C (^{303}CCCCCCCTCCCCC^{315}, 7CT5C) in the mtDNA *HV1* and *HV2* regions, respectively. The analysis was based on separation via capillary electrophoresis using an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems) and Gene Scan Analysis Software (version 3.1, Applied Biosystems). TA cloning for the confirmation of mtDNA minisatellite alterations was carried out according to a previously published protocol [6, 23]. We compared this mtMSI data of the patients with AML with our previously published data from healthy Korean donors [6].

Determination of mtDNA copy number

The linearity of the quantitative mtDNA assay was assessed using the cloned mtDNA *CYTB* standard, which had also been serially diluted to prepare a series of calibrators with known concentrations. The determination of mtDNA copy number was performed using our previously published methods [7, 8].

Classification of mtDNA haplogroups

The mtDNA sequences of control and coding regions were assigned to identify haplogroups according to a classification previously proposed [24, 25]. We compared the frequencies and distributions of the haplogroups of the patients with AML with those of control subjects.

Statistical analysis

The significance of observed differences in proportions was tested by Fisher's exact test. To analyze the AML risk, the odds ratio (OR) with 95% confidence interval (CI) was tested. The Mann-Whitney U test was used to determine significance between differences of the medians. Overall survival (OS) was calculated as the time from diagnosis until death from any cause, with the observation censored at the date of the last follow-up for patients last known to be alive. Event-free survival (EFS) was defined as the time from complete remission (CR) or bone marrow transplantation/peripheral blood stem cell transplantation (BMT/PBSCT) until relapse or death from any cause. In EFS analyses, only patients who achieved CR or who underwent BMT/PBSCT were included. Log-rank (Mantel-Cox) test was used to estimate OS and EFS. Cox regression analyses of specific parameters were performed to calculate univariate and multivariate *P*-values and hazard ratios (HR), considering patient characteristics such as age, white blood cell count, blast percentage, prognostic group based on cytogenetic study, and secondary AML. All statistical computations were performed using PASW 18.0 (SPSS, Chicago, IL, USA). *P*-value <0.05 was considered to indicate statistically significant differences.

Table 2. Characteristics of the patients enrolled in this study.

Characteristics	N	%
Gender		
Male	39	53
Female	35	47
Age, years		
17–59	51	69
60–77	23	31
WBC, ×10^9/L, median (range)	15.2 (0.2–165.4)	·
BM blasts, %, median (range)	49 (1–100)	·
Biology		
De novo AML	68	92
Secondary AML	6	8
FAB type		
M0	1	1
M1	7	9
M2	22	30
M3	13	18
M4	13	18
M5	6	8
M6	6	8
M7	2	3
Uncertain	4	5
Cytogenetics		
Abnormal karyotypes	35	47
Favorable	24	32
Intermediate	37	50
Adverse	12	16
Not tested	1	1

Abbreviations: FAB, French-American-British; N, number; WBC, white blood cells.

RESULTS

Patient characteristics

The patient cohort included diagnostic bone marrow samples from 74 adult patients with AML. The ages varied from 17 to 77 years, and the average was 48 years. The male and female ratio was 1.1:1. Six patients had secondary AML, and 68 patients had *de novo* mutations resulting in AML. The characteristics of the patients enrolled in this study are shown in Table 2.

mtDNA sequence variants in AML patients

We established mtDNA polymorphism databases by analysis of *HV1* and *HV2* control regions and the *tRNA^leu1* and *CYTB* genes. Representative sequencing chromatograms revealing mtDNA mutations are shown in Fig. 1. In AML patients, a total number of 963 variants at 134 different positions were found, with a median of 12 variants per patient (Supplementary Table 1). In control subjects, 920 variants at 113 different positions were found, and the median of variants was 13. Most variations were single nucleotide substitutions, and only a small portion of insertions/deletions was detected (Supplementary Table 1). In patients with AML, 57 mtDNA variants were identified, which were absent in control subjects. We detected two novel variants, A16162T and G366A. The A16162T variant was found in three patients with AML (4.1%) and two control subjects (2.9%). G366A was observed only in one patient with AML (Supplementary Table 1). The variants of G16129A, A16182C, A16183C, T16189C, T489C, and G3010A showed statistically significant differences between patients with AML and control subjects. However, all five variants in *HV1* and the variation in *tRNA^leu1* appeared more frequently in control subjects than in patients with AML, and only the T489C variant

in *HV2* was dominant in patients with AML.

