AML1 mutation and its coexistence with different transcription factor gene families in *de novo* acute myeloid leukemia: redundancy or synergism

***AML1* mutations were identified in 6.3% of AML patients with chromosomal translocations involving *CBF, PML-RARα, HOX*, or *ETS* transcription factor (TF) gene families. Rare chromosomal abnormalities, t(16;21) and t(7;11), were also found. This study represents the first series to demonstrate the coexistence of known and novel *AML1* mutations with different TF gene mutations. Although the occurrence of two TF gene mutations may appear unnecessary, the possible synergistic mechanism between different TF gene families cannot be excluded and needs to be further explored.**

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Different families of transcription factor genes have been functionally characterized in AML including core binding factor (*CBF*), retinoic acid receptor α (*RARα*), and members of the homeobox (*HOX*) and *ETS* gene family.[1-4] Although loss of function of transcription factor leads to impaired differentiation, a single transcription factor gene mutation by itself is not sufficient to cause acute leukemia in animal models.[1] The human *AML1* gene encodes the major α subunit of the heterodimeric *CBF* complex that plays an important role in normal hematopoiesis.[1,2] Translocation and non-translocation mechanism of *AML1* deregulation have been reported.[2, 5-6] This study evaluates whether *AML1* point mutation could be an additional genetic event associated with leukemic transformation in 80 *de novo* AML cases with chromosomal translocations involving *CBF, PML-RARα, HOX* or *ETS* gene families. Thirty-eight were males and 42 were females with a median age of 41 years (range 15-83 years). Chromosomal abnormalities were described according to the International System for Cytogenetic Nomenclature (ISCN).[7] Exons 3-5 covering the most commonly mutated region of the *AML1* gene were investigated by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) techniques.[5] PCR amplifications of *AML1-ETO* and PML-RARα variants were also performed.[8,9] *FLT3* internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutations were examined in cases with mutated *AML1* using our previously established protocol.[10] *AML1* mutations were found in 6.3%. Three novel mutations were identified including c.412_413insTTTTG, c.292delC, and c.359C→A. All *AML1* mutations occurred in exon 4. 3.2% (1/31) of patients with t(8;21)/*AML1-ETO* and 5.4% (2/37) of patients with t(15;17)/*PML-RARα* cases had *AML1* mutations. *AML1* mutations were also found in 2 patients with rare karyotypic abnormalities, i.e. t(7;11)(p15;p15) involving *HOX* gene family and t(16;21)(p11;q22) involving *ETS* gene family as shown in Table 1. Four out of five patients carrying *AML1* mutations and transcription factor fusion genes were males and three of them were over sixty years old. The patient with t(7;11)(p15;p15) and a novel *AML1* mutation was a 78-year-old woman (no. 875) who also had trisomy 8 and *FLT3-TKD* mutation as shown in Figures 1A. Another novel *AML1* mutation was found in a patient carrying *AML1-ETO* fusion gene (no. 597) as shown in Figure 1B. The presence of bcr1 gene variant is shown in Figure 1C in two patients with *AML1* mutations. Figure 1D shows the sequencing analysis of *AML1-ETO* fusion gene that coexisted with AML1 mutation (c.359C→A), indicating that both mutations occurred in the same allele.

A two-hit model of AML emphasizes the clear collaboration between inactivating mutations of transcription factors and mutations affecting receptor tyrosine kinases. In this study, we challenge the above model by looking for collaboration between two transcription factors from different gene families. Interestingly, despite its relatively low incidence (6.3%), *AML1* mutation could be identified across all different families of transcription factor genes. This present study is unique in many aspects. It is the first to report (i) three novel *AML1* mutations in non-M0 *de novo* AML patients, (ii) the coexistence of *AML1* mutation with *PML-RARα,* and (iii) the coexistence of *AML1* mutation with rare karyotypes involving *HOX* or *ETS* gene family including t(7;11)(p15;p15) and t(16;21)(p11;q22). Although this study represents a small and selective cohort, it is interesting to find such co-occurrence of *AML1* mutation with different members of transcription factor gene families. It could be speculated that abnormalities in more than one transcription factor genes result in the activation of cellular pathways that could together initiate and propagate the leukemic transformation without help from a different class of gene mutations such as tyrosine kinases or their downstream effectors. The fact that rare karyotypes

Table 1. Frequency of AML1 mutation in *de novo* AML patients with chromosome translocations involving transcription factor genes.

