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Letter to the editor

Germline *CEBPA* mutations in Korean patients with acute myeloid leukemia

To the Editor,

CCAAT/enhancer binding protein alpha (*CEBPA*) mutations occur in 7.5%–12.8% of de novo acute myeloid leukemia (AML) [1–4]. Two types of mutations are possible: an N-terminal frame-shift mutation causing a 42-kDa isoform truncation and 30-kDa isoform overproduction and a C-terminal in-frame mutation in the basic leucine zipper (bZIP) region disrupting the DNA binding and dimerization of *CEBPA* [5]. *CEBPA* double mutations (*CEBPA*dm) usually contain an N-terminal and a C-terminal mutation, which is now known to be a favorable prognostic factor [3]. The majority of mutations are somatic, while 7%–11% are germline mutations that have recently been included in the World Health Organization (WHO) classification as the subgroup 'Acute myeloid leukemia with germline *CEBPA* mutation' [1,3,6,7].

Herein, we evaluated *CEBPA* mutations in 851 patients diagnosed with *de novo* AML at Seoul St. Mary's Hospital in Seoul from January 2014 to June 2017. The median age of the patients was 49 years (range 0–87) and the male to female ratio was 1.2. Detail demographics and diagnosis of the patients are provided in the supplementary Table S1 based on the 2008 WHO classification. The *CEBPA* mutations were analyzed by Sanger sequencing using initial bone marrow aspirates. Two primer pairs were used to cover the whole single exon *CEBPA* gene: for PP1 (576 bp), 5′-TCGCCATGCCGGGAG AACTCTAAC-3′ and 5′-CTGGTAAGGGAAGAGGCCGGCCAG-3′; for PP2 (703 bp), 5′-CCGCTGGTGATCAAGCAGGA-3′ and 5′-CACGGTCT GGGCAAGCCTCGAGAT-3′. Evaluation of obtained sequences was described based on GenBank Accession No. NM_004364.4. This study was approved by the Institutional Review Board of The Catholic University of Korea.

CEBPA mutations were detected in 94 patients (11.0%), 34 of which (36.2%) were *CEBPA* single mutations (*CEBPA*sm) and 60 (63.8%) were *CEBPA*dm, including 2 cases with triple mutations (Fig. 1). Among patients with *CEBPA*sm, 18 (52.9%) had an N-terminal mutation and 16 (47.1%) had a C-terminal mutation. Of 60 patients with *CEBPA*dm, most (n = 56, 93.3%) had both N- and C-terminal mutations, except one (1.7%) with 2 C-terminal mutations and three (5.0%) with homozygous mutations. By Sanger sequencing of buccal mucosa specimens and/or bone marrow aspirates in complete remission from the 94 patients with *CEPBA*mutations, we identified four patients with germline mutations (4.3%; according to Reviewer 2's opinion Table 1 and Supplementary Fig S1–4). Two patients (cases 1 and 3) carried germline N-terminal frameshift mutations, and one had an additional somatic C-terminal in-frame mutation (case 3). The other 2 patients (cases 2 and 4) carried germline C-terminal mutations, and one (case 4) had an additional somatic N-terminal frameshift mutation. The age of onset of AML in patients with germline *CEBPA* mutations was significantly lower than that in patients with somatic mutations (median; 15.5 years (range, 11–35) vs. 46 years (range, 3–80), $P = 0.010$, Mann–Whitney U test using IBM SPSS Statistics 24). This finding is consistent with results from a previous study demonstrating that germline *CEBPA* mutations are typically found in children or young adults with AML [8]. It is known that the germline N-terminal mutations are frame-shift mutations and cause familial AML with near complete penetrance [1,4,9]. On the other hand, the reported germline C-terminal mutations are mostly in-frame missense mutations, including a large family showing 46% penetrance and 3 patients without family history [3,10]. One case of germline C-terminal frame-shift mutation without family history has been reported [11]. It is interesting that we identified a patient (case 2) with a novel frame-shift C-terminal *CEBPA* mutation (c.994_998dup, p.Glu334Alafs*90) with a history of familial AML. The patient's mother had AML and is now disease-free after hematopoietic stem cell transplantation (HSCT). Case 2 carried an *NPM1* mutation, which is also included in WHO classification. In addition, we performed high-throughput sequencing, fragment analysis, and Sanger sequencing and identified additional somatic mutations in *WT1* (n = 2), *FLT3* (n = 2), *GATA2*, *NPM1*, *TET2*, *PTPN11*, and *KIT* genes. We detected an *FLT3* internal tandem duplication in 2 patients; however, the mutant burden was low (10.0% and 5.5%). It is known that secondary mutations in patients with germline *CEBPA* mutation are similar to those observed in sporadic *CEBPA*dm AML. Additional somatic *CEBPA* mutations were found in most patients, and *GATA2*, *WT1*, *EZH2*, *TET2*, and *NRAS* mutations frequently occurred in those patients [8]. Although studies are currently limited, our study combined with previous data revealed convergence on secondary mutations such as *CEBPA*, *GATA2*, and *WT1*. Further studies are needed to scrutinize the association of later mutations and familial leukemogenesis.