Distribution of mtDNA minisatellite instability (mtMSI)

The overall frequency of mtMSI in patients with AML was 100% (N=74). Gene scan analyses of poly C-stretch region at nucleotide position (np) 303–315, 16184–16193, and 514–515(CA)$_5$ repeats are shown in Fig. 2. All patients with AML had mtMSI values of 303 poly-C, and 70.3% (N=52) of the patients had mtMSIs in 16189 poly-C (Table 3). In our previous report of healthy Korean donors, the pattern number was 6 in 303 poly-C and 8 in 16189 poly-C [6]. However, in this study, the number of the patterns increased into 11 and 11, respectively.

mtDNA copy number

The mean mtDNA copy number of 70 control subjects was 45.1, and the range was from 3.6 to 142.3. Among the 74 patients with AML involved in this study, the copy number analysis was not available in six patients. The mean mtDNA copy number of 68 patients with AML was 503.1, and the range was from 4.0 to 7901.7. The mean mtDNA copy number of patients with AML increased approximately nine-fold compared to normal control subjects ($P < 0.0001$).

mtDNA haplogroup in patients with AML

There were no statistically significant differences in the distribution of mtDNA haplogroup frequencies between patients with AML and control subjects, except for haplogroup D4 (Supplementary Table 2). Haplogroup D4 was found to have a higher frequency in patients with AML, 31.0% versus 15.7% ($P=0.046$), as compared to that in control subjects. This difference translated into an OR of 2.408 for AML (95% CI, 1.064–5.452).
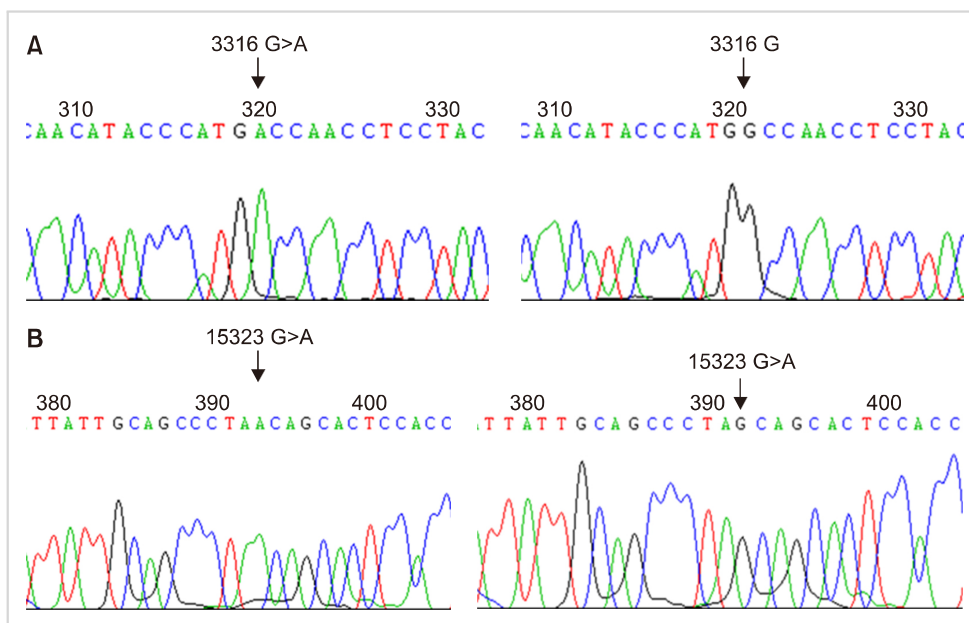

