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t(11;17)(q23;q21)와 *KMT2A/MLLT6* 유전자의 융합을 동반한 급성단구성백혈병 1례

Acute Monoblastic Leukemia with t(11;17)(q23;q21): Fusion of the *KMT2A(MLL)* and *MLLT6(AF17)* Genes

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The *KMT2A* (formerly *MLL*) gene is associated with at least 10% of all cases of acute leukemia. More than 80 translocation partner genes of *KMT2A* have been discovered to date, six of which have been identified on the long arm of chromosome 17. Among these, the *MLLT6* (formerly *AF17*) gene is located at 17q12 and fuses with the *KMT2A* gene in rare cases of acute leukemia. We report here a case of AML with a *KMT2A/MLLT6* fusion that was confirmed using molecular genetic methods. According to a literature review, this is the first reported case of AML with a *KMT2A/MLLT6* fusion in Korea.

Key Words: Acute monoblastic leukemia, AML, *KMT2A, MLL, MLLT6*

INTRODUCTION

The lysine K-specific methyltransferase 2A (*KMT2A*) gene, formerly known as the *MLL* gene, is located on chromosome 11q23.3 [1], and its chromosomal rearrangement is implicated in at least 10% of all acute leukemia (AL) cases of various types, including ALL, AML, biphenotypic AL, treatment-related leukemia, and infant leukemia [2]. The KMT2A protein acts as a transcriptional regulator in normal hematopoiesis; AL caused by *KMT2A* translocation tends to exhibit a more aggressive clinical course than that caused by other etiologies [3].

According to the French-American-British (FAB) classification of AML that is based on cytomorphology and cytochemistry [4], AL caused due to a *KMT2A* translocation is strongly associated with the monocytic lineage, including an association with M5a in 38.5% of cases, M5b or M4 in 21.2% of cases each, and M2 in 9.6% of cases [5].

More than 80 translocation partner genes (TPGs) of the *KMT2A* gene have been described to date, many of which have already been cloned and analyzed at the molecular level [2]. Six TPGs located on the long arm of chromosome 17 have been reported: *KMT2A/GAS7* (17p13), *KMT2A/ACACA* (17q21), *KMT2A/LASP1* (17q21), *KMT2A/MLLT6* (17q21), *KMT2A/RARA* (17q21), and *KMT2A/SEPT9* (17q25) [1].

MLLT6, a PHD finger containing (*MLLT6*) gene, formerly known as *AF17*, is located at 17q12, proximal to *RARA*, which has also been detected in 17q21 [6]. Fusion of the *KMT2A* and *MLLT6* genes is quite rare in AML etiology, as only 2.85% (54 in 1,897) AML cases were confirmed to harbor *KMT2A* rearrangement [5], and 1.55% (9 in 579) cases of *KMT2A*-related AML showed a *KMT2A/MLLT6* fusion [7]. Herein, we report a case of AML with the *KMT2A/MLLT6*

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fusion resulting in a monoblastic morphology, representing the first such case in Korea, and provide context for the literature on AML showing a *KMT2A/MLLT6* fusion reported to date.

CASE REPORT

A 69-year-old female with a history of hypertension and atherosclerosis after having a fever for one week, accompanied by headache, malaise, and epigastric pain, presented to our hospital. Physical examination showed an afebrile state, without hepatosplenomegaly or abdominal tenderness. A complete blood count revealed a leukocyte count of 6.3×10^9/L, hemoglobin at 9.6 g/dL, and the platelet count at 5.4×10^9/L. A peripheral blood smear and manual differential count showed 64% blasts and 26% monocytes. The bone marrow aspirate showed 86.9% large leukemic blasts of an FAB M5a morphology (no faggot cells, Fig. 1A and 1B). Thus, it was diagnosed to be AML. In cytochemistry, the cells were positive for Periodic acid–Schiff stain, and negative for myeloperoxidase, Sudan black B, and dual esterase (<3%). Bone marrow biopsy showed 90% cellularity, and immunohistochemistry showed focal positivity for myeloperoxidase and negativity for terminal deoxynucleotidyl transferase. Immunophenotyping was positive for CD45, CD34, HLA-DR, CD13, CD33, CD64, and CD117, while CD14 was negative.