To our knowledge, the prognosis of germline *CEBPA* mutations is superior to that of somatic *CEBPA* mutations. We cannot generalize these findings to all germline mutations since a previous study was limited to *CEBPA*dm patients with germline N-terminal and somatic C-terminal mutations [8]. Allogeneic hematopoietic stem cell transplantation (HSCT) following salvage therapy is suggested to mitigate the risk of recurrence, because over 50% of familial patients develop AML recurrence, often occurring as a late event [12]. In this study, 2 patients received HSCT from a family member and have been disease-free for 17 and 10 months. A patient with *CEBPA*dm achieved complete remission after chemotherapy and has maintained a stable hematologic condition for 4 years. A patient with N-terminal *CEBPA*sm failed induction therapy three times and expired 5 months after diagnosis due to complications from infection. Until now, there was no recurrence among the above patients.

In this study, we demonstrated a new germline C-terminal frameshift mutation in a familial AML patient, indicating the need for further establishment of the characteristics and significance of AML with germline *CEBPA* mutation in a large number of cases. Also, it is

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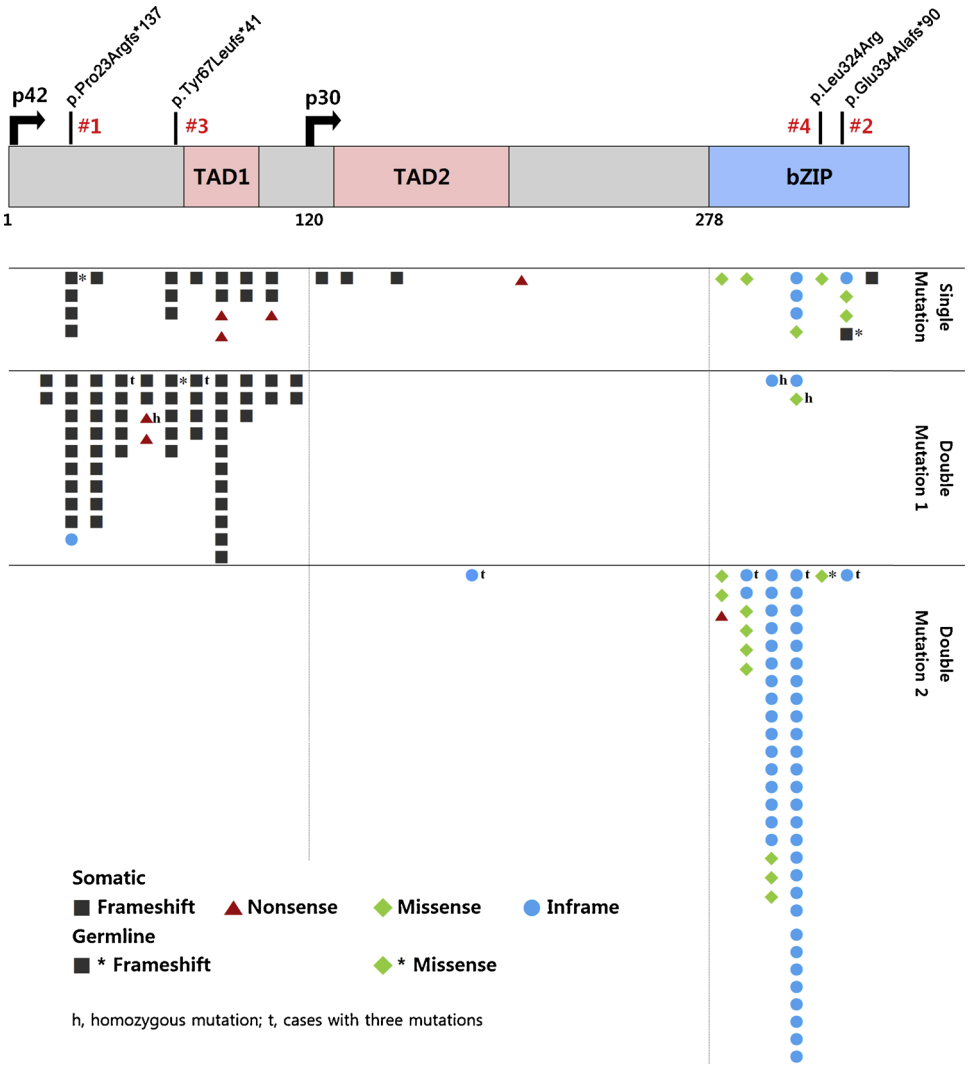

Fig. 1. Locations of 4 germline and 149 somatic *CEBPA* mutations in 68 Korean AML patients.

Table 1
Characteristics of the 4 AML Patients with germline *CEBPA* mutation.