Fig. 1. Representative sequencing chromatograms revealing mtDNA mutations in *tRNA^leu1* gene (AML No.112 patient) and *CYTB* gene (AML No.50 patient) as nonsynonymous mutations. **(A)** mtDNA *tRNA^leu1* gene mutation-affected amino acid change (Ala→Thr) for AML patient No.112. **(B)** mtDNA *CYTB* gene mutation-affected amino acid change (Ala→Thr) for AML patient No.50.
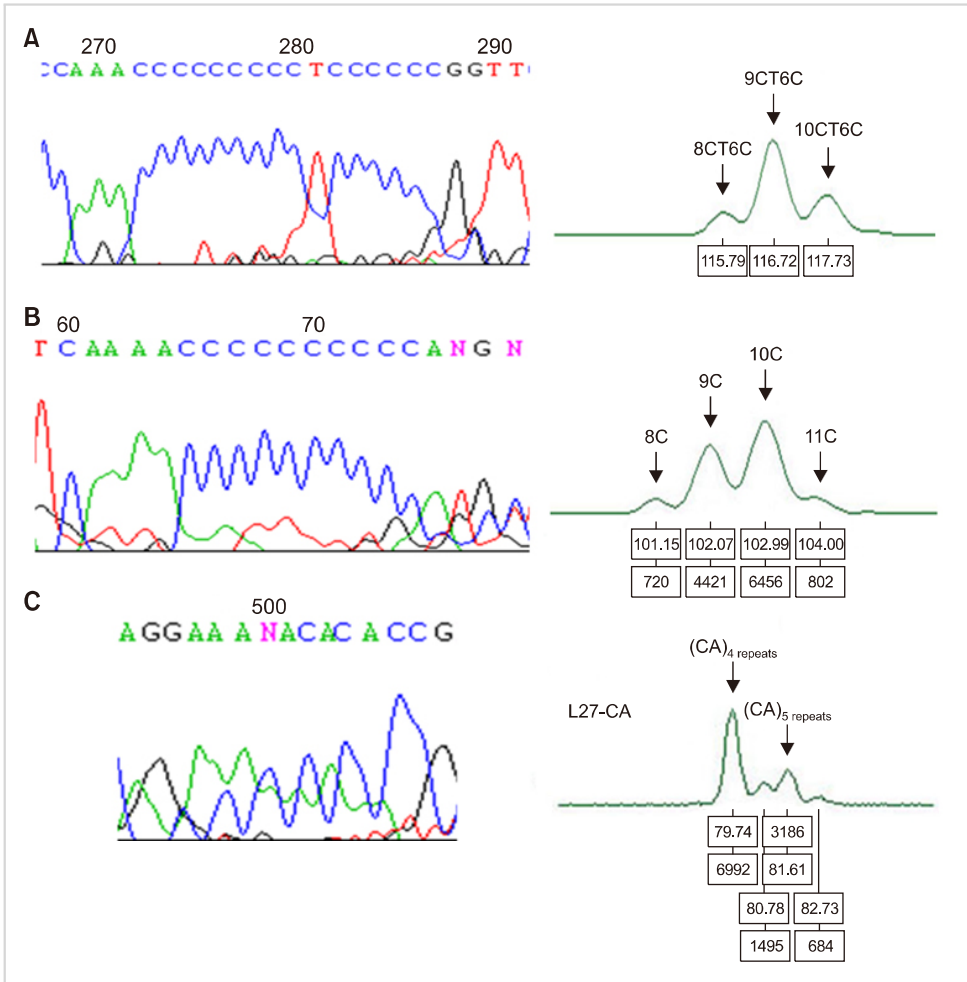
Fig. 2. Gene scan analysis of poly C-stretch region at nucleotide position (np) 303–315, 16184–16193, and 514–515(CA)$_5$ repeats. **(A)** The mtDNA D-loop *HV2* (303 poly-C) region. Capillary electropherogram of the np 303 poly-C region from AML patient No.123 showed 9CT6C as a C insertion type. **(B)** The mtDNA D-loop *HV1* (16189 poly-C) region. Capillary electropherogram of np 16184–16193 poly-C region from samples of AML No.29 patient also disclosed the profile of several mtDNA types (heteroplasmic mutations). **(C)** The mtDNA D-loop *HV2* 514–515(CA)$_5$ repeats region. Gene scan analysis of CA repeats starting at np 514 (AML No.27 patient) also disclosed heteroplasmic mutations, which suggest the coexistence of wild-type and mutant mtDNA in the same patient.

Impact of mtDNA sequence variant, mtMSI, mtDNA copy number, and haplogroup on outcomes in patients with AML

Fifty-seven of 74 patients were treated at our hospital, and 40 (70.2%) patients achieved CR with an induction of chemotherapy or underwent stem cell transplantation. Twenty-one (52.5%) patients relapsed or died after achieving remission. According to the aforementioned results, patients with a higher number (above the median) of sequence variants, T489C sequence alteration, new mtDNA MSI patterns, higher copy number (above the median), or haplogroup D4 were included to estimate the HR of EFS and OS. However, the survival data analyzed by Cox regression (univariate or multivariate) showed no statistical significance (Table 4).