Chromosomal analysis showed t(11;17)(q23;q21) (Fig. 2), and the reverse transcription (RT)-PCR test (HemaVision-28N, DNA Diagnostics A/S, Risskov, Denmark) was positive for the *KMT2A/MLLT6* fusion (Fig. 3). Treatment was initiated with cytarabine and daunorubicin. After one month of therapy, follow-up bone marrow aspiration showed a hypocellular marrow with 2.49% blasts. No chromosomal analysis was conducted at this point due to the lack of mitotic cells; however, FISH analysis (*KMT2A* Break-apart, Cytocell, Cambridge, UK) identified a rearrangement of the *KMT2A* gene in 2.5% of interphase cells (Fig. 4). No further chemotherapy was administered due to neutropenia. Four months after the initial diagnosis, the leukemia relapsed, and the patient ultimately died from septic shock. Next-generation sequencing (NGS) of the frozen RNA sample (taken at initial diagnosis) and a gene panel test which can identify the gene fusion (Oncomine Myeloid Research Assay, Thermo Scientific Inc., Waltham, MA,

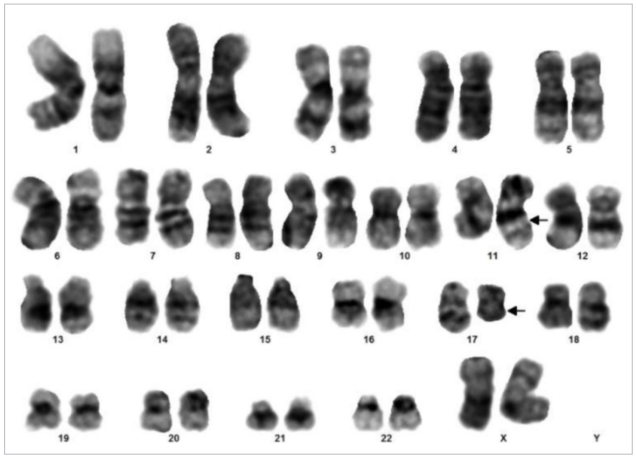

Fig. 2. Chromosomal analysis showed the translocation, 46,XX,t(11;17)(q23;q21)[19]/46,XX[1] (trypsin-Giemsa G-band).

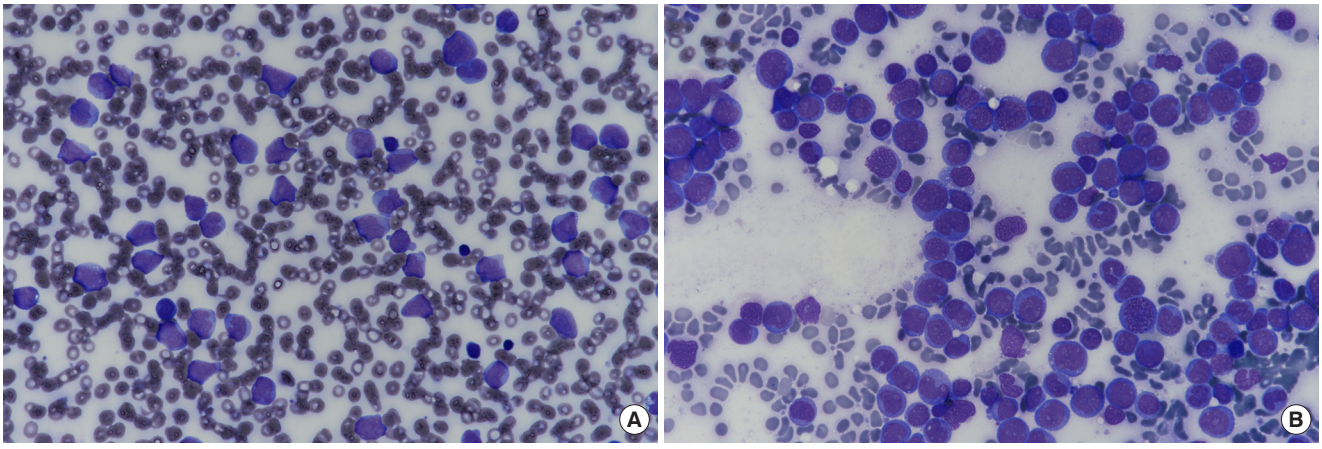

Fig. 1. Peripheral blood and bone marrow smears of the patient. (A) A peripheral blood smear shows monoblasts with abundant cytoplasm and prominent nucleoli (Wright stain, ×400). (B) A bone marrow aspiration smear shows uniform proliferation of monoblasts which have round/folded nuclear and abundant cytoplasm (Wright stain, ×400).