Case No.	Age /Sex	Initial Diagnosis	*CEBPA* mutations		Family history	Other accompanied gene somatic mutation	Current status
			N-terminal	C-terminal			
1	35/F	AML with maturation	**Germline c.68del (p.Pro23Argfs*137)**	None	None	*WT1* (c.1138_1139 in.) *PTPN11* (c.227 A > G)	Expired 5 months after diagnosis in a refractory state
2	17/F	AML with MRC	None	**Germline c.994_998dup (p.Glu334Alafs*90)**	Mother (AML)	*NPM1* (c.860_863dup) *TET2* (c.4075C > T) *FLT3*-ITD (mutant: 10.0%)	Alive with no disease for 17 months after HSCT from sibling (Diagnosed 22 months ago)
3	14/F	AML without maturation	**Germline c.198dup (p.Tyr67Leufs*41)**	Somatic c.934_936dup	None	None	Continuous complete remission for 4 years (Diagnosed 4 years 2 months ago)
4	11/F	AML without maturation	Somatic c.97_112del	**Germline c.971 T > G (p.Leu324Arg)**	None	*WT1* (c.1221_1222insC) *GATA2* (c.953C > T) *KIT* (c.1914 G > C) *FLT3*-ITD (mutant: 5.5%)	Alive with no disease for 10 months after HSCT from mother (Diagnosed 14 months ago)

AML: acute myeloid leukemia; **MRC:** myelodysplasia-related changes; **ITD:** internal tandem duplication; **HSCT**: hematopoietic stem cell transplantation.

important to recognize the presence of familial mutations when preparing HSCT from a related donor. Identification of germline *CEBPA* mutation should be continued because we have yet to determine the prognostic significance, especially for germline *CEBPA*sm and/or germline C-terminal *CEBPA* mutation.