DISCUSSION

Several studies on mtDNA genome variations in acute leukemia have been reported. He *et al.* [26] compared the entire mitochondrial genome from both normal tissue and leukemic samples from 24 leukemia patients. Grist *et al.* [19] sequenced the D loop (nt 16111–190) of 48 patients with leukemia. Yao *et al.* [27] analyzed the mtDNA control region sequence variation in single cells from 18 leukemia patients. Sharawat *et*

al. [21] evaluated the entire D loop region in 44 pediatric patients with AML by direct sequencing. Silkjaer *et al.* [22] investigated the entire mtDNA in 56 AML patients. Finally, Han *et al.* [28] analyzed mutations and mtMSI in the mtDNA D-loop region in BM cells of 19 patients with acute leukemia.

In this study, we collected 74 patients with AML and 70 control subjects, and studied not only the mtDNA sequence variants, but also the mtMSI, copy number, and haplogroup. The median number of the mtDNA sequence variant was not different in the patients with AML and control subjects. The changed sequence also showed no significant difference between the two groups. He *et al.* [26] demonstrated that somatic mtDNA mutations were present in approximately 40% of patients, and they found the A15296G mutation as a leukemia-specific marker. In our study, no participants had a mutation on nucleotide (nt) 15296. Silkjaer *et al.* [22] reported that 21% of the AML patients harbored the T16311C variant, and this variant tended to be associated with chromosomal abnormalities of favorable prognosis. In this study, we found the T16311C variant in six control subjects (8.5%) and three patients with AML (4%). Among the three patients with AML, two patients had normal karyotypes and one had t(15;17). However, the sample size was too small to analyze for the statistical sig-

Table 3. Distribution of mtDNA minisatellite instability of hypervariable regions in AML patients.

Markers	Reference sequence	Patterns	No.	%
303 poly-C	CCCCCCCTCCCCCC (7CT6C)	7CT6C+8CT6C	22	29.7
		8CT6C+9CT6C	22	29.7
		9CT6C+10CT6C	5	6.8
		8CT6C+9CT6C+10CT6C	6	8.1
		9CT6C+10CT6C+11CT6C	5	6.8
		7CT6C+8CT6C+9CT6C+10CT6C[a]	6	8.1
		8CT6C+9CT6C+10CT6C+11CT6C[a]	3	4.1
		9CT6C+10CT6C+11CT6C+12CT6C[a]	1	1.4
		7CT6C+8CT6C+9CT6C+10CT6C+11CT6C[a]	1	1.4
		15C, 16C, 17C[a]	2	2.7
		16C, 17C, 18C, 19C[a]	1	1.4
Subtotal			74	100.0
16189 poly-C	CCCCCTCCCC (5CT4C)	4CT4C[a]	1	1.9
		3CT4C+4CT3C[a]	1	1.9
		5CT4C+5CT3C	29	55.8
		5CT4C+6CT4C[a]	1	1.9
		5CT4C+6CT4C+7CT4C[a]	2	3.8
		3CT4C+4CT4C+5CT4C+6CT4C[a]	2	3.8
		9C+10C+11C	1	1.9
		8C+9C+10C+11C[a]	1	1.9
		9C+10C+11C+12C	12	23.1
		10C+11C+12C+13C[a]	1	1.9
		9C+10C+11C+12C+13C	1	1.9
Subtotal			52	100.0

[a]Newly appeared patterns in AML patients involved in this study compared to normal controls of the previous study [6].

Table 4. Cox regression analyses of outcome data.

Parameter	EFS				OS			
	Univariate		Multivariate		Univariate		Multivariate	
	P	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)
No. of sequence variation, grouped by median (12)	0.736	1.119 (0.582–2.148)	0.971	0.985 (0.433–2.241)	0.911	0.969 (0.562–1.671)	0.321	0.726 (0.386–1.366)
T489C	0.766	0.903 (0.460–1.770)	0.946	1.035 (0.388–2.756)	0.845	1.062 (0.583–1.935)	0.515	1.312 (0.580–2.966)
New mtDNA MSI pattern	0.888	0.945 (0.433–2.064)	0.359	1.609 (0.583–4.447)	0.573	1.192 (0.647–2.197)	0.289	1.444 (0.732–2.848)
mtDNA copy number, grouped by median (175.9)	0.529	1.247 (0.628–2.477)	0.258	1.537 (0.730–3.236)	0.994	1.002 (0.575–1.748)	0.322	1.352 (0.774–2.458)
Haplogroup D4	0.450	0.749 (0.354–1.585)	0.768	0.864 (0.328–2.278)	0.592	0.849 (0.467–1.545)	0.405	0.718 (0.330–1.565)

Abbreviations: CI, confidence interval; EFS, event-free survival; OS, overall survival.

nificance of the relationship and chromosome abnormality. We also found the T489C variant, which appeared in a higher proportion (73%) of patients with AML than in control subjects (50%), but it showed no impact on outcomes. All patients of the haplogroup D4 had the T489C variant.