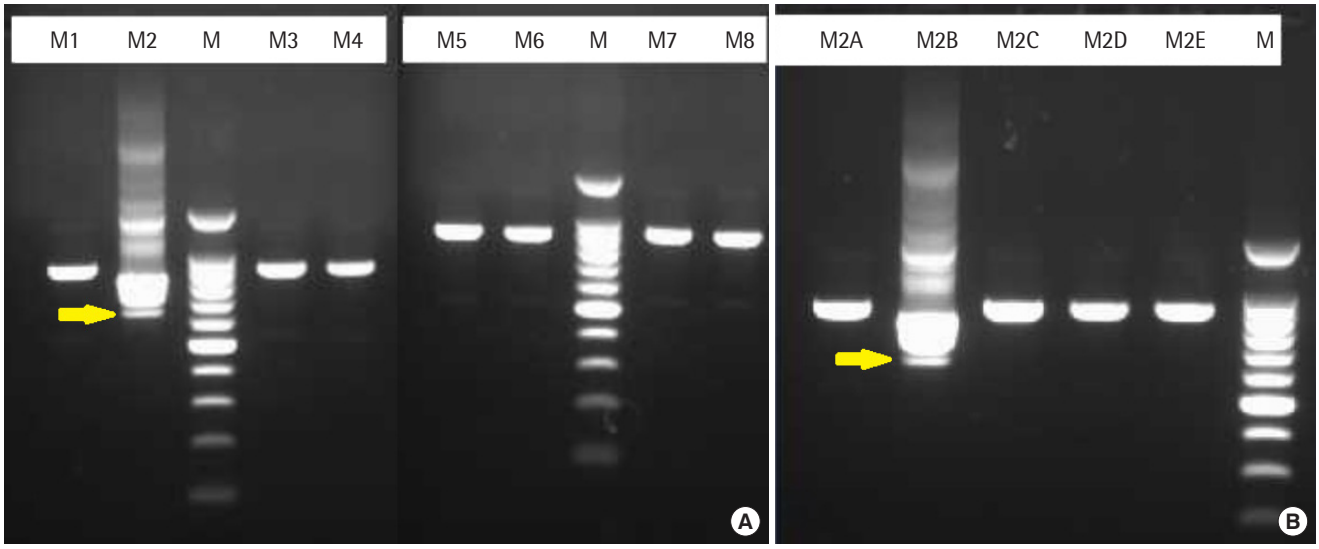

Fig. 3. (A) A reverse transcription-PCR assay shows a band in the second lane (M2). (B) Split-out image shows a band (approximately 600 base pairs) in lane 2B, which indicates *KMT2A/MLLT6* fusion transcripts.