Authors' disclosures of potential conflicts of interest

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

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.leukres.2018.12.003.

References

[1] T. Pabst, M. Eyholzer, S. Haefliger, J. Schardt, B.U. Mueller, Somatic CEBPA mutations are a frequent second event in families with germline CEBPA mutations and familial acute myeloid leukemia, J. Clin. Oncol. 26 (2008) 5088–5093, https://doi.org/10.1200/jco.2008.16.5563.

[2] C.L. Green, K.K. Koo, R.K. Hills, A.K. Burnett, D.C. Linch, R.E. Gale, Prognostic significance of CEBPA mutations in a large cohort of younger adult patients with acute myeloid leukemia: impact of double CEBPA mutations and the interaction with FLT3 and NPM1 mutations, J. Clin. Oncol. 28 (2010) 2739–2747, https://doi.org/10.1200/jco.2009.26.2501.

[3] E. Taskesen, L. Bullinger, A. Corbacioglu, M.A. Sanders, C.A. Erpelinck, B.J. Wouters, et al., Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for CEBPA double mutant AML as a distinctive disease entity, Blood 117 (2011) 2469–2475, https://doi.org/10.1182/blood-2010-09-307280.

[4] I.H. Hollink, M.M. van den Heuvel-Eibrink, S.T. Arentsen-Peters, M. Zimmermann, J.K. Peeters, P.J. Valk, et al., Characterization of CEBPA mutations and promoter hypermethylation in pediatric acute myeloid leukemia, Haematologica 96 (2011) 384–392, https://doi.org/10.3324/haematol.2010.031336.

[5] M.L. Smith, J.D. Cavenagh, T.A. Lister, J. Fitzgibbon, Mutation of CEBPA in familial acute myeloid leukemia, N. Engl. J. Med. 351 (2004) 2403–2407, https://doi.org/10.1056/NEJMoa041331.

[6] A. Corbacioglu, S. Fröhling, C. Mendla, K. Eiwen, M. Habdank, H. Döhner, et al., CEBPA germline mutation screening in cytogenetically Normal acute myeloid leukemia with somatically acquired CEBPA mutations, Blood 110 (2007) 363.

[7] D.A. Arber, A. Orazi, R. Hasserjian, J. Thiele, M.J. Borowitz, M.M. Le Beau, et al., The 2016 revision to the world health organization classification of myeloid neoplasms and acute leukemia, Blood 127 (2016) 2391–2405, https://doi.org/10.1182/blood-2016-03-643544.

[8] K. Tawana, J. Wang, A. Renneville, C. Bodor, R. Hills, C. Loveday, et al., Disease evolution and outcomes in familial AML with germline CEBPA mutations, Blood 126 (2015) 1214–1223, https://doi.org/10.1182/blood-2015-05-647172.

[9] C. Owen, M. Barnett, J. Fitzgibbon, Familial myelodysplasia and acute myeloid leukaemia–a review, Br. J. Haematol. 140 (2008) 123–132, https://doi.org/10.1111/j.1365-2141.2007.06909.x.

[10] A. Pathak, K. Seipel, A. Pemov, R. Dewan, C. Brown, S. Ravichandran, et al., Whole exome sequencing reveals a C-terminal germline variant in CEBPA-associated acute myeloid leukemia: 45-year follow up of a large family, Haematologica 101 (2016) 846–852, https://doi.org/10.3324/haematol.2015.130799.

[11] J.A. Gutman, B. Hoffner, A novel CCAAT/enhancer binding protein alpha germline variant in a case of acute myeloid leukemia, Leuk. Lymphoma 53 (2012) 1006–1007, https://doi.org/10.3109/10428194.2011.638718.

[12] K. Tawana, A. Rio-Machin, C. Preudhomme, J. Fitzgibbon, Familial CEBPA-mutated acute myeloid leukemia, Semin Hematol 54 (2017) 87–93, https://doi.org/10.1053/j.seminhematol.2017.04.001.

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