In the present study, most patients with AML had mtMSI in 303 poly-C and 16189 poly-C (Table 3), and the pattern number of the mtMSI was greater in patients with AML than in control cohorts. These greater heteroplasmy lengths

in patients with AML might be associated with the clinical implication. Mitochondrial genomic instability has been strongly correlated with a high incidence of circular mtDNA molecules (of multiple lengths) in human leukemic cells, and a positive correlation has been observed between the frequency of these molecules and the disease severity [29].

Meanwhile, Grist et al. [19] found mtDNA mutations in 36% of AML patients. Yao et al. [27] found that some patients at relapse presented a complex shift in major haplotypes.

In our study, the haplogroup D4 was found at a higher frequency in patients with AML compared to that in control subjects, and this difference resulted in an OR of 2.408 for AML (P=0.046). Moreover, in the report by Sharawat *et al.* [21], some variations in HV-1 were associated with inferior EFS. Silkjaer *et al.* [22] suggested that alterations in cytochrome c oxidase subunit I and II have an adverse impact on prognosis. In addition, they reported that the T16311C variant tended to be associated with chromosomal abnormalities.

There are also limitations in this study. Since we compared the mtDNA from patients with AML with that from control subjects, there could be the possibility that the sequence changes between the patients with AML and controls may simply reflect extensive sequence variation within the population [30]. As shown in this study, there were no significant differences in mtDNA sequence variation between patients with AML and control subjects. However, even though the variants could not be compared with respect to the potential role in malignancy formation, mtDNA mutations might still contribute to genomic instability.

In conclusion, AML cells revealed more heterogeneous patterns in mtMSI markers and had increased mtDNA copy numbers. These findings implicate mitochondrial genome instability in primary AML cells. In addition, the mtDNA haplogroup D4 might be associated with AML risk among Koreans.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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Supplementary Table 1. Sites of variations in *HV1* and *HV2* regions, *tRNA*[leu1] and *CYTB* gene of mtDNA (74 AML patients, 70 control subjects).