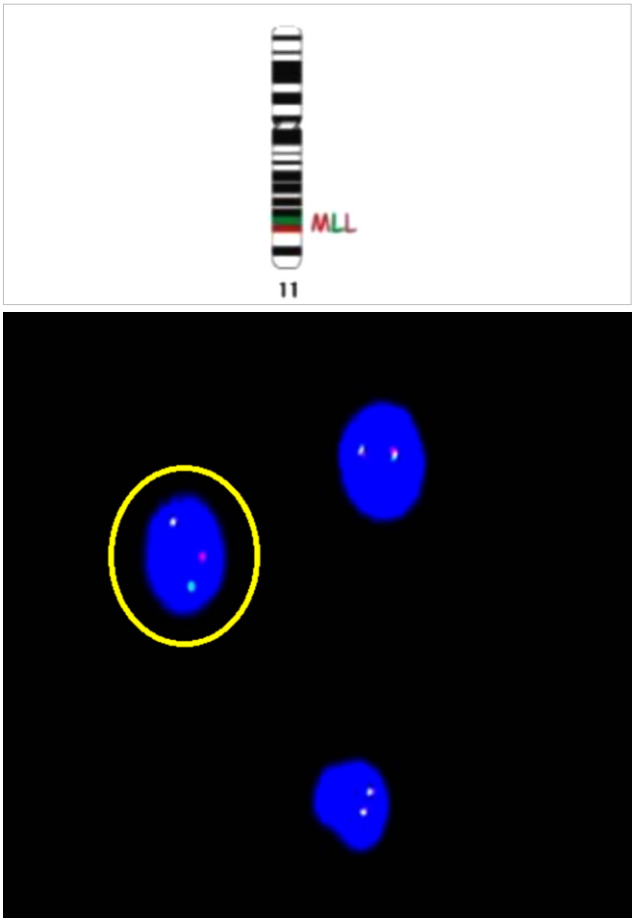

Fig. 4. FISH analysis of a follow-up bone marrow sample with dual color (green and red) probes of *KMT2A*. The circled cell shows one yellow, one green and one red signal, which indicate *KMT2A* rearrangement (in 5/200 interphase cells, reference range: < 2%).

USA) were negative for the fusion gene.

DISCUSSION

A total of 13 cases of AML with the *KMT2A/MLLT6* fusion have been reported to date [2, 8-13] (Table 1). Prasad et al. [8] reported the first such case involving the fusion of *KMT2A* exon 5 and an unknown exon of *MLLT6* based on RT-PCR and the Sanger sequencing method. Suzukawa et al. [9] found a breakpoint at *KMT2A* exon 6 and *MLLT6* exon 9, and confirmed the *KMT2A* rearrangement by FISH analysis. Moore et al. [10] used a gene-specific probe to confirm the *KMT2A/MLLT6* fusion in FISH analysis, whereas Grossman et al. [12] applied NGS for identification of this fusion for the first time with the pyrosequencer (454 FLX, Roche Diagnostics, Rotkreuz, Switzerland).

The *MLLT6* gene is located on chromosome 17q12, but karyotype analysis has provided diverse results such as 17q21 [9-12 and present case] or 17q12-21 [2] in previous literature. This discrepancy is likely due to resolution differences in the karyotype analyses [10] or reflects the molecular heterogeneity of AML patients [11].

In our case, we used NGS sequence analysis (S5 XL, Thermo Scientific Inc.) and a gene panel test for hematologic malignancy (Oncomine Myeloid Research Assay, Thermo Scientific Inc.) to identify the gene fusion, but were unable to find the gene fusion using these methods. *KMT2A* is included in the RNA fusion gene list of the assay [14], but the sample was stored for about two years

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Table 1. Reported cases* with confirmed *KMT2A/MLLT6* fusions and their cytogenetic characteristics