Chromosome translocation	*No. of cases*	*Transcription factor fusion gene*	*Frequency of AML1 mutation (%)*	*Type of AML1 mutations*	*Other abnormalities*
t(8;21)(q22;q22)	31	AML1/ETO	1 (3.2)	c.359C→A[a]	–
inv(16)(p13;q22)	5	CBFβ/SMMHC	0 (0)		–
t(15;17)(q22;q11)	37	PML/RARα	2 (5.4)	c.210delC, c.292delC[a]	Monosomy 7
t(5;17)(q31;q11)	1	NPM/RARα	0 (0)	–	–
t(11;17)(p13;q11)	1	PLZF/RARα	0 (0)	–	–
t(7;11)(p15;p15)	2	NUP98/HOXA9	1 (50)	c.412_413ins5[a]	Trisomy 8, FLT3-TKD
t(16;21)(p11;q22)	3	FUS/ERG	1 (33.3)	c.343_364dup22	–
Total	80		5 (6.3)		

[a]*novel mutations.*

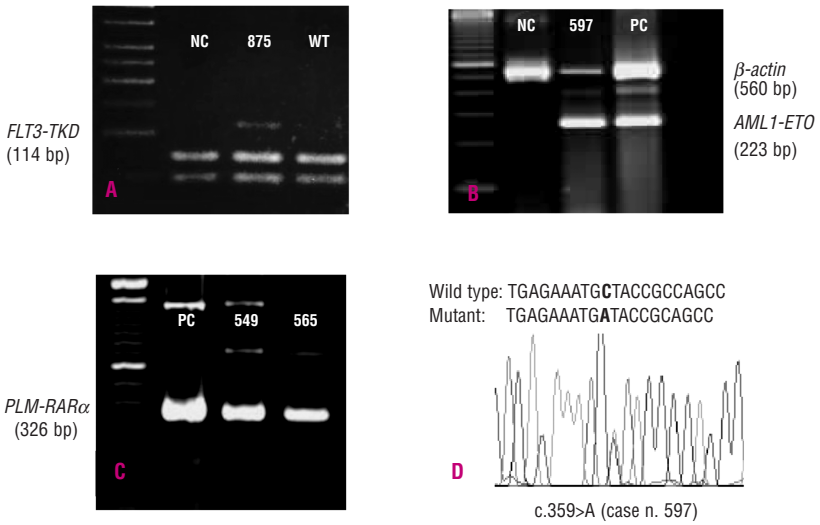

Figure 1. A. *FLT3-TKD* mutation in a patient (case no. 875) with t(7;11) and trisomy 8. B. *AML1-ETO* fusion gene in a patient (case no. 597) with co-existing AML1 mutation. C. PCR products of *PML-RARα* fusion gene (bcr1) in 2 patients with coexisting *AML1* mutations. D) sequencing analysis of *AML1-ETO* fusion gene with co-existing *AML1* mutation (c.359C→A) in the same allele. NC represents a negative control, PC represents a positive control, and WT represents a wild type.

such as t(7;11)(p15;p15) and t(16;21)(11;q22) were identified in this series of Southeast Asian AML patients is also of interest. This phenomenon has been reported to be more frequent in Asian populations and results in the replacement of the transcriptional regulatory region of *HOXA9* by a region of *NUP98*.[3] In this study, *AML1* mutation also occurred in 1 out of 3 patients with t(16;21)(p11;q22). The t(16;21)(p11.2;q21) leads to the production of the *FUS-ERG* fusion gene.[4] Given that t(16;21)(p11;q22) is such a rare karyotype and only <50 cases were reported in literature, it is interesting that *AML1* mutation occurred frequently in this subgroup. In conclusion, rare karyotypes and three novel *AML1* mutations were identified in this study. Although an emerging paradigm of AML emphasizes the concept of collaboration between transcription factors and tyrosine kinases, the collaborative mechanism between various different transcription factor gene families may exist and requires further studies.

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