Region	S. No.	Position of nucleotide	Base variation	No. of patients (%)	No. of control subjects (%)	P	Region	S. No.	Position of nucleotide	Base variation	No. of patients (%)	No. of control subjects (%)	P
Control region (*HV1*)	1	16129	G → A	1 (1.4)	11 (15.7)	0.002	Control region (*HV2*)	67[a]	61	C → T	1 (1.4)	0 (0)	1.000
	2[a]	16134	C → T	1 (1.4)	0 (0)	1.000		68	65	T → G	1 (1.4)	4 (5.7)	0.200
	3	16140	T → C	1 (1.4)	3 (4.3)	0.356		69[a]	72	T → C	1 (1.4)	0 (0)	1.000
	4	16162	A → T	3 (4.1)	2 (2.9)	1.000		70	73	A → G	73 (98.6)	68 (97.1)	0.612
	5[a]	16171	A → T	1 (1.4)	0 (0)	1.000		71	93	A → G	1 (1.4)	1 (1.4)	1.000
	6	16172	T → C	6 (8.1)	9 (12.9)	0.149		72	94	G → A	2 (2.7)	1 (1.4)	1.000
	7	16174	C → T	3 (4.1)	3 (4.3)	1.000		73	103	G → A	1 (1.4)	2 (2.9)	0.612
	8	16176	C → T	3 (4.1)	1 (1.4)	0.620		74[a]	109	G → A	1 (1.4)	0 (0)	1.000
	9	16182	A → C	3 (4.1)	14 (20.0)	0.004		75	143	G → A	1 (1.4)	1 (1.4)	1.000
	10	16183	A → C	7 (9.5)	18 (25.7)	0.015		76	146	T → C	8 (10.8)	7 (10.0)	1.000
	11[a]	16184	C → A	2 (2.7)	0 (0)	0.497		77	150	C → T	17 (23.0)	20 (28.6)	0.453
	12	16187	C → T	1 (1.4)	5 (7.1)	0.109		78[a]	151	C → T	1 (1.4)	0 (0)	1.000
	13[a]	16188	C → ins	1 (1.4)	0 (0)	1.000		79	152	T → C	18 (24.3)	16 (22.9)	0.847
	14	16189	T → C	17 (23.0)	27 (38.6)	0.048		80[a]	153	A → G	1 (1.4)	0 (0)	1.000
	15[a]	16192	C → T	1 (1.4)	0 (0)	1.000		81[a]	184	G → A	1 (1.4)	0 (0)	1.000
	16[a]	16193	C → T	1 (1.4)	0 (0)	1.000		82[a]	185	G → A	1 (1.4)	0 (0)	1.000
	17[a]	16195	T → C	1 (1.4)	0 (0)	1.000		83	189	A → G	1 (1.4)	1 (1.4)	1.000
	18[a]	16196	G → A	1 (1.4)	0 (0)	1.000		84	194	C → T	4 (5.4)	2 (2.9)	0.682
	19	16209	T → C	3 (4.1)	2 (2.9)	1.000		85	195	T → C	6 (8.1)	3 (4.3)	0.495
	20	16217	T → C	7 (9.5)	6 (8.6)	1.000		86[a]	198	C → T	1 (1.4)	0 (0)	1.000
	21	16223	C → T	60 (81.1)	48 (68.6)	0.088		87	199	T → C	5 (6.8)	3 (4.3)	0.720
	22[a]	16228	C → T	1 (1.4)	0 (0)	1.000		88	200	A → G	2 (2.7)	2 (2.9)	1.000
	23	16232	C → A	2 (2.7)	3 (4.3)	0.675		89	204	T → C	2 (2.7)	5 (6.8)	0.266
	24	16234	C → T	5 (6.8)	2 (2.9)	0.443		90	207	G → A	2 (2.7)	4 (5.7)	0.432
	25	16235	A → G	1 (1.4)	1 (1.4)	1.000		91[a]	214	A → G	1 (1.4)	0 (0)	1.000
	26[a]	16237	A → G	1 (1.4)	0 (0)	1.000		92	228	G → A	2 (2.7)	2 (2.9)	1.000
	27[a]	16240	A → G	1 (1.4)	0 (0)	1.000		93	235	A → G	8 (10.8)	6 (8.6)	0.781
	28	16243	T → C	1 (1.4)	4 (5.7)	0.200		94[a]	237	A → G	1 (1.4)	0 (0)	1.000
	29[a]	16248	C → T	1 (1.4)	0 (0)	1.000		95	249	A → del	8 (10.8)	7 (10.0)	1.000
	30	16249	T → C	4 (5.4)	5 (7.1)	0.740		96[a]	260	G → C	1 (1.4)	0 (0)	1.000
	31	16257	C → A	4 (5.4)	7 (10.0)	0.358		97	263	A → G	72 (97.3)	69 (98.6)	1.000
	32	16260	C → T	1 (1.4)	1 (1.4)	1.000		98[b]	303	C→delins(n)	40 (54.1)	33 (47.1)	1.000
	33	16261	C → T	6 (8.1)	11 (15.7)	0.199		99[a]	317	C → A	1 (1.4)	0 (0)	1.000
	34	16265	A → C	3 (4.1)	1 (1.4)	0.620		100[a]	318	T → C	1 (1.4)	0 (0)	1.000
	35	16266	C → G	2 (2.7)	5 (7.1)	0.266		101[a]	320	C → T	1 (1.4)	0 (0)	1.000
	36[a]	16270	C → T	1 (1.4)	0 (0)	1.000		102[a]	324	C → T	3 (4.1)	0 (0)	0.245
	37[a]	16271	T → C	1 (1.4)	0 (0)	1.000		103[a]	325	C → T	1 (1.4)	0 (0)	1.000
	38	16274	G → A	4 (5.4)	2 (2.9)	0.682		104[a]	326	A → G	1 (1.4)	0 (0)	1.000
	39	16278	C → T	7 (9.5)	4 (5.7)	0.534		105[a]	356	C → ins	1 (1.4)	0 (0)	1.000
	40[a]	16280	A → G	1 (1.4)	0 (0)	1.000		106[a]	366	G → A	1 (1.4)	0 (0)	1.000
	41[a]	16284	A → G	1 (1.4)	0 (0)	1.000		107[a]	411	C → A	2 (2.7)	0 (0)	0.497
	42	16290	C → T	8 (10.8)	8 (11.4)	1.000		108	456	C → T	5 (6.8)	1 (1.4)	0.210
	43	16295	C → T	2 (2.7)	1 (1.4)	1.000		109	489	T → C	54 (73.0)	35 (50.0)	0.006
	44[a]	16296	C → T	1 (1.4)	0 (0)	1.000		110[a]	495	C → T	1 (1.4)	0 (0)	1.000
	45	16297	T → C	1 (1.4)	1 (1.4)	1.000	*tRNA*[leu1]	111	3010	G → A	7 (9.5)	19 (27.1)	0.009
	46	16298	T → C	7 (9.5)	2 (2.9)	0.167		112	3107	C → del	74 (100)	70 (100)	NA
	47	16304	T → C	5 (6.8)	6 (8.6)	0.760		113	3206	C → T	4 (5.4)	3 (4.3)	1.000
	48[a]	16309	A → G	1 (1.4)	0 (0)	1.000		114[a]	3394	T → C	1 (1.4)	0 (0)	1.000
	49	16311	T → C	3 (4.1)	9 (12.9)	0.073		115	3396	T → C	1 (1.4)	2 (2.9)	0.612
	50[a]	16316	A → G	1 (1.4)	0 (0)	1.000		116	3497	C → T	2 (2.7)	1 (1.4)	1.000

Supplementary Table 1. Continued.

Region	S. No.	Position of nucleotide	Base variation	No. of patients (%)	No. of control subjects (%)	P	Region	S. No.	Position of nucleotide	Base variation	No. of patients (%)	No. of control subjects (%)	P
	51[a]	16318	A → G	1 (1.4)	0 (0)	1.000	CYTB	117[a]	14944	C → T	1 (1.4)	0 (0)	1.000
	52	16319	G → A	10 (13.5)	8 (11.4)	0.803		118[a]	14978	A → G	1 (1.4)	0 (0)	1.000
	53	16324	T → C	3 (4.1)	1 (1.4)	0.620		119	14979	T → C	3 (4.1)	3 (4.3)	1.000
	54	16325	T → C	3 (4.1)	4 (5.7)	0.713		120[a]	15038	A → G	1 (1.4)	0 (0)	1.000
	55[a]	16327	C → T	2 (2.7)	0 (0)	0.497		121	15043	G → A	45 (60.8)	31 (44.3)	0.066
	56[a]	16335	A → G	1 (1.4)	0 (0)	1.000		122[a]	15046	A → G	1 (1.4)	0 (0)	1.000
	57[a]	16342	T → G	1 (1.4)	0 (0)	1.000		123	15067	T → C	3 (4.1)	4 (5.7)	0.713
	58	16344	C → T	1 (1.4)	3 (4.3)	0.356		124[a]	15139	T → C	1 (1.4)	0 (0)	1.000
	59	16357	T → C	2 (2.7)	2 (2.9)	1.000		125[a]	15204	T → C	1 (1.4)	0 (0)	1.000
	60[a]	16359	T → C	1 (1.4)	0 (0)	1.000		126[a]	15211	C → T	1 (1.4)	0 (0)	1.000
	61	16362	T → C	37 (50.0)	34 (48.6)	0.869		127[a]	15226	A → C	1 (1.4)	0 (0)	1.000
	62	16399	A → G	1 (1.4)	1 (1.4)	1.000		128	15235	A → G	1 (1.4)	2 (2.9)	0.612
	63[a]	16445	T → C	1 (1.4)	0 (0)	1.000		129[a]	15263	C → T	1 (1.4)	0 (0)	1.000
	64[a]	16463	A → G	1 (1.4)	0 (0)	1.000		130	15292	C → T	3 (4.1)	2 (2.9)	1.000
	65	16497	A → G	2 (2.7)	3 (4.3)	0.675		131	15301	G → A	45 (60.8)	32 (45.7)	0.094
	66	16519	T → C	31 (41.9)	30 (42.9)	1.000		132	15323	G → A	3 (4.1)	4 (5.7)	0.713
								133	15326	A → G	72 (97.3)	70 (100)	0.497
								134	15346	G → A	4 (5.4)	2 (2.9)	0.682

[a]Variations, only found in AML patients, not in control subjects. [b]303-315 poly-C.
Abbreviation: NA, not available.

Supplementary Table 2. Haplogroup distribution in AML patients and odds ratio for AML according to haplogroup.

Haplogroup	No. of patients (%)	No. of control subjects (%)	Odds ratio (OR)	95% CI	P [b]
A	2 (2.8)	4 (5.7)	0.478	0.085＜OR＜2.699	0.441
A4	2 (2.8)	0 (0.0)	1.000	NA	0.497
A5	1 (1.4)	2 (2.9)	0.486	0.043＜OR＜5.482	0.620
B	0 (0.0)	4 (5.7)	1.000	NA	0.058
B4	4 (5.6)	2 (2.9)	0.423	0.360＜OR＜11.456	0.681
B4a	1 (1.4)	4 (5.7)	0.236	0.026＜OR＜2.164	0.209
B5a	0 (0.0)	1 (1.4)	1.000	NA	0.497
B5b	0 (0.0)	1 (1.4)	1.000	NA	0.497
C	2 (2.8)	0 (0.0)	1.000	NA	0.497
D	0 (0.0)	2 (2.9)	1.000	NA	0.245
D4 [a]	**22 (31.0)** [a]	**11 (15.7)** [a]	**2.408** [a]	**1.064＜OR＜5.452** [a]	**0.046** [a]
D4a	1 (1.4)	2 (2.9)	0.486	0.043＜OR＜5.482	0.620
D4b1	1 (1.4)	1 (1.4)	0.986	0.060＜OR＜16.075	1.000
D4b2	2 (2.8)	0 (0.0)	1.000	NA	0.497
D5	1 (1.4)	4 (5.7)	0.236	0.026＜OR＜2.164	0.209
D5a	0 (0.0)	2 (2.9)	1.000	NA	0.245
D5b	1 (1.4)	1 (1.4)	0.986	0.060＜OR＜16.075	1.000
F	1 (1.4)	3 (4.3)	0.319	0.032＜OR＜3.144	0.366
F1	1 (1.4)	0 (0.0)	1.000	NA	1.000
F1a	3 (4.2)	1 (1.4)	3.044	0.309＜OR＜29.995	0.620
F1b	1 (1.4)	2 (2.9)	0.486	0.043＜OR＜5.482	0.620
F3a	0 (0.0)	1 (1.4)	1.000	NA	0.497
G1a	3 (4.2)	4 (5.7)	0.728	0.157＜OR＜3.378	0.719
G2a	0 (0.0)	2 (2.9)	1.000	NA	0.245
G2a1	2 (2.8)	0 (0.0)	1.000	NA	0.497
G3	0 (0.0)	1 (1.4)	1.000	NA	0.497
G3a	1 (1.4)	0 (0.0)	1.000	NA	1.000
M	4 (5.6)	1 (1.4)	4.119	0.449＜OR＜37.813	0.366
M10	3 (4.2)	0 (0.0)	1.000	NA	0.245
M7a1	0 (0.0)	1 (1.4)	1.000	NA	0.497
M7b2	2 (2.8)	1 (1.4)	1.000	NA	1.000
M7c1	3 (4.2)	0 (0.0)	2.000	0.177＜OR＜22.571	0.245
M8	2 (2.8)	0 (0.0)	1.000	NA	0.497
M8a	1 (1.4)	0 (0.0)	1.000	NA	1.000
M9a	0 (0.0)	4 (5.7)	1.000	NA	0.058
N9a	3 (4.2)	3 (4.3)	0.985	0.192＜OR＜5.056	1.000
N9a1	0 (0.0)	1 (1.4)	1.000	NA	0.497
N9a2	1 (1.4)	3 (4.3)	0.319	0.032＜OR＜3.144	0.366
Y1	0 (0.0)	1 (1.4)	1.000	NA	0.497
Total in groups					
A	5 (7.0)	6 (8.6)	0.808	0.235＜OR＜2.780	0.764
B	5 (7.0)	12 (17.1)	0.446	0.157＜OR＜1.265	0.075
C	2 (2.8)	0 (0.0)	1.000	NA	0.497
D	28 (39.4)	23 (32.9)	1.331	0.668＜OR＜2.651	0.484
F	6 (8.5)	7 (10.0)	0.831	0.265＜OR＜2.608	0.779
G	6 (8.5)	7 (10.0)	0.831	0.265＜OR＜2.608	0.779
M	15 (21.1)	7 (10.0)	2.411	0.917＜OR＜6.338	0.103
N	4 (5.6)	7 (10.0)	0.537	0.150＜OR＜1.924	0.366
Y	0 (0.0)	1 (1.4)	1.000	NA	0.497

[a]Statistical significance in bold type. [b]P-values were calculated with Fisher's exact test.
Abbreviations: CI, confidence interval; NA, not available.