Case No.	Sex	Age	FAB	Chromosome	RT-PCR	FISH	NGS	Reference
1	NA	NA	NA	t(11;17)(q23;q21)	*KMT2A/MLLT6*	NT	NT	[8]
2	M	40	M5b	46,XY,t(11;17)(q23;q21)	*KMT2A/MLLT6*	*KMT2A*†	NT	[9]
3	M	36	M5b	46;XY,t(11;17)(q23;q21)	*KMT2A/MLLT6*	*KMT2A/MLLT6*	NT	[10]
4	M	15	M5a	46,XY,t(11;17)(q23;q12)[10]/46,XY,?t(1;11;17)(p?;q23;q12),der(2)t(2;9)(p?;q?)[7]	*KMT2A/MLLT6*	*KMT2A/MLLT6*	NT	[11]
5	M	63	NA	46,XY,t(11;17)(q23;q21)[6]	*KMT2A/MLLT6*	*KMT2A/MLLT6*	NT	[11]
6	F	30	M5	46,XX,t(11;17)(q23;q21)[16]/47,XX,+8,t(11;17)(q23;q21)[4]	*KMT2A/MLLT6*	*KMT2A/MLLT6*	NT	[11]
7	F	9	M4	46,XX,t(11;17)(q23;q21)[20]	*KMT2A/MLLT6*	ND	NT	[11]
8	M	14	M4	47,XY,+8,t(11;17)(q23;q21)[20]	*KMT2A/MLLT6*	*KMT2A/MLLT6*	NT	[11]
9	M	23	M5a	46,XY,t(11;17)(q23;q21)[4]/46,XY[16]	*KMT2A/MLLT6*	*KMT2A/MLLT6*	NT	[11]
10	F	NA	M4	46;XX,t(11;17)(q23;q12)	*KMT2A/MLLT6*	*KMT2A*†	*KMT2A/MLLT6*	[12]
11	F	41	M5a	47,XX,+8,t(11; 17)(q23; q12)[18]/48,XX,+i(8)(q10),+i(8)(q10),t(11; 17)(q23; q12)[3]	*KMT2A/MLLT6*	*KMT2A/MLLT6*	NT	[13]
12	M	19	M5	46,XY,t(11; 17)(q23; q21)[23]	*KMT2A/MLLT6*	*KMT2A/MLLT6*	NT	[13]
13	M	21	M5b	46,XY,t(11;17)(q23;q12-21)[20]	*KMT2A/MLLT6*	*KMT2A*†	NT	[2]
Present case	F	69	M5a	46,XX,t(11;17)(q23;q21)[19]46,XY[1]	*KMT2A/MLLT6*	*KMT2A*†	ND	Present case

*Cases are listed chronologically; †Only the *KMT2A* rearrangement was confirmed.
Abbreviations: FAB, French-American-British classification of leukemia; RT-PCR, reverse transcription polymerase chain reaction; NGS, next-generation sequencing; NA, not available; NT, not tested; ND, not detected.

at -20°C. Since ribonuclease can remain active at this temperature [15], the low sample quality might explain this negative result.

This is the first case of *KMT2A/MLLT6* fusion in a patient with AML reported in Korea. Kim et al. [16] reported a case of AML M5 with t(11;17)(q23;21) in 2006. In this case, FISH analysis revealed that the breakpoint in chromosome 17 was proximal to the *RARA* gene, but the authors did not perform a RT-PCR test to confirm the *KMT2A* gene rearrangement. Several cases of AML have been reported to be associated with rearrangement of the *KMT2A* gene with the region proximal to the *RARA* gene [17, 18]; however, these might not involve the *KMT2A/MLLT6* fusion, because two other TPGs (besides *MLLT6* and *RARA*) are located in that region [1] and should thus be confirmed by more sensitive assays such as RT-PCR or NGS.

Unlike the FAB classification based on morphological findings, the WHO classification uses cytogenetic characteristics for classification. AML with the *KMT2A/MLLT6* fusion is not yet accepted as a full entity in the WHO classification [19], but the aggressive disease course shown in the present case emphasizes the importance of molecular genetic assays in addition to morphology, cytochemistry and cytogenetic analyses. King et al. [20] reported 12% (7/57) of cases of acute leukemia which had translocation were positive using the RT-PCR method and negative using the conventional cytogenetic assay. Therefore, for patients newly diagnosed as having AL, RT-PCR or FISH should be performed to find chromosomal aberrations not detected by conventional cytogenetic assays.

요 약

라이신메틸전달효소 유전자(*KMT2A, MLL*)는 10%의 급성 백혈병 발병과 관련되어 있고 현재까지 80개 이상의 결합파트너 유전자가 밝혀져 있다. 17번 염색체 장완에는 6개의 결합파트너 유전자가 알려져 있는데 이 중 *MLLT6* 유전자는 17q12에 위치하며 *KMT2A/MLL6* 융합이 동반된 급성골수성백혈병은 소수의 증례가 보고되었다. 이에 저자들은 단핵모구성 양상의 급성골수성백혈병에서 분자유전학적 방법으로 *KMT2A/MLLT6* 융합이 확인된 증례에 대해 보고하고자 한다. 문헌 고찰에 의하면 본 증례는 한국에서 *KMT2A/MLLT6* 융합이 확인된 첫 번째 급성 백혈병 증례이다